

Review Article

Intervention during pregnancy and allergic disease in the offspring

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The etiology of allergy is multifactorial, with many variables contributing to the final expression of atopic disease. Three breeding grounds are needed to develop allergic disease: the appropriate genetic background, contact with the allergen(s) and environmental factors. Timing and dosing of allergen(s) are of major importance. Contact with (dietary) allergens and various agents such as tobacco smoke and infections occur not only during post-natal life, but also perinatally and even pre-natally. A critical review of published evidence regarding the impact of maternal exposure to antigens during pregnancy on later development of allergy in the offspring can only conclude that more research is urgently needed. Contact with multiple dietary allergens should be in general of benefit to the fetus to develop tolerance. Current knowledge suggests that pregnant women should have a normal diversified diet, avoiding toxic agents such as tobacco and alcohol. The role of maternal intake of polyunsaturated fatty acids on the development of atopy in the infants needs to be further evaluated. If parental history would be insufficient to determine the fetal risk, preventive measurements would be advisable for all fetuses.

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Why is the prevention of allergy important?

In 1903 the terms allergy was introduced to define 'altered responses' to certain substances (1). In the last 20–30 yr allergic manifestations impressively increased in prevalence (2) and food allergy is reported early in life in 4–6% of children (3).

In this review, attention will be focused on the impact of (different factors of) intervention during pregnancy on allergic disease in the offspring.

Infections before or during pregnancy

Maternal vaginal colonization with *Ureaplasma urealyticum* during pregnancy is associated with infant wheezing [odds ratio (OR) 2.0], but not with asthma at 5 yr (4). Conversely, maternal colonization with Staphylococci (OR: 2.2) and use of antibiotics in pregnancy (OR: 1.7) are associated with asthma at 5 yr of life (4).

Maternal seroconversion for *Toxoplasma gondii*, *Cytomegalovirus*, *Parvovirus B19*, *Respiratory syncytial virus*, and *influenza A* and *B* during pregnancy or at delivery did not differ significantly among infants with an increased or an undetectable cord-blood IgE level (5).

Mode of delivery

In a register study children born by cesarean section showed significantly higher incidence of asthma at the age of 7 (4.2% vs. 3.3%) and more positive allergy tests (22% vs. 11%) compared to those vaginally delivered (6). However, a recent study including 24,690 births could not find a significant increase of allergic disease in babies born by cesarean, forceps or breech delivery (7). Only among children whose mothers were allergic, cesarean section was associated with a sevenfold increased risk of parentally perceived reactions to egg, fish or nuts and a fourfold

increases risk of confirmed egg allergy (8). Babies born by caesarean section present different flora and infants with food allergy do have different gut flora (9). However, modification of flora could be related to other factors than mode of delivery such as mucosa factors or use of antibiotics.

Antibiotics

Exposure to antibiotics in utero has been associated with an increased risk of asthma and hay fever in a dose-related manner in children (7) but not associated with an increased risk of food allergy (8).

The effect of pollen on fetal development

During pregnancy, maternal exposure to high levels of birch pollen results in an increased risk of sensitization to the same allergen and atopic manifestation in children with atopic heredity. Post-natal early exposure determines a further higher effect (10).

Being born some weeks before the peak of the pollen season favors the Th₂ phenotype in infants and increases the risk for sensitization to pollen (11, 12). Raised proliferative responses to house dust mite at birth, subsequent to high maternal exposure especially during the third trimester of pregnancy, has been also reported in infants (with atopic parent) developing allergic symptoms and positive skin prick test by 1 yr (13).

The effect of smoking on fetal development

High exposure to tobacco smoke is associated with an increased risk for pre-term birth, deficits in lung function, cord blood IgE increase, early wheezing illness in infancy, childhood asthma and airway hyper-responsiveness (14–18). Cytokine production by placental trophoblasts is also sensitive to maternal smoking (19).

Genetic influence

Cooke and Coca reported 100 yr ago on the importance of genetic components in allergy (20, 21) but genetic factors alone cannot explain the recent rapid increase of atopic diseases. Familial clustering and higher concordance rates in monozygotic twin pairs compared with dizygotic twins are strong arguments in favor of a genetic contribution to allergy (22, 23) but many atopic children are without atopic heredity (24).

Nowadays it is recognized that allergy is a complex disorder, with numerous contributing

genes interacting with each other and with environmental factors such as allergen exposure, parental smoking, pollutants and infections (25, 26).

More than 20 different loci on different chromosomes have been linked to asthma/atopy (25–32) (Table 1). Evaluation of polymorphism and/or mutations included genes that regulate IgE production, mast cell growth factors, pro- and anti-inflammatory cytokines, receptors (antigen receptors, IgE and T-cells receptors,...) and nuclear transcription factors (AP-1, NF-IL-2, STAT-1/2,...) (25). Total serum IgE levels and airways hyper-responsiveness seem to be controlled by different chromosomes but not all studies could replicate the same findings (29–35). Associations between certain HLA-related alleles and sensitization to pollen allergens are also established (36). Linkage of markers close to the T-cell receptor gene with sensitization to house dust mite allergens were identified on chromosome 14q (37).

The fact that so many loci/genes are involved in allergy not only reflects the complex nature of this disorder, but also the differences in study populations (ethnic background, different environmental factors, definition of allergy, and sample size,...).

Elevated maternal, but not paternal, total IgE levels (> 150 kU/L) correlated with increase of cord blood and infant IgE levels (both $p < 0.001$), and infant eczema ($p = 0.008$) (38). The major maternal (vs. paternal) atopic influence in newborns development of allergy may indicate a crucial role either of maternal alleles or

Table 1. Genes and atopy

Locus	Candidate gene	Phenotype
1p36	Unknown	Asthma
2q21-23, q33	Unknown	Specific IgE, asthma
3q21	CD80–CD86	Total IgE, severe eczema
4q35	Endothelin receptor	Asthma, atopy
5q23-31, q33	IL-4, IL-13, MHC, CD14, glucocorticoid, and β 2-adrenergic receptors	Total IgE, asthma, eczema
6p21.3, 22-24	MHC class II, TNF- α	Asthma, atopy, specific IgE
7p15, q33	T-cell receptor	Asthma, total IgE
8p21-23, q13	Unknown	Asthma, specific IgE
9q22	Unknown	Asthma
11q13	FC ϵ R1, Clara cell 16 gene	Asthma, IgE, atopy
12q15-q24.1	IGF1, SCF, IFN γ , NOS1	Asthma, total IgE, atopy
13q14	Unknown	Asthma
14q11.2, q32	TCR- α	Specific IgE, eczema, atopy
16p11.2-12.1	IL-4 REC	Total IgE, asthma
17	Rantes	Eczema
19p13	Unknown	Asthma
21	Unknown	Atopy

intrauterine milieu. Future studies are certainly needed to further delineate the genetic basis of atopy but also to create opportunities for more individual-based treatments (25).

Immunology: allergy and tolerance

Tolerance is a complex phenomenon involving different mechanisms depending on the characteristics of the antigen (nature, size, dose, and timing and frequency of exposure) and of the microenvironment (intestinal flora and inflammatory stimuli) (39). Central and peripheral tolerance also develops in a different way (40).

The maturation of the immune system during fetal life is still a matter of research (40). The intrauterine milieu is normally skewed towards Th₂ immunity, which seems the necessary pattern for successful pregnancy (41, 42). After birth a Th₁ polarization normally occurred. A post-natal Th₂ predominance persists in (some) subjects with atopic diseases. Both a Th₁ maturation and a Th₁ trigger defect may contribute to a dual Th₁ allergy theory (43). However, the fetus that mounts a generalized immaturity or a decreased production of both Th₁ and Th₂ cytokines is also at high risk of developing allergy, as these cytokines are important for healthy placental function, which in turn may positively affect maturation of immune competence in the developing fetus. Cord blood cytokines mirrored cytokine production of peripheral-blood T-cells and showed an inverse relation between concentrations of IL-4, IFN- γ , and TNF- α and the risk of asthma, atopy, or both at the age of 6 yr (19). Newborns from atopic families have a decreased IFN- γ production (44). It is not clear whether the alterations in cytokine levels represent maternal influence rather than events having a basis solely within the infant. The principal cytokines involved in the allergic cascade are listed in Table 2.

Recent findings suggest that the allergic phenomenon represents a primary failure to establish tolerance and also support the hypothesis that allergic sensitization may be due to inadequate regulatory response rather than Th₁/Th₂ imbalance (45, 46).

Stimulation with specific allergens produced higher number of cord blood T-cells in newborns that later develop allergic symptoms to those allergens (47, 48).

Infants of atopic mothers have a decreased CM antigen-specific IL-4 and a significantly lower TGF- β response compared to infants of non-atopic mothers. This strongly suggests an immune dysregulation to dietary antigens in utero and some deficiency in Th₃ cells and/or Tr cells (making IL-10) in children of atopic mothers (49).

Cord blood IgA level is higher in infants born in the developing than in the developed world (50) and deficiency of IgA had been related to allergic sensitization (51).

Confounding factors

More asthma and other allergic diseases are reported in children when maternal health complications occurred during pregnancy, labor or delivery (47). The association between pregnancy complications and the risk of asthma (OR: 1.82) still remain with data corrected for plurality, geographical origin, maternal atopy and education (52).

Newborns with a greater head circumference, corresponding to disproportionate fetal growth, have higher serum total IgE at birth (53) and in adulthood (54) and higher risk of asthma (55). There is also slightly increased risk with high birth weight (>3800 g), placental weight (>750 g) or weight gain (56).

However, also impaired fetal growth is a risk factor for adult asthma (57). The OR for asthma

Table 2. Principal cytokines involved in the allergic cascade

Cytokine	Cell sources	Main functions
IL-2	Th ₁	Increase proliferation of Th and B lymphocytes
IL-4	Th ₂ , Th ₀ , mast cells	Increase production of IgE, development and recruitment of eosinophils, development of mast-cells
IL-5	Th ₂ , mast cells	Increase development and recruitment of eosinophils
IL-9	Th ₂	Increase production of IgE, development and recruitment of eosinophils, development of mast-cells
IL-10	Th ₂ , Tr ₁	Inhibit Th ₁ , down-regulate inflammation
IL-12	APC, Th ₀	Increase proliferation Th ₁ and production of IFN- γ , reduce IL-4
IL-13	Th ₂	Increase production of IgE, development and recruitment of eosinophils, development of mast-cells
IFN- γ	Th ₁	Increase expression of HLA molecules, phagocytosis and NK cytotoxic functions, inhibit Th ₂
TGF- β	Th ₃	Down-regulate inflammation, stimulate IgA production? Induce tolerance
TNF- α	APC, Th ₁	Stimulate APC, Th, neutrophils, adhesion molecules expression, increase acute phase proteins...

APC, antigen presenting cells; NK, natural killer.

in young adult born with a birth weight below 2500 g or before 34 gestational weeks was 1.5 and 0.8, respectively. The OR for atopic dermatitis was 3.0 and 0.3, respectively, indicating that fetal growth retardation more than pre-term delivery is the predominant associated factors to subsequent atopic manifestation (58). Another survey found less atopy (clinical manifestations, IgE levels, skin prick test) at the age of 10 yr in the children born pre-term than at term (59).

Higher presence of food allergy has also been related to older maternal age (over 30 yr) at delivery (60). In the PIAMA study, season of birth, birth weight, mode of delivery, gestational and maternal age, and older siblings were not associated with a high neonatal total IgE or sensitization. Pets at home during pregnancy were negatively associated with a high neonatal total IgE and sensitization at 12 months, but not at 4 yr (for cat) (61). High maternal exposure to volatile organic compounds (smoking, new carpets and home renovation) increases atopic disease in the offspring (62).

What reported above illustrates the great difficulty or the virtual impossibility to control all the variables influencing atopic disease. Different approaches may be also needed for different forms of atopic diseases (24). Furthermore post-natal measures of prevention are also complex and crucial to determine the final atopic destiny (24, 63).

Intervention during pregnancy?

The ideal situation would be to bring the individual in contact with levels of antigens capable of inducing tolerance but not causing allergic reactions. Allergy prevention can be directed at three potential stages: primary prevention, which inhibits immune sensitization; secondary prevention, which avoids disease expression subsequent to sensitization and tertiary prevention, which suppresses symptoms after disease expression (64). As a consequence, intervention during pregnancy concerns primary prevention. A prevention strategy may be allergen specific (antigen avoidance) or non-specific (environmental effect) (65).

In utero exposure to (food and inhalant) allergens

Fetus is exposed to circulating antigens through the amniotic fluid and the placenta (66). Human amniotic fluid at 16–18 wk of gestation may contain intact IgE (proportionally with maternal circulating levels) that may bind to low-affinity IgE receptors (CD23+ cells) expressed in

lymphoid follicles of the fetal gut (67). The fetus can also mount (own) specific IgE responses to foods (68, 69). The ability of a molecule to traverse the placenta is associated with its molecular weight, lipid solubility, polarity and ionization, and specific transport mechanisms (66). The transplacental passage of common allergens such as β -lactoglobulin, ovalbumin and major birch pollen allergen Bet v1 occurs from before 26 wk of gestation using an *ex vivo* model of transfer (66) but cord blood mononuclear cell reactivity to allergens was observed as early as 20 wk of gestation (70–72). In one study, proliferative response of cord blood mononuclear cells to specific inhalant allergens (birch rBet v1 and timothy grass rPhl p1) was much higher when maternal pollen exposure occurred at the end of the first trimester of pregnancy (73). However, because in humans the migration of T-cell precursors to the epithelial thymus takes place at 7–8.5 wk of gestation and CD3+ and TCR- β + cells appear at 8.5 and 9.5 wk, priming should not be obtained in the first 2 months of pregnancy (74, 75). However pre-natal allergen priming seems similar in atopy-prone and non-atopy-prone subjects (70). The importance of lymphocyte proliferation in the clinical manifestation of allergic symptoms still needs to be fully elucidated.

Maternal-fetal IgG transport begins at 16 wk of gestation, rapidly increasing from 22 wk with fetal IgG1 exceeding maternal levels (76). The *ex vivo* trans-placental transport of CM β -lactoglobulin is enhanced by the addition of human immunoglobulin, and is essential for the transfer of Bet v1 (69). Evidence for *in utero* sensitization to inhalant allergens is currently controversial (69, 77, 78).

The role of diet and nutrition

Evidence from Japan suggests that maternal BMI and intake of lipids (fat and vegetable oil) and vegetables relate positively, and intake of protein, carbohydrates and milk and its products correlate negatively with allergic diseases in the infants ($p < 0.05$) (79). Atopic mothers have a higher intake of total and saturated fat and a lower intake of carbohydrate than non-atopic mothers ($p = 0.017$, 0.050 , and 0.004 , respectively). During breastfeeding, maternal intake of saturated fat is associated with atopic sensitization of the infant (OR = 1.16, $p = 0.048$), irrespective of the maternal atopic status (80). Famine exposure during gestation affected neither the concentrations of total or specific IgE (against mite, pollen and cat) The prevalence of

obstructive airways disease increased if famine was in mid gestation (AOR: 1.7) or early gestation (OR: 1.5) suggesting that fetal lungs can be permanently affected by nutritional challenges during periods of rapid growth (81).

Maternal dietary antigen avoidance

Lovegrove showed that maternal milk-free diet during late pregnancy and lactation significantly reduced ($p < 0.04$) the allergy incidence at 12 and 18 months of life in the atopic group (82). However maternal diet during the third trimester also failed to reduce food allergy, or any other atopic manifestation up to the age of 5 yr (63, 64, 83–86). Chandra et al. observed a reduction of infant atopic eczema if mother's followed a diet restricted in milk, dairy products, egg, fish, beef and peanut during pregnancy and lactation (87) or just during lactation (88). Restricted diet during pregnancy and/or lactation did not produce conclusive evidence of benefit (24, 84, 89–91). A Cochrane Review (92) including four controlled trials did not suggest any protective effect of maternal dietary antigen avoidance during pregnancy on the incidence of atopic eczema [relative risk (RR) 0.94] during the first 12–18 months of life in high-risk infants. Data on allergic respiratory manifestations were insufficient to draw meaningful conclusions. A CM free diet in the first or second trimester, or an increased exposure during pregnancy has not been tried. However, the elimination of early contact of the fetal immune system with antigens of dietary proteins may compromise the development of tolerance.

American and European guidelines recommend normal feeding during pregnancy and lactation (93, 94). A low allergen content diet is currently being considered as more disadvantageous for possible maternal and fetal malnutrition than eventual advantageous for allergic prophylaxis. However, the situation may be different if specific allergens are avoided such as egg or peanut. Grimshaw et al. reports a significantly reduced sensitization rate to egg and inhalant allergens in 18-month-old infants with strict maternal egg avoidance during pregnancy and lactation (95). Hourihane reported an association between a self-reported increase in maternal consumption of peanut products during pregnancy and lactation and an earlier age of onset of peanut allergy during childhood (96). The consumption of peanuts more than once a week during pregnancy increased the sensitization to peanut in the child (97). Interestingly, there is no significant relation between peanut

consumption during lactation and peanut sensitization. However, peanuts and peanut butter are introduced into the child's diet from a significant younger age in the peanut-sensitized subjects (97). Further data are currently necessary before recommending a peanut or egg-free diet in pregnancy.

A potential role of anti-inflammatory polyunsaturated fatty acids (PUFAs) in the prevention of allergic disease has been suggested (98). Newborns at risk of atopic disease showed significantly lower levels of PUFAs in umbilical cord blood (99). Breast milk of mothers with children with atopic dermatitis contains reduced levels of prostaglandin E (100). Maternal fish oil supplementation in pregnancy reduces IL-13 levels in cord blood of infants at high risk of atopy (101). A diet intervention (oil supplement, margarines, and cooking oils containing high levels of ω -3 fatty acids) in 616 pregnant women resulted at the age of 18 months in a 9.8% reduction ($p = 0.02$) of wheezing but it had no effect on serum IgE, atopy, or doctors' diagnosis of asthma (102). At the age of 3 yr there was a significant 10% reduction in the prevalence of cough in atopic children in the active diet group ($p = 0.003$) and a 7.2% reduction of sensitization to house dust mite without significant differences in wheezing (103). A randomized controlled trial in 83 atopic pregnant women receiving fish oil (3.7 g ω -3 PUFAs per day) from 20 wk gestation showed lower cytokine response (IL-5, IL-13, IL-10, and IFN- γ), a 3-fold reduction of positive skin prick test to egg at 1 yr of age (OR = 0.34, $p = 0.055$) but no difference in atopic dermatitis in the supplemented group (104).

Microflora and probiotics

The possible benefit of probiotic and/or prebiotic supplementation during pregnancy (and lactation) is based on the differences reported in the flora of atopic versus non-atopic subjects, on the immunological effects of the intestinal flora and on promising results of the first's interventional studies. Germ-free animals showed impaired tolerance mechanism (105, 106). Atopic human populations have a high prevalence of Clostridia, coliforms and *Staphylococcus aureus* vs. Lactobacilli and Bifidobacteria (bifidum) (9, 107, 108). An ideal 'good' maternal flora might influence the establishment of a similar neonatal flora through vaginal delivery and first nursing (109) thus conditioning subsequent colonization and decreasing the likelihood of allergy in later life.

Lactobacillus GG has been shown to induce a NF- κ B-mediated response in human macrophages (110). Additionally, specific probiotics including Lactobacillus GG may generate anti-inflammatory IL-10 and TGF- β (111). If specific components of selected probiotics are sufficient and efficient to exert immunological effects and if the change in maternal flora does have an influence on the fetus still has to be demonstrated.

A Finnish placebo-controlled, randomized clinical trial was the first to attempt the prevention of food allergy through supplementation with Lactobacillus GG for 2 wk before delivery and for 6 months post-natally to the at-risk infant. A twofold reduction (23% vs. 46%) in the incidence of atopic dermatitis by age 2 and 4 yr was reported. No benefits were noted, however, on the total IgE levels, specific IgE levels to foods, skin prick tests (112), seasonal allergic rhinitis and asthma (113).

Current recommendations and the future

Avoidance of multiple food allergens during pregnancy severely restricts what mothers can eat and is not justified by the current scientific evidence. The importance of balanced fat and probiotic supplementation still needs to be confirmed. Smoking exposure should be strongly discouraged not only for allergic prevention but also for lung development and function. Potential immunological strategies for allergy prevention may include modulation of (atopic-prone) cytokines by competitive cytokines or by specific bacterial/probiotic stimulation or use of monoclonal antibodies.

Conclusion

The development of atopic disease is influenced by post-natal, perinatal and very likely pre-natal factors. Tolerance or allergic sensitization in newborns depends on the genetic profile in combination with the immunological balance and environmental factors. It can be speculated that the impact of interventions during pregnancy may have a significant effect only when combined with adequate perinatal and post-natal interventions.

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