

Review article

Can allergic diseases be prevented prenatally?

Allergic diseases continue to increase in prevalence, and now affect over a third of the population in many countries. There is evidence that the increase in such diseases has its origins in early life exposures. Pregnancy or early childhood may therefore be critical periods for preventing the onset of allergic disease, and prenatal interventions are an attractive possibility for a population-based preventive approach. Here we review the data suggesting that prenatal exposures are important in the development of allergic disease, and that interventions during this time might be effective in prevention. We find evidence from both animal and human studies that prenatal interventions can influence the future development of allergic disease. There are a number of mechanisms through which such interventions may act to prevent allergic sensitization. We conclude that prenatal interventions have the potential to reduce the burden of allergic disease, and merit continued investigation. Further research in this area may lead to significant public health initiatives.

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The prevalence of allergic diseases such as asthma, eczema and rhinoconjunctivitis increased rapidly in the second half of the 20th century (1, 2). Such diseases now represent a major burden to human health. For example, in Australia, asthma is now the commonest chronic disease of childhood and the commonest cause of hospital admission in children (3). Over a third of children report symptoms of asthma, eczema or allergic rhinitis in many countries (4). A wide range of reports have demonstrated this rapid increase in the prevalence of allergic diseases (5). For example, repeated cross sectional surveys in Aberdeen, Scotland found that 10% of school children had a recent history of wheezing in 1964, 20% in 1989, 25% in 1994 and 28% in 1999 (2). Hospital admissions for systemic allergic disorders such as anaphylaxis increased in the UK up to 7-fold between 1990/2001 and 2000/2001 (6). An increase has also been shown in the prevalence of sensitization and reactivity to specific allergens (7). For example, a 2-fold increase in peanut allergy was reported in both the United States and United Kingdom in recent years (8). Changes in the prevalence of eczema and allergic rhinitis have been less thoroughly evaluated, but many studies report a similar increase in these diseases (2). The reason for this increase in allergic diseases is not yet clear, and it is interesting to note that the prevalence of asthma now appears to be stabilizing in many countries, at least for adults (9). Such diseases certainly have a significant genetic component to their aetiology—twin studies suggest that up to 73% of the variation in asthma risk between individuals can be

explained by heritable factors (10). However, the increase in allergic disorders in the second half of the 20th century has occurred too rapidly for germline genetic changes to underlie this trend. It must be explained by changes in one or more environmental exposures, perhaps acting through epigenetic mechanisms (11). If these environmental exposures can be identified and modified, then prevention of a major part of the global burden of allergic disease may be possible.

Several environmental factors have been consistently associated with risk of developing allergic disease. These include birth order, family size, and some microbiological exposures. In many cases exposure in the first years of life appears to be important for influencing the risk of allergic disease. Studies of the effects of early childcare and of international adoptees suggest that the early postnatal period is important (12, 13). However, in other studies, the relative importance of the prenatal and early postnatal periods is unclear (14). Understanding the role of prenatal influences in the development of allergic disease is of particular relevance to the implementation of prevention strategies. Prenatal approaches have considerable advantages in terms of convenience and compliance when compared with postnatal infant or child-directed interventions. Moreover, if the prenatal period is important in the genesis of allergic disease, then postnatal preventive measures may have only limited efficacy. In this article, we review the evidence that prenatal exposures are important in the development of allergic

disease, and that preventive interventions during this period may be effective.

Are prenatal exposures important in the development of allergic disease?

Allergic diseases have been reported in infants as young as 8 days, and in premature infants (15). Clinical experience therefore suggests that the fetal immune system may be primed for the development of allergic disease before birth. Research evidence that prenatal exposures may influence the future development of allergic disease comes from epidemiological and immunological studies.

Epidemiological studies

A consistent finding from epidemiological studies is that the presence of older siblings is associated with reduced risk of developing allergic disease (16). A number of studies now suggest that this protective effect may act prenatally – for example, low birth order is associated with raised IgE at the time of birth (17). This sibling effect may be mediated by maternal influences on the developing fetus, as there is also a direct impact of pregnancy on maternal allergic markers. Cross-sectional surveys have found total maternal IgE levels and the risk of maternal allergic rhinitis to be inversely related to the number of live births they have delivered, and a cohort study suggested that both allergic sensitization and allergic rhinitis may wane in women who have further pregnancies (18–20). If pregnancies protect the mother against allergic sensitization and disease, then this may modify the *in utero* environment for future pregnancies, in turn reducing the fetus' risk of allergic sensitization and disease. A number of other epidemiological findings suggest that prenatal exposures may be important in the development of allergic disease. These include the increased risk of allergic disease seen with prenatal infections and antibiotic exposures in some studies, the positive association of head circumference at birth with total IgE level in later life, and the established effects of maternal smoking during pregnancy on lung growth and early wheezing (14, 21–24).

Immunological studies

A wide range of immunological abnormalities have been reported in cord blood which are associated with the subsequent development of allergic disease [reviewed in (25)]. While some of these cord blood abnormalities may reflect the individual's genetic heritage, it is likely that *in utero* fetal exposures also play a role. The most consistent abnormality reported by our laboratory and others is reduced mitogen and allergen-induced IFN γ secretion in cord blood mononuclear cells (26–28). The defect in IFN γ secretion appears in many studies to be

independent of atopic family history (29, 30). A similar defect in IFN γ secretion has been found later in life in those with allergic diseases such as eczema and asthma and in those with high total IgE levels (31). Prescott et al. found the maturation of IFN γ responses to be delayed in those with allergic disease, and our laboratory has found that resolution of asthma in adulthood is associated with a normalization of IFN γ responses (26, 32). It therefore seems that the defective IFN γ responses found in the cord blood of those who develop allergic disease are closely related to the cellular mechanisms underlying such diseases. A second early association of allergic disease is raised cord blood IgE level. The positive predictive value of raised cord blood IgE for the future development of allergic disease varies from 35% to 72% in different studies (33). A relationship between raised cord blood IgE and defective IFN γ secretion has also been reported by some investigators (34). The fact that a range of immune abnormalities are already present at the time of birth suggests interventions aimed at preventing allergic disease may need to occur in the prenatal period.

Can prenatal interventions prevent the development of allergic disease?

We have seen that prenatal exposures may be important in the development of allergic disease. If this is the case then the prenatal period may represent an ideal time for the implementation of interventions to prevent allergic disease. Evidence from intervention studies supporting this concept is summarized in Table 1, and discussed in more detail below.

Human intervention studies

A number of clinical trials have explored the efficacy of prenatal interventions in preventing allergic disease.

Omega 3 fatty acids. Omega 3 fatty acids are found in high concentration in oily fish, and are known to have potent *in vitro* anti-inflammatory effects mediated by a decrease in arachidonic acid-derived mediators (35). Animal studies suggest that maternal and neonatal dietary fatty acid composition may affect the development of neonatal tolerance (36). Two intervention trials to date have examined the role of omega 3 fatty acids in the early prevention of allergic disease. A large randomized controlled trial of combined pre and postnatal dietary supplementation with omega 3 fatty acids in childhood did not have significant effects on the development of wheeze or allergic sensitization at 3 years (37). The intervention in this study commenced at 36 weeks gestation, and many infants did not have direct dietary supplementation until 6 months postnatal age so there was only limited prenatal intervention here. A smaller randomized controlled trial used a much higher dose of

Table 1. Selected intervention studies suggesting that prenatal approaches may be effective in preventing allergic sensitization and disease

Study	Subjects	Intervention	Principal outcomes
Dunstan et al. (39)	Humans	Fish oil supplementation from 20 weeks gestation to delivery	Reduced prevalence of severe eczema in offspring at 1 year (2.5% vs 11.6% controls)
Kalliomaki et al.* (40)	Humans	Probiotic supplementation from 36 weeks gestation to 6 months postnatal age	Reduced prevalence of eczema at 2 and 4 years age (RR 0.57 at 4 years)
Falth-Magnusson and Kjellman (43)	Humans	Strict egg and cow's milk avoidance from 28 weeks gestation to delivery	Increased egg allergy in offspring at 5 years (egg avoidance group 7% vs controls 0%)
Woodcock et al.* (44)	Humans	Strict house dust mite avoidance from 16 weeks gestation through early childhood	Increased sensitization to house dust mite in offspring at 3 years age (RR 2.85)
Jarrett and Hall (45)	Rats	Intraperitoneal egg albumin in aluminium hydroxide adjuvant prior to conception	50% reduction in specific IgE response of offspring to postnatal egg albumin sensitization
Telemo et al. (47)	Guinea pigs	Cow's milk protein fed to mothers from birth and throughout pregnancy	Antigen-specific hyporesponsiveness to cow's milk protein in the offspring postnatally
Melkild et al. (78)	NIH/OlaHsd mice	Intraperitoneal ova in aluminium hydroxide adjuvant at different times during pregnancy	Reduced ova specific IgE response to intraperitoneal ova postnatally in the offspring of mothers treated in early pregnancy
Victor et al. (49)	A/Sn mice	Intraperitoneal derp1 in aluminium hydroxide adjuvant prior to conception	Reduced derp1 specific IgE response to intraperitoneal derp1 postnatally in offspring
Uthoff et al. (48)	BALB/c mice	Intraperitoneal ova in aluminium hydroxide adjuvant prior to conception, and ova aerosol every second day through pregnancy	Complete abrogation of ova specific IgE response to intraperitoneal ova injection postnatally
Herz et al. (50)	BALB/c mice	Intraperitoneal ova in aluminium hydroxide adjuvant prior to conception, and ova aerosol every second day through pregnancy	Reduced IFN γ secretion by neonatal monocytes
Blumer et al. (52)	BALB/c mice	Intraperitoneal LPS prior to conception, and intranasal LPS every third day through pregnancy	(i) 66% reduction in IgE response of offspring to postnatal ova sensitization (ii) 75% reduction in IL5 and IL13 responses of offspring to postnatal ova sensitization (iii) Reduction in airway eosinophil response of offspring to the induction of experimental asthma

*Relative importance of pre- and postnatal intervention is unclear in these studies.

omega 3 fatty acids in the form of fish oil, and the supplement was only given prenatally (38). In this study, fish oil supplementation in women with allergic disease from 20 weeks gestation to delivery was associated with reductions in both egg sensitization and severe eczema in their infants at 1 year age (39). This small study supports the concept of prenatal prevention of allergic disease.

Probiotic supplementation. Probiotic supplementation has also been studied in the early prevention of allergic disease. The first such study was a randomized controlled trial of the probiotic *Lactobacillus* GG (LGG) in the prevention of eczema. LGG or placebo was given daily to women from 36 weeks gestation to delivery, and then continued postnatally for 6 months either directly to the infant or to the breast feeding mother (40). There was a 50% reduction in eczema at 2-year follow-up, and 43% reduction at 4 years. There was also a reduction in exhaled nitric oxide levels at 4 years age, suggesting a possible protective effect against inflammatory airway disease (41). It is not clear whether pre or postnatal LGG was more important for this protective effect. However, a protective role for prenatal treatment is suggested because a treatment benefit was seen even in those infants who did not receive LGG directly in the postnatal period (42). In

infants who were breastfed LGG was administered to their mother postnatally, and only formula fed infants received LGG directly in the postnatal period. In breastfed infants, LGG treatment led to a 68% reduction in the risk of developing eczema. In formula fed infants LGG treatment led to a 34% reduction in eczema risk. Therefore prenatal LGG may have provided the greatest protective benefit against eczema. It is also likely that breastfeeding enhanced the protective effects of LGG treatment. Further studies to explore the relative roles of pre- and postnatal probiotic supplementation in eczema prevention are warranted.

Allergen avoidance. Some prenatal interventions have been shown to *increase* the risk of allergic disease or allergic sensitization. These include some human studies of allergen avoidance during pregnancy. For example, in a Swedish trial of strict egg and cow's milk avoidance in the last trimester of pregnancy, an increase in egg allergy at 5 years age was found in those born to mothers in the active treatment group (43). A similar effect of prenatal allergen avoidance was found in the Manchester Asthma and Allergy Study, which investigated pre- and postnatal avoidance of inhaled allergens. Here stringent house dust mite avoidance measures were undertaken from 16 weeks

gestation and continued throughout childhood in a high risk cohort – the avoidance measures reduced house dust mite allergen levels in maternal bedding by 97% prenatally and overall house dust mite allergen exposure during the first 3 years of life was reduced by over 50% (44). Although there was a reduction in a measure of airway resistance at 3 years age, the Manchester study found no difference in the overall risk of allergic disease in the first 3 years. Moreover, there was an increase in allergic sensitization to house dust mite (risk ratio 2.85) in the intervention group at this time (44). These studies suggest that prenatal allergen avoidance increases the risk of postnatal sensitization to the same allergen. This provides further support for the importance of prenatal exposures in influencing the development of allergic disease or sensitization.

Animal intervention studies

Allergen exposure. Animal work investigating the dose, route and timing of antigen exposure in the development of allergic immune responses supports the importance of prenatal antigen exposures in the development of allergic disease. Such work suggests that modulation of prenatal antigen exposure may be an effective way to prevent allergic disease. Over 20 years ago, the induction of maternal antigen-specific IgG antibodies in rats was first found to protect the offspring against subsequent allergic sensitization (45). Postnatal administration of immune serum to neonates born of unimmunized mothers, and feeding of such neonates by immunized mothers have also resulted in antigen-specific IgE suppression in offspring (46). Telmo and colleagues showed in a guinea pig model that maternal dietary antigen experience, and gestational antigen exposure are both important for the development of immunological tolerance to milk proteins (47). Guinea pigs born to mothers who were fed cow's milk from birth and throughout pregnancy had a high degree of tolerance to a milk challenge. If milk exposure was ceased during late pregnancy, or the introduction of milk to the mother's diet was delayed to adulthood then the offspring could be sensitized to cow's milk (47). Murine studies have similarly shown that antigen exposure preconceptually with or without continuous exposure through pregnancy protected offspring against postnatal IgE mediated sensitization in an antigen-specific manner (48). These effects are likely to be related to increased maternal antigen-specific IgG, which has an inhibitory effect in the neonate on the development of an IgE response (49). A caution regarding these studies is that murine T lymphocytes are known to mature later in pregnancy than human T lymphocytes, and the relative importance of prenatal and postnatal immune stimulation may differ between mice and humans (50). However, the studies do demonstrate that prenatal exposures can modify subsequent allergic responses in a range of animals, by inducing maternal antigen-specific IgG.

LPS exposure. A second line of animal work which supports the efficacy of prenatal interventions is the study of prenatal LPS exposure. Experiments in adult rodents have shown that LPS exposure prior to or during ova sensitization can protect against the development of ova-specific IgE (51). As initial allergen exposure often occurs *in utero*, this work suggested that prenatal exposure to bacterial components such as LPS may prevent allergic sensitization. Subsequently, Blumer and colleagues confirmed the protective effect of prenatal LPS exposure. They found that prenatal LPS exposure in mice led to an attenuation of the offspring's IgE, IL-5, IL-13 and airway eosinophil responses to ova sensitization (52). Prenatal LPS treatment was also associated with an increase in neonatal IFN γ response, a well-recognized marker in humans for low risk of subsequent allergic disease. Interestingly, there was no effect on airway hyper-responsiveness after ova sensitization, however, this is consistent with the studies of LPS exposure in adult mice and rats (51). These studies suggest that in rodents prenatal LPS exposure via maternal exposure has similar immune effects on the fetus to direct LPS exposure in the adult animal. Both forms of exposure protect against the development of allergic immune responses to novel antigens. This provides strong support for the efficacy of prenatal microbial interventions in preventing allergic sensitization in rodents. However, it should be noted that high-dose LPS exposure in pregnant mice was associated with high rates of pregnancy loss, and this may limit the application of such an approach in humans (52).

What are the mechanisms by which prenatal interventions may prevent allergic disease?

It is clear from the evidence cited above that prenatal interventions may impact on the future development of allergic disease, at least in animal models. In order to develop effective human prenatal interventions, an understanding of the mechanisms through which these interventions act would be helpful. A number of prenatal interventions may impact on fetal and neonatal immune development, and these can be broadly divided into antigen-specific interventions and non antigen-specific interventions. Possible mechanisms underlying the effects of such interventions are discussed below, and presented in Fig. 1.

Prenatal antigen exposures may modify infant immune responses

Fetal immune responses to allergens. Food allergens and to a lesser extent aeroallergens are actively and selectively transferred across the human placenta (53). For example, it has been shown in an *ex-vivo* placental model that around 1 in 1000 ovalbumin molecules cross the placenta (54). This is supported by *in vivo* studies demonstrating that in the majority of pregnant women with detectable

Antigen-Specific Mechanisms 1. Direct stimulation of fetal immune response via transplacental allergen exposure. 2. Induction of maternal antigen-specific IgG, transferred transplacentally to the developing fetus/neonate. Non Antigen-Specific Mechanisms 1. Stimulation of fetal immune responses via placental pattern-recognition molecules e.g. LPS stimulation of placentally expressed TLR4. 2. Stimulation/inhibition of fetal immune responses via micronutrients passing transplacentally to the fetal compartment e.g. omega 3 fatty acids. 3. Induction of maternal soluble immune mediators, acting on the fetus transplacentally or via breast milk excretion. 4. Alteration of the maternal microbial environment, with consequent alteration of the microbial environment influencing neonatal immune development.

Figure 1. Mechanisms by which prenatal interventions may prevent allergic sensitization or disease.

circulating ovalbumin, ovalbumin is also detectable in umbilical cord blood at birth (55). Aeroallergens such as Derp1 have also been detected in human umbilical cord blood, and allergen specific fetal lymphocyte responses from 22 weeks gestation (56). The presence of antigen presenting cells expressing costimulatory molecules in the fetal intestine from 16 weeks gestation suggests that the fetal immune response is mature enough at this stage to respond to transplacentally acquired antigens (57). Indeed it is well established from studies of helminth infection and from vaccine studies that prenatal microbial exposures can result in fetal antigen-specific tolerance, antigen-specific priming or fetal IgM responses depending on the nature of the prenatal stimulus (58–60). *In utero* T cell priming to allergens has also been shown to occur commonly (61). It has been hypothesized that the small populations of allergen-specific T cells found in the newborn may be the basis for postnatal responses to allergen exposure which can lead to long-term allergic sensitization (62). However, these cells behave in a distinct way to allergen-specific T cells from adults, and recent work suggests that early T cell responses to allergens usually fail to lead to typical memory T cell development (63). *In utero* allergen priming of humoral responses also occurs and can be clinically significant. For example, prospective studies have shown that up to 76%

of infants with IgE mediated cow's milk allergy already have low levels of cow's milk-specific IgE present at birth (64). It is therefore clear that the human fetus is both exposed to allergen and is able to mount allergen-specific immune responses *in utero*. These immune responses may be related to the future development of allergic disease, and have the potential to be modulated by prenatal interventions.

Induction of maternal IgG. The level of antigen-specific IgG in humans is related to their level of exposure to the same antigen (65). A prospective study in humans found that high levels of maternally transferred IgG to inhalant allergens in cord blood may protect against the future development of allergen specific IgE and atopic disease (66). This suggests a mechanism through which reduced prenatal exposure may lead to increased postnatal allergic sensitization in an antigen-specific manner. The study accords with the animal intervention studies described above, and with epidemiological studies showing that those born just before the birch and grass pollen seasons (and therefore likely to have low levels of maternally transferred pollen-specific IgG) are at higher risk of developing birch pollen sensitization as children, and birch and grass pollen allergies as adults (67–69). Reviews of allergic outcomes in children whose mothers received grass pollen immunotherapy during pregnancy are also of interest. Based on animal data one might expect immunotherapy to protect the offspring against allergic sensitization through the transfer of maternal allergen-specific IgG. However, these studies have yielded conflicting data, and their design is not robust enough to draw firm conclusions regarding the effects of prenatal exposure to immunotherapy (70, 71).

While maternal allergen-specific IgG does clearly protect offspring from allergic sensitization, at least in animals, the mechanism for this is not clear. The protective effect may be related to the antibodies themselves, or to the formation of allergen-antibody complexes, which have been commonly found in human cord blood (72). Maternal IgG may bind to FcγRIIb on neonatal B cells, and subsequent cross-linking of FcγRIIb and the B cell receptor by antigen may lead to inhibition of B cells. Maternal IgG may also mask antigenic determinants, and IgG coated antigen may be cleared and destroyed by phagocytosis. Transplacental antigen exposure *per se* has different effects on the developing fetal immune system to those of maternal antigen-specific IgG, and the ways in which these two effects interact are not well understood.

Other prenatal exposures may also modify infant immune responses

Fetal immune responses to nonantigen-specific exposures. Prenatal exposure to maternally ingested nutrients or microbial components may influence the developing

fetal immune system in a nonantigen-specific manner. For example, prenatal exposure to LPS may act on the fetal compartment of immunity via placental pattern recognition molecules. The human placenta expresses high levels of TLR4, which is important in the recognition of LPS (73). This high level of TLR4 expression may allow maternal LPS exposure to influence the developing fetal immune system as seen in the animal models described above. It has recently been shown that the circulating mononuclear cells of high risk neonates have a reduced expression of TLR2 and TLR4 relative to their mothers – if this has relevance to placental expression patterns, then it suggests that maternal allergy may reduce the effect of microbial products on the infant's developing immune system (74). Maternally ingested nutrients may also impact on the fetal immune system transplacentally. For example, maternal antioxidant intake during pregnancy may have protective effects against fetal development of allergic disease (75). In one study, omega 3 fatty acid supplementation during pregnancy led to increased levels of cord blood erythrocyte omega 3 fatty acid levels in the neonate (39). Prenatal supplementation also led to significant immune effects in the neonate, where cord blood cytokine responses to a range of stimuli were reduced, as were baseline levels of the Th2 cytokine IL13 (38).

Indirect effects via a modified maternal environment. Some prenatal interventions that are not antigen-specific may act indirectly via modulation of the maternal environment, which strongly influences infant development in both the fetal and neonatal periods. For example, prenatal interventions may induce regulatory or immunostimulatory cytokine secretion in the pregnant mother. Soluble factors may then pass transplacentally and influence fetal immune development, or may act on the infant postnatally via breast milk secretion. Probiotic supplementation has been shown to increase secretion of the regulatory cytokine TGF β 2, and this may affect the infant's immune responses either via transplacental effects or via increased breast milk

secretion of TGF β 2 (42). One concern surrounding prenatal interventions designed to act via maternal immune stimulation is that the secretion of some cytokines such as IFN γ is strongly associated with pregnancy loss. The safety of such prenatal interventions should therefore be carefully evaluated. A final mechanism that may mediate the effects of prenatal interventions relates to live microbial supplementation. Maternal probiotic supplementation has been shown to influence the neonatal intestinal microbiota postnatally, and the neonatal intestinal microbiota in turn is a strong influence on postnatal immune development including IgE responses (76, 77). So maternal probiotic supplementation may indirectly affect the development of allergic immune responses in the neonate by altering their intestinal microbiota.

Conclusions

The prenatal period is an attractive candidate for primary interventions aimed at reducing the growing burden of allergic disease. There are considerable logistical advantages inherent in many prenatal interventions when compared with infant or early childhood interventions, which means they have the potential to be applied at a population level. Indeed they may be more effective than postnatal interventions, because immune abnormalities are already present at birth in those who develop allergic disease. Here we have reviewed the current evidence that prenatal approaches are realistic – we find evidence that early life exposures are important in the development of allergic disease, and present mechanisms by which prenatal interventions can influence immune development in the infant. A number of intervention studies in animals and humans support the concept that allergic disease might be prevented prenatally. Prenatal approaches to preventing allergic disease are an exciting area of study with the potential to lead to significant public health interventions, and further investigation is warranted in this area.

References

1. Stewart AW, Mitchell EA, Pearce N, Strachan DP, Weilandon SK. The relationship of per capita gross national product to the prevalence of symptoms of asthma and other atopic diseases in children (ISAAC). *Int J Epidemiol* 2001;**30**:173–179.
2. Devanny A, Wassall H, Ninan T, Omran M, Khan SD, Russell G. Respiratory symptoms and atopy in children in Aberdeen: questionnaire studies of a defined school population repeated over 35 years. *BMJ* 2004;**329**:489–490.
3. AIHW. Asthma in Australia 2005. Canberra: AIHW Australian Centre for Asthma Monitoring, 2005.
4. 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.
5. Woolcock AJ, Peat JK. Evidence for the increase in asthma worldwide. *Ciba Found Symp* 1997;**206**:122–134; discussion. 134–139, 157–159.
6. Gupta R, Sheikh A, Strachan D, Anderson HR. Increasing hospital admissions for systemic allergic disorders in England: analysis of national admissions data. *BMJ* 2003;**327**:1142–1143.

7. Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005;**116**:377–383.
8. Grundy J, Matthews S, Bateman B, Dean T, Arshad SH. Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol* 2002;**110**:784–789.
9. von Hertzen L, Haahela T. Signs of reversing trends in prevalence of asthma. *Allergy* 2005;**60**:283–292.
10. Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T. Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. *Eur Respir J* 1999;**13**:8–14.
11. Bousquet J, Jacot W, Yssel H, Vignola AM, Humbert M. Epigenetic inheritance of fetal genes in allergic asthma. *Allergy* 2004;**59**:138–147.
12. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;**343**:538–543.
13. Hjern A, Rasmussen F, Hedlin G. Age at adoption, ethnicity and atopic disorder: a study of internationally adopted young men in Sweden. *Pediatr Allergy Immunol* 1999;**10**:101–106.
14. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;**358**:1129–1133.
15. Swart JF, Ultee K. Rectal bleeding in a preterm infant as a symptom of allergic colitis. *Eur J Pediatr* 2003;**162**:55–56.
16. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;**299**:1259–1260.
17. Karmaus W, Arshad H, Mattes J. Does the sibling effect have its origin *in utero*. Investigating birth order, cord blood immunoglobulin E concentration, and allergic sensitization at age 4 years? *Am J Epidemiol* 2001;**154**:909–915.
18. Forastiere F, Sunyer J, Farchi S, Corbo G, Pistelli R, Baldacci S et al. Number of offspring and maternal allergy. *Allergy* 2005;**60**:510–514.
19. Karmaus W, Arshad SH, Sadeghnejad A, Twiselton R. Does maternal immunoglobulin E decrease with increasing order of live offspring? Investigation into maternal immune tolerance. *Clin Exp Allergy* 2004;**34**:853–859.
20. Harris JM, White C, Moffat S, Mills P, Newman Taylor AJ, Cullinan P. New pregnancies and loss of allergy. *Clin Exp Allergy* 2004;**34**:369–372.
21. McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: a birth cohort study using the West Midlands General Practice Database. *Am J Respir Crit Care Med* 2002;**166**:827–832.
22. Xu B, Jarvelin MR, Pekkanen J. Prenatal factors and occurrence of rhinitis and eczema among offspring. *Allergy* 1999;**54**:829–836.
23. Xu B, Pekkanen J, Jarvelin MR, Olsen P, Hartikainen AL. Maternal infections in pregnancy and the development of asthma among offspring. *Int J Epidemiol* 1999;**28**:723–727.
24. Leadbitter P, Pearce N, Cheng S, Sears MR, Holdaway MD, Flannery EM et al. Relationship between fetal growth and the development of asthma and atopy in childhood. *Thorax* 1999;**54**:905–910.
25. Allam JP, Zivanovic O, Berg C, Gembruch U, Bieber T, Novak N. In search for predictive factors for atopy in human cord blood. *Allergy* 2005;**60**:743–750.
26. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999;**353**:196–200.
27. Tang ML, Kemp AS, Thorburn J, Hill DJ. Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet* 1994;**344**:983–985.
28. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994;**24**:423–430.
29. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon-gamma production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995;**96**(5 Pt 1):652–660.
30. Hagendorens MM, Van Bever HP, Schuerwegh AJ, De Clerck LS, Bridts CH, Stevens WJ. Determination of T-cell subpopulations and intracellular cytokine production (interleukin-2, interleukin-4, and interferon-gamma) by cord blood T-lymphocytes of neonates from atopic and non-atopic parents. *Pediatr Allergy Immunol* 2000;**11**:12–19.
31. Smart JM, Kemp AS. Increased Th1 and Th2 allergen-induced cytokine responses in children with atopic disease. *Clin Exp Allergy* 2002;**32**:796–802.
32. Smart JM, Horak E, Kemp AS, Robertson CF, Tang ML. Polyclonal and allergen-induced cytokine responses in adults with asthma: resolution of asthma is associated with normalization of IFN-gamma responses. *J Allergy Clin Immunol* 2002;**110**:450–456.
33. Michel FB, Bousquet J, Greillier P, Robinet-Levy M, Coulomb Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1980;**65**:422–430.
34. Kondo N, Kobayashi Y, Shinoda S, Takenaka R, Teramoto T, Kaneko H et al. Reduced interferon gamma production by antigen-stimulated cord blood mononuclear cells is a risk factor of allergic disorders – 6-year follow-up study. *Clin Exp Allergy* 1998;**28**:1340–1344.
35. Calder PC. N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* 2003;**38**:343–352.
36. Korotkova M, Telemo E, Hanson LA, Strandvik B. Modulation of neonatal immunological tolerance to ovalbumin by maternal essential fatty acid intake. *Pediatr Allergy Immunol* 2004;**15**:112–122.
37. Peat JK, Miharshahi S, Kemp AS, Marks GB, Tovey ER, Webb K et al. Three-year outcomes of dietary fatty acid modification and house dust mite reduction in the Childhood Asthma Prevention Study. *J Allergy Clin Immunol* 2004;**114**:807–813.
38. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clin Exp Allergy* 2003;**33**:442–448.
39. Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG et al. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003;**112**:1178–1184.
40. 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.

41. 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.
42. Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breastfeeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 2002;**109**:119–121.
43. Falth-Magnusson K, Kjellman NI. Allergy prevention by maternal elimination diet during late pregnancy—a 5-year follow-up of a randomized study. *J Allergy Clin Immunol* 1992;**89**:709–713.
44. Woodcock A, Lowe LA, Murray CS, Simpson BM, Pipis SD, Kissen P et al. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am J Respir Crit Care Med* 2004;**170**:433–439.
45. Jarrett EE, Hall E. The development of IgE-suppressive immunocompetence in young animals: influence of exposure to antigen in the presence or absence of maternal immunity. *Immunology* 1984;**53**:365–373.
46. Roberts SA, Turner MW. Specific suppression of rat IgE responses with milk from immunized females and with feeds of serum antibody. *Immunology* 1983;**48**:195–199.
47. Telemo E, Jakobsson I, Westrom BR, Folkesson H. Maternal dietary antigens and the immune response in the offspring of the guinea-pig. *Immunology* 1987;**62**:35–38.
48. Uthoff H, Spenner A, Reckelkamm W, Ahrens B, Wolk G, Hackler R et al. Critical role of preconceptional immunization for protective and nonpathological specific immunity in murine neonates. *J Immunol* 2003;**171**:3485–3492.
49. Victor JR, Jr, Fusaro AE, Duarte AJ, Sato MN. Preconception maternal immunization to dust mite inhibits the type I hypersensitivity response of offspring. *J Allergy Clin Immunol* 2003;**111**:269–277.
50. Herz U, Joachim R, Ahrens B, Scheffold A, Radbruch A, Renz H. Prenatal sensitization in a mouse model. *Am J Respir Crit Care Med* 2000;**162**(3 Pt 2):S62–S65.
51. Tulic MK, Wale JL, Holt PG, Sly PD. Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;**22**:604–612.
52. Blumer N, Herz U, Wegmann M, Renz H. Prenatal lipopolysaccharide-exposure prevents allergic sensitization and airway inflammation, but not airway responsiveness in a murine model of experimental asthma. *Clin Exp Allergy* 2005;**35**:397–402.
53. Szepefalusi Z, Loibichler C, Pichler J, Reisenberger K, Ebner C, Urbanek R. Direct evidence for transplacental allergen transfer. *Pediatr Res* 2000;**48**:404–407.
54. Edelbauer M, Loibichler C, Nentwich I, Gerstmayr M, Urbanek R, Szepefalusi Z. Maternally delivered nutritive allergens in cord blood and in placental tissue of term and preterm neonates. *Clin Exp Allergy* 2004;**34**:189–193.
55. Vance GH, Lewis SA, Grimshaw KE, Wood PJ, Briggs RA, Thornton CA et al. Exposure of the fetus and infant to hens' egg ovalbumin via the placenta and breast milk in relation to maternal intake of dietary egg. *Clin Exp Allergy* 2005;**35**:1318–1326.
56. Holloway JA, Warner JO, Vance GH, Diaper ND, Warner JA, Jones CA. Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood. *Lancet* 2000;**356**:1900–1902.
57. Jones CA, Vance GH, Power LL, Pender SL, Macdonald TT, Warner JO. Co-stimulatory molecules in the developing human gastrointestinal tract: a pathway for fetal allergen priming. *J Allergy Clin Immunol* 2001;**108**:235–241.
58. Steel C, Guinea A, McCarthy JS, Ottesen EA. Long-term effect of prenatal exposure to maternal microfilariemia on immune responsiveness to filarial parasite antigens. *Lancet* 1994;**343**:890–893.
59. 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**:1759–1766.
60. Gill TJ, Jr, Karasic RB, Antoncic J, Rabin BS. Long-term follow-up of children born to women immunized with tetanus toxoid during pregnancy. *Am J Reprod Immunol* 1991;**25**:69–71.
61. Van Duren-Schmidt K, Pichler J, Ebner C, Bartmann P, Forster E, Urbanek R et al. Prenatal contact with inhalant allergens. *Pediatr Res* 1997;**41**:128–131.
62. Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD et al. 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.
63. Thornton CA, Upham JW, Wikstrom ME, Holt BJ, White GP, Sharp MJ et al. Functional maturation of CD4 + CD25 + CTLA4 + CD45RA + T regulatory cells in human neonatal T cell responses to environmental antigens/allergens. *J Immunol* 2004;**173**:3084–3092.
64. Host A, Husby S, Gjesing B, Larsen JN, Lowenstein H. Prospective estimation of IgG, IgG subclass and IgE antibodies to dietary proteins in infants with cow milk allergy. Levels of antibodies to whole milk protein, BLG and ovalbumin in relation to repeated milk challenge and clinical course of cow milk allergy. *Allergy* 1992;**47**:218–229.
65. Vance GH, Grimshaw KE, Briggs R, Lewis SA, Mullee MA, Thornton CA et al. Serum ovalbumin-specific immunoglobulin G responses during pregnancy reflect maternal intake of dietary egg and relate to the development of allergy in early infancy. *Clin Exp Allergy* 2004;**34**:1855–1861.
66. Jenmalm MC, Bjorksten B. Cord blood levels of immunoglobulin G subclass antibodies to food and inhalant allergens in relation to maternal atopy and the development of atopic disease during the first 8 years of life. *Clin Exp Allergy* 2000;**30**:34–40.
67. Kihlstrom A, Lilja G, Pershagen G, Hedlin G. Exposure to high doses of birch pollen during pregnancy, and risk of sensitization and atopic disease in the child. *Allergy* 2003;**58**:871–877.
68. Bjorksten F, Suoniemi I, Koski V. Neonatal birch-pollen contact and subsequent allergy to birch pollen. *Clin Allergy* 1980;**10**:585–591.
69. Aalberse RC, Nieuwenhuys EJ, Hey M, Stapel SO. 'Horoscope effect' not only for seasonal but also for non-seasonal allergens. *Clin Exp Allergy* 1992;**22**:1003–1006.
70. Settupane RA, Chafee FH, Settupane GA. Pollen immunotherapy during pregnancy: long-term follow-up of offspring. *Allergy Proc* 1988;**9**:555–561.
71. Glovsky MM, Ghekiere L, Rejzek E. Effect of maternal immunotherapy on immediate skin test reactivity, specific rye I IgG and IgE antibody, and total IgE of the children. *Ann Allergy* 1991;**67**:21–24.
72. Casas R, Bjorksten B. Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers. *Pediatr Allergy Immunol* 2001;**12**:59–64.

73. Holmlund U, Cebers G, Dahlfors AR, Sandstedt B, Bremme K, Ekstrom ES et al. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. *Immunology* 2002;**107**:145–151.
74. Amoudruz P, Holmlund U, Malmstrom V, Trollmo C, Bremme K, Scheynius A et al. Neonatal immune responses to microbial stimuli: is there an influence of maternal allergy? *J Allergy Clin Immunol* 2005;**115**:1304–1310.
75. Devereux G, Seaton A. Diet as a risk factor for atopy and asthma. *J Allergy Clin Immunol* 2005;**115**:1109–1117; quiz 1118.
76. Schultz M, Gottl C, Young RJ, Iwen P, Vanderhoof JA. Administration of oral probiotic bacteria to pregnant women causes temporary infantile colonization. *J Pediatr Gastroenterol Nutr* 2004;**38**:293–297.
77. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997;**159**:1739–1745.
78. Melkild I, Groeng E-C, Leikvold RB, Granum B, Lovik M. Maternal allergen immunization during pregnancy in a mouse model reduces adult allergy-related antibody responses in the offspring. *Clin Exp Allergy* 2002;**32**:1370–1376.