Developmental origins of asthma and related allergic disorders

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Introduction

Asthma is a chronic disorder affecting the conducting airways, in which genetic and environmental factors interact to produce both inflammation and structural changes in the airway wall (Tattersfield et al. 2002). The consequence of these pathological changes is variable airflow limitation which is manifested by recurrent cough and wheeze. Recent asthma guidelines have emphasised the importance of treating the underlying inflammatory response as well as relieving the symptoms of asthma, but beyond the use of inhaled corticosteroids (ICS) and beta-2 adrenoceptor agonists, which were introduced 30–40 years ago, there has been very little new to add to the therapeutic algorithm (British Thoracic Society 2003). While utilisation of these two pharmacotherapies is highly effective in controlling symptoms and improving quality of life, there is no evidence that these therapies either alter the natural history of the disease or ever affect a cure (Martinez 2003). With the possible exception of immunotherapy no treatment has been shown to modify the natural course of the disease and no cure has been identified (Durham et al. 1999).

Most asthma has its origins in early life, and the best predictors of continuation into adulthood are an early age of onset, sensitisation to house dust mites (in environments where this is the major allergen), reduced lung function, and increased bronchial hyper-responsiveness (BHR) in early life (Sears et al. 2003). Even employment of ICS at a very early stage in the disease evolution does not influence outcomes (Covar et al. 2004). Thus it becomes imperative to understand the early-life origins of the disease in order to identify targets for prevention and early intervention.

There has been a progressively increasing prevalence of asthma and related allergic diseases such as atopic eczema and allergic rhinoconjunctivitis over the last 30–40 years. The increases have occurred far too rapidly for this to be accounted for by a genetic change in the population. It is more likely to have been due to a shift in environmental influences acting on a pre-existing genetic susceptibility (Holgate 1998). While there are two basic components which contribute to airflow limitation in asthma, namely airway inflammation and altered airway wall structure, most studies of the environmental influences on the disease have concentrated on allergic sensitisation. It has now become clear that concentrating on the cellular and mediator pathways producing an allergic inflammatory response falls short of explaining the full pathology of the disease (Holgate et al. 2004). Hitherto it has been assumed that allergic inflammation is the main cause of the structural changes and that one follows the other. However, genetic and environmental factors can affect the airway-wall structure independent of allergic sensitisation. Indeed increased BHR, which may well

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represent changes to airway wall structure, can be detected as early as 4 weeks of age and its presence is predictive of asthma at 6 years of age independent of allergic sensitisation (Palmer et al. 2001). The origins of asthma will therefore be discussed both in relation to the early-life origins of allergy and its influence on airway inflammation, and in relation to the evolution of changes to airway wall structure. While there are distinct genetic and environmental influences on the two components of the disease, common pathways exist for environmental factors to affect both components simultaneously, thereby leading to the disease we know as asthma.

**Epidemiology of allergy**

It has long been known that allergy is one of the most important risk factors for asthma. Its presence is associated with an increased risk of developing the disease (Van Asperen and Mukhi 1994), and once the disease is manifest it predicts persistence from childhood through adolescence and into adulthood (Ross et al. 1995). Successive studies have demonstrated that both the prevalence and the severity of asthma has increased in many countries around the world. In the United Kingdom surveys amongst 12- to 14-year-olds have shown an increase in point prevalence from 1973 to 1988 and finally to 1996 of 4%–9%–20.9% respectively (Burr et al. 1989, Kaur et al. 1998). This increase has occurred simultaneously with increases in all other allergic diseases. Thus eczema over the same time period has increased 5%–16%–16.4%, and hay fever 9%–15%–18.2% (Austin et al. 1999). Similar increases have been seen in four consecutive studies conducted on children aged 9–11 years in Aberdeen, UK (Fig. 26.1) (Ninan et al. 2000). Diagnostic transfer does not explain this as studies had used virtually identical ascertainment at each time-point. There has also been a considerable increase in hospital admissions for childhood asthma over the last 15 years with no reduction in severity on admission, no increase in readmission ratio and no evidence of diagnostic transfer (Anderson 1989). A relatively small shift in popu-

![Figure 26.1](image-url)

**Figure 26.1** There has been a considerable increase in the prevalence rates of all allergic conditions over the last 30 years. This is shown for seasonal rhinitis, asthma and eczema between 1964 and 1999 in four successive studies conducted on Aberdeen schoolchildren aged 9–11 years. From Ninan et al. (2000).

lation susceptibility due to changes in the environment could account for the increased frequency of disease and particularly of severe disease. It is, however, important to note that very recent studies are suggesting that the increase in prevalence of asthma may be coming to an end (Hess and de Jongste 2004), at least in developed countries.

Many environmental factors which have been linked to the rising trends of allergic diseases have embraced the concept of an alteration in the balance of immune responses between (a) those which are associated with an allergic pattern, (b) those associated with protection against infection and (c) those which regulate all responses.

**The allergic immune response**

The immunological paradigm associated with allergic disease is the expression of T-lymphocyte cell-mediated immunity to common environmental allergens that is biased towards T-helper 2 (Th2) lymphocyte activity. Th2 lymphocytes release peptide
regulatory factors (cytokines) which orchestrate the production of immunoglobulin E (IgE), which is the archetypal allergy-promoting antibody, and activate inflammatory cells such as eosinophils which are commonly associated with allergic inflammation. The relevant cytokines and their effects are shown in Fig. 26.2, namely interleukins (IL)4, IL5, IL9 and IL13. The counter-regulatory pathways include those generated by a normal immune response to infection orchestrated by the T-helper 1 (Th1) lymphocytes which generate the cytokines interferon gamma (IFNγ) and IL2 (Romagnani 1991). The other pathway involves a group of T-lymphocyte regulators which have an influence on both Th1 and Th2 activity by either cell–cell contact or the generation of IL10 and transforming growth factor beta (TGFβ) (Romagnani 2004) (Fig. 26.2). Based on this paradigm it becomes clear that either overexpression of Th2 activity or a failure of control by Th1 or T-regulatory function will result in a higher probability of the development of allergy and allergic inflammation.

The pattern of response of T lymphocytes is dictated by the nature of the signalling from antigen-presenting cells (APCs). APCs are affected by the nature of the antigen exposure (Rothoeft et al. 2003), which influences their interaction with T lymphocytes (Kruse et al. 2003). These cells generate IL12, 15, 18 and 23 which predominantly though not exclusively stimulate Th1 responses, while IL10 from regulatory T cells inhibits IL12 and therefore favours Th2 activity.

**Genetic polymorphisms and allergy**

Twin and family studies have shown that asthma is highly heritable (Duffy et al. 1990). A plethora of single nucleotide polymorphisms (SNPs) have been associated with allergy and are clearly associated with effects on Th2 pathways. The greatest focus has been on the cytokine gene cluster on the long arm of chromosome 5 (5q31–33) (Liu et al. 2003). This chromosome region contains the genes for many Th2 cytokines, IL 3, 5, 9 and 13, GM-CSF and also the gene for an endotoxin receptor known as CD14 (Baldini et al. 1999). This latter molecule will be discussed in relation to the hygiene hypothesis. However, the associations between polymorphisms in this chromosome region are much more strongly associated with allergy rather than asthma (Holloway and Holgate 2004). Other associations have been with a common subunit of the IL4 and IL13 receptor on chromosome 16p12; those for IFNγ on 12q; the major histocompatibility complex molecules, tumour necrosis factor alpha and beta, on 6p21; the IgE receptor on 11q12–13; and IL18 on 11q22 (Holloway and Holgate 2004). The latter is particularly interesting because IL18 has complex interactions in initiating both Th1 and Th2 activity, dependent on the antigenic stimulus (Kruse et al. 2003). Thus all these polymorphisms have a credible mechanistic association with a higher probability of developing allergy and allergic inflammation.
The impact of the polymorphisms may only be apparent at critical stages during development of the immune response as their presence does not inevitably mandate the development of allergy. Relatively minor perturbations of the balance between Th1 and Th2 activity, particularly in early life, is likely to have significant effects on outcomes.

**Ontogeny of immune responses in the fetus**

Observations of an altered immune response at birth in children at risk of developing allergy by virtue of a family history of allergic disease, and similar alterations in the subset who go on to develop allergy and disease, highlights the contribution of fetal life (Jones et al. 2000). There has been a commonly held misconception that the newborn baby is immunologically naive. However, it is very clear that the capacity to mount a significant immune response is present from very early gestation. There are stem cells present in the human yolk sack at 21 days, with the first lymphocytes being seen in the thymus by the ninth week of gestation. The lymphocytes are detectable in many organs including the lungs and gut by 14 weeks, and by 16 weeks circulating B cells can be detected with surface immunoglobulin M (Hayward 1998).

The neonate is known to be able to mount an antibody response to a wide array of antigens following specific exposure of the mother during pregnancy. Fetal IgM antibody responses have been demonstrated after maternal immunisation with tetanus toxoid (Gill et al. 1983), and of course they are well recognised by paediatricians in association with maternal infection with rubella, toxoplasmosis, cytomegalovirus and human immunodeficiency virus early in pregnancy Naot et al. 1981, (Daffos et al. 1984, Clerici et al. 1993, Jones et al. 2002a). Studies have shown that direct immunisation of the baboon fetus with recombinant hepatitis B surface antigen can initiate a specific fetal IgG antibody response where there is no such response detected in the mother. The resulting baboon infant has an enhanced responsiveness to hepatitis B surface antigen when immunised again postnataally (Watts et al. 1999). This indicates that the complete process of immune sensitisation must have occurred, commencing with the pickup and processing of antigen by APCs. The subsequent presentation of the antigen to T lymphocytes and thereafter stimulation of the B lymphocyte to produce specific immunoglobulin has also occurred. Thus the ability to detect antigen-specific responses at birth should not be considered unusual.

It has, therefore, been possible to demonstrate that circulating blood mononuclear cells are capable of mounting a specific proliferative response to allergens such as ovalbumin from hens’ eggs and even the major allergen of house dust mite from as early as 22 weeks gestation (Jones et al. 1996). As the pregnancy proceeds a progressively higher percentage of fetuses mount specific responses to common environmental factors. By birth the majority, if not all, demonstrate such responses (Prescott et al. 1998). Furthermore, a number of studies have shown that a high proliferative response to an allergen at birth is associated with a higher probability of allergic reaction to that factor and of allergic disease later in childhood (Kondo et al. 1992).

**The immunology of pregnancy**

Normal pregnancy can only proceed if the maternal cell-mediated immune response to fetopaternal antigens is suppressed. Initial studies of murine pregnancies (Wegmann et al. 1993), but more recently also in humans, have shown that a range of tissues in the fetoplacental unit produce cytokines which have a profile similar to that associated with the Th2 phenotype (Tang et al. 1992, Warner et al. 1994, Piccinni et al. 1996, Jones et al. 2000). These cytokines shift the balance of the maternal immune response away from cell-mediated activity towards a less damaging humoral immune response (Raghupathy 1997). Such cytokines almost certainly also have additional properties in promoting fetal growth. Thus it has been shown that granulocytemacrophage colony-stimulating factor (GM-CSF)
has an effect on surfactant homeostasis by stimulating differentiation and proliferation of the type 2 pneumonocytes (Whitsett and Wert 1998). Based on the Th1/Th2 paradigm, it is very likely that the Th2-cytokine bias of normal pregnancy promotes a successful outcome by suppressing Th1 responses. The latter are clearly associated with pregnancy loss or intrauterine growth retardation (Raghupathy 1997, Piccinni et al. 1998). IL4 and the regulatory cytokine IL10 have an effect on cell maturation and therefore directly and indirectly affect lung structure and function. IL10 is worthy of specific mention because of its regulatory properties. It will diminish the production of a range of cytokines including IL12 from antigen-presenting cells which would otherwise increase IFNγ production (Fickenscher et al. 2002).

Decidual tissues have been shown to be a source of IL1 alpha and beta, IL6, GM-CSF, IL4, IL10 and IL13 (Jones et al. 1998a). IL13 immune reactivity can be detected in the placenta between 16 and 27 weeks gestation but not thereafter. From 27 weeks until 34 weeks IL13 can be found spontaneously released from fetal mononuclear cells. Thereafter it is only released on stimulation of cells (Williams et al. 2000). Thus there must be a very subtle regulation of production of this and other cytokines with an interaction between the mother, the placenta and fetus. These Th2 and regulatory cytokines will not only modulate the maternal immune response but will also have an impact on the fetus. It is, therefore, not surprising that there is a universally Th2-biased immune response to antigens detectable in all newborn babies. Rapid postnatal maturation of Th1 responsiveness normally counterbalances this (Prescott et al. 1999).

The Th2 and T-regulatory cytokines IL4, IL10, IL13 and TGFβ can be detected in amniotic fluid during the second trimester of pregnancy (Jones et al. 1998a). Such cytokines will be swallowed by the fetus. Indeed the protein turnover in amniotic fluid occurs at a rate of 70% each day, with much of this removal being via fetal swallowing (Gitlin et al. 1972). The fetus, of course, also has a highly permeable skin (Hardman et al. 1999) and there is some aspiration of amniotic fluid into the respiratory tract during fetal respiratory movements (Schittny et al. 2000). Thus factors in the amniotic fluid have the capacity to gain access to mucosal surfaces and to influence immune responses at that level.

The fetal gut has been well studied. There are many HLA-DR-positive cells comprising macrophages, B lymphocytes and dendritic cells detectable in the submucosa from as early as 11–12 weeks gestation. By 14 weeks gestation HLA-DR-positive APCs are found in lymphoid follicles of the rudimentary Peyer’s patches of the fetus. Surface markers on these cells suggest that antigen presentation can occur with appropriate costimulatory signals in order to promote a sensitising immune response in T lymphocytes. Both T lymphocytes and B lymphocytes can be detected in the lymphoid follicles from 14–16 weeks gestation. They contain the relevant ligands for co stimulatory signalling. Furthermore, the location of such receptors to one side of the cells, a phenomenon known as capping, suggests that communication is occurring between APCs and T cells (Jones et al. 2001). The mere presence of dendritic cells (the most professional of APCs) in lymphoid follicles expressing maturation markers implies that these cells have already picked up antigen and migrated to the follicles. The only likely route of exposure to antigen will have been through swallowed amniotic fluid. It has been possible to demonstrate both food and inhalant allergen in some amniotic fluids. Hen-egg ovalbumin and the major allergen of house dust mites have been detected at levels of about 10% of those levels found in the maternal circulation (Holloway et al. 2000). Thus all the ingredients for allergen sensitisation with a Th2 bias are present within the fetal gut. To date it has not been possible to demonstrate similar maturity of immune active cells in the fetal skin, airway or circulation. It is likely that the maturation of cells in the fetal gut is a consequence of exposure to the cytokines in the amniotic fluid.

Amniotic fluid also contains IgG and IgE antibodies of maternal origin, at levels about 10% of that found in the maternal circulation, from as early as 16 weeks gestation. IgG and IgE receptors can be detected on cells within the lamina propria of the
fetal gut (Thornton et al. 2003). Therefore there is the potential for these immunoglobulins to facilitate the pick-up of antigen by APCs. This is a phenomenon known as antigen focusing. With IgE on relevant low- or high-affinity IgE receptors it is possible to achieve sensitisation to concentrations of allergen 100–1000 times lower than would be achieved without the immunoglobulin present (Maurer et al. 1995).

While the likely route of primary sensitisation to allergen in pregnancy is via the fetal gut during the second trimester, there is also exposure to allergen via the fetal circulation in the third trimester. This is predominantly as a consequence of active transport of IgG antibody across the placenta complexed with antigens and allergens. What evidence exists would suggest that the higher the IgG antibody to specific allergens the less the likelihood of subsequent sensitisation to those allergens (Casas and Björkenstén 2001). This indicates that the timing of exposure to allergen in pregnancy and the concentration of exposure will have subtly different influences on outcome (Jenmalm and Björkenstén 2000). Thus one study of birch and timothy grass pollen exposure via the pregnant mother suggested that the fetus only mounted a sensitising cellular response if the pollen season occurred during the first six months of pregnancy, with later exposure resulting in tolerance (Van Duren-Schmidt et al. 1997).

Environmental factors influencing antenatal immune responses

It is well known from many studies that if the mother is allergic herself the infant is far more likely to show allergy and allergic disease from an early stage in life, compared with merely inheriting allergy genes from the father, where the subsequent prevalence of disease is lower (Ruiz et al. 1992). This implies that maternal allergy in some way primes the fetus to be more likely to react in an allergic way. Clearly if the mother is allergic she has a higher level of IgE in her circulation. This in turn means there is more IgE in the amniotic fluid and therefore a greater probability of antigen focusing producing sensitisation to very-low-concentration allergen exposure in the second trimester of pregnancy. Furthermore, the amniotic fluid of allergic mothers has higher levels of IL10 than that of non-allergic mothers (Warner et al. 1997). It is interesting to speculate why such a sophisticated immune response should be in place so early in gestation. It is likely that it facilitates a fetal response to maternal helminth infection (Jones et al. 1998b). Certainly infants born to helminth-infected mothers have specific Th2-biased immune responses to helminth antigen and detectable IgE antibodies to those antigens at birth (Malhotra et al. 1997). Furthermore, although the newborn baby has an obligate exposure to its mother’s parasites it is exceedingly rare for the infant to become infected by those parasites, implying a very mature immune response preventing such infection (D’Alauro et al. 1983). In this era of low parasite infestation it is likely that certain properties of allergens have counterparts of parasite antigens leading to stimulation of the same immune response (Stewart and Thompson 1996). These mechanisms would certainly explain the higher probability of IgE-sensitisation of the baby born to an IgE-sensitised (i.e. allergic) mother.

As suggested, the timing and concentration of allergen exposure during pregnancy could also influence outcomes. Low-dose exposure in the second trimester appears to be more likely to sensitisé, while high-dose exposure has the converse effect. The latter might be explained by the generation of high levels of IgG allergen-specific antibody in the mother (Vance et al. 2004). One study of the children of mothers who had undergone rye-grass-allergen immunotherapy during pregnancy and consequently had high IgG antibody levels compared to children born to rye-grass-allergic mothers not receiving immunotherapy showed fewer positive skin tests to rye-grass 3–12 years later in the offspring (Glovsky et al. 1991). Newborn babies with high levels of IgG antibody to cats and/or pollens have been shown to have a lower probability of generating IgE antibodies to those allergens up to eight years later (Jenmalm and Björkenstén 2000). This might explain the recent observations that children born into families where there is a high exposure to dogs have less subsequent sensitisation to these allergens.
Asthma and related allergic disorders

These observations imply that attempts to reduce allergen exposure in pregnancy might have an adverse rather than a favourable effect. Indeed two recent publications have suggested that this may be the case. While at 1 year of age house-mite avoidance has been shown to be associated with somewhat less wheezing, by 3 years of age there was increased sensitisation to house dust mite (Custovic and Simpson 2004). Most studies have failed to demonstrate any consistent effect of house-mite avoidance in preventing sensitisation or asthma, and low-level exposure to house mite has been associated with a greater risk of IgE sensitisation and asthma than higher levels (Cullinan et al. 2004). Studies from my own lab have suggested that there is a bell-shaped curve of risk of allergic sensitisation in relation to allergen exposure in pregnancy. Thus very low and very high-dose exposure protects, and it is in the middle range of exposures that sensitisation occurs (Vance et al. 2004). Any attempt to reduce exposure will shift those in the high-dose tolerance range into the sensitising range and will of course also move a number from the sensitising range into the very-low-dose no-sensitisation range. However in the majority of circumstances the overall result for a population will be of no effect on the prevalence of sensitisation and disease (Fig. 26.3).

Fetal growth and nutrition may well also have an impact on the ontogeny of immune responses. There have been some unexpected associations between large head circumference at birth and levels of total IgE at birth (Oryszczyn et al. 1999), in childhood (Gregory et al. 1999) and even in adulthood (Godfrey et al. 1994). More importantly, there is an association between large head circumference at birth and asthma requiring medical attention (Gregory et al. 1999). It has been hypothesised that large head circumference at birth is indicative of a rapid fetal growth trajectory, because of good nutrient supplies in early pregnancy. The fetus is subsequently programmed to continue on a rapid growth trajectory, but of course also retains a high nutrient demand. If this is not met in the later stages of pregnancy there is continuing head growth at the expense of relatively poor nutrition to the body, with consequent effects on immune responses (Fig. 26.4). The key question raised by these observations is whether there are any specific nutrients of importance in protecting immune responsiveness. The focus has hitherto been on antioxidants. Certainly reduced intake of fresh fruit and vegetables is associated with a higher rate of allergic sensitisation when different populations around Europe are compared (Butland et al. 1999). However, the prevailing view is that reduced antioxidant intake has an impact on asthma severity rather than on asthma prevalence. Low cord-blood selenium and iron have been associated with a higher subsequent risk of persistent wheeze for the former, and both wheeze and eczema for the latter nutrient (Shaheen et al. 2004). Trials of supplementation in pregnancy are now required.

Recent studies have focused on lipids as perhaps also being important in immune ontogeny (Peat et al. 2004). Indeed fatty acids have a crucial role as a source of energy, as the principal component of cell membranes, and as signalling molecules for synthesis of prostaglandins and leukotrienes. Minor

![Figure 26.3 The potential effects of allergen avoidance, based on the assumption that there is a bell-shaped distribution of risk of allergy in relation to the dose of allergen exposure. The 20% of the population having either very low-dose or very high-dose exposure are not sensitised. Shifting the population by half a standard deviation results in the majority in the high-dose tolerance range moving into the lower-dose sensitisation range, with an equivalent number moving from the mid-range into the very-low-dose no-sensitisation range. The end result on the whole population is to achieve no reduction in allergy prevalence.](image-url)
Figure 26.4 A hypothesis to explain the association between a large head circumference at birth and allergy. Affluent societies where good nutrition predominates have an impact on programming of fetal growth. Early in pregnancy, with good nutrient delivery, the fetus begins a rapid growth trajectory. This must be sustained throughout life. Nutrient need exceeds delivery in the third trimester, resulting in a brain-sparing reflex with continued growth of the brain and head but selected compromise of rapidly growing tissues, which will have a particular impact on immune responsiveness and may selectively compromise Th1 activity. This leads to the association between a big head and allergy and could explain some of the demographic trends in allergy prevalence.

Postnatal influences on allergic immune responses

Studies have shown that newborn infants have allergen-reactive T cells which are of fetal and not maternal origin (Prescott et al. 1998). The responses are characterised by the dominant production of Th2 rather than Th1 cytokines. Postnatally these responses are modulated, with a progressive increase in Th1 activity and associated reduction in Th2 activity. However, those infants that go on to have allergic symptoms have a very different pattern of response with an age-associated increase in Th2 activity (Prescott et al. 1999). Figure 26.5 shows the different outcomes arising from the interaction of postnatal allergen exposure and infection. It is also possible to detect differences in allergen-induced cytokine production at birth in infants who have subsequently developed allergic disease (Kondo et al. 1992). There is a generally reduced capacity in such infants to generate both IFNγ, the archetypal Th1 cytokine (Warner et al. 1994), and IL13, a characteristic Th2 cytokine (Williams et al. 2000). To what extent this represents a form of immunological immaturity or immune suppression as a consequence of
overactive regulatory T cells remains to be established. Factors that are thought to influence the evolution of the allergic immune response postnatally include the route and dose of allergen exposure, and exposure to the adjuvantising effect of infection or particular gut flora (Holt et al. 2004).

Longitudinal cohort studies following atopic and non-atopic children through early childhood have demonstrated a biphasic pattern of change in IgE antibodies with significant differences in responses to dietary allergens which does not occur in response to inhalant allergens. There is an initial peak of IgE antibody production against foods in infancy followed by a decline by 1–2 years of age, but with higher peak levels in those who are allergic. There is an implication that very high levels of dietary allergen exposure will eventually stimulate so-called high-zone tolerance by mechanisms involving T-cell depletion and/or anergy. In contrast, the IgE antibody response to inhalants is much slower and takes many years to decline, with a significant percentage having a persistence of the antibody, particularly those who manifest inhalant, allergy and asthma (Holt et al. 1995). Clearly exposure to inhalants, predominantly in the respiratory rather than the gastrointestinal tract, is at much lower concentrations and therefore is unlikely to reach the levels associated with high-zone tolerance. Again, therefore, the implication is that rather than allergen avoidance, high-dose allergen exposure as a form of immunotherapy may be more appropriate. This indeed is borne out by a few immunotherapy trials. One such study involved the administration of pollen immunotherapy to children with allergic rhinitis but not asthma. This was associated with a lower period prevalence of asthma over the three years of the study compared with contemporaneous controls who were not given immunotherapy (Møller et al. 2002). Furthermore, studies have shown that children given immunotherapy when they have a single inhalant allergy have a much lower probability of subsequently developing new inhalant allergies, again compared with untreated contemporaneous controls (Des Roches et al. 1997).
Birth order
- Early day care
- Early antibiotics
- Farmers’ children
- Early cat or dog ownership
- Inverse relationship with measles, tuberculin positivity etc
- Gut flora

Figure 26.6 Factors which have been suggested as explaining the so-called ‘hygiene hypothesis’, which states that early exposure to microbial factors is associated with a lower subsequent prevalence of allergy and allergic disease.

The hygiene hypothesis

One of the most compelling explanations for the increasing prevalence of allergic diseases in the developed world is the so-called ‘hygiene hypothesis’ (Fig. 26.6). Exposure to microbes might be expected to up-regulate Th1 activity, and therefore reduced exposure to microbes might be predicted to be associated with a persistence of Th2 activity and a higher probability of allergy and allergic disease (Folkerts et al. 2000). Many studies have shown an inverse relationship between the prevalence of certain infections and allergy (Strachan 2000). For example the infectious diseases typhoid and tuberculosis occur rarely in countries with a high prevalence of allergic disease, and it has been suggested that this explains the geographical variation in disease prevalence.

The hypothesis was first proposed in 1989 with the demonstration of an inverse relationship between birth order in families and the subsequent prevalence of allergic rhinoconjunctivitis (Strachan 1989). Whether this is an ante- or a postnatal effect could be disputed, as the sibling effect may also have an impact on cord-blood IgE levels (van Gool et al. 2004, Karmaus et al. 2001). Studies have also shown an inverse relationship between tuberculin responsiveness, previous hepatitis A or measles infection and allergy (Shaheen et al. 1996, Shirakawa et al. 1997). This is further reinforced by the observation that BCG, which will produce a positive tuberculin test, can suppress local Th2 immune responses in a murine model (Folkerts et al. 2000). However BCG (Annus et al. 2004) has not been shown to have a significant effect on the subsequent development of allergy in Europe, although it may have an effect in developing countries (Aaby et al. 2000).

The hygiene hypothesis has been used to explain differences in the development of allergy and allergic disease in children born and raised on farms (Radon et al. 2004) or adopting an anthroposophic lifestyle (Alm et al. 1999). It has been suggested that the farming effect is a consequence of exposure to Toxoplasma gondii (Radon et al. 2004).

There is an inverse relationship between antibiotic usage, both during pregnancy (McKeever et al. 2002) and in infancy (Farooqi and Hopkin 1998), and the prevalence of allergy. Such antibiotic usage will have a potent influence on the type of organisms in the maternal gastrointestinal tract, which in turn will lead to a difference in those organisms colonising the newborn infant’s gut. The composition of gastrointestinal flora has been shown to be different in allergic compared with non-allergic infants (Björksten et al. 1999). This has led to one study administering probiotics in a pregnancy cohort study where parents had allergy and therefore the infant was at high risk of developing allergic sensitisation and disease (Kalliomaki et al. 2001). In a parallel-group double-blind placebo-controlled study where the probiotic Lactobacillus GG was administered to the mother during pregnancy and the infant postnally there was less atopic eczema up to 2 years of age than in the group receiving placebo. Unexpectedly, however, there was no difference in the degree of allergic sensitisation between the two groups. Many more and larger studies are required to elaborate on this. It is perhaps naive to think that the administration of a single so-called probiotic organism will change outcomes. The gut flora is a complex combination of up to 200 different bacteria. The range of colonising organisms varies with age and at present it is not possible to say which are more or less important in relation to modulating immune responses (Berg 1996). Furthermore, as probiotic bacteria do not achieve long-lasting colonisation
it may also be important to modulate the diet in order to achieve permanent colonisation, utilising so-called prebiotics (Gibson and Roberfroid 1995). Children brought up in families with an anthroposophic lifestyle, where there is avoidance of immunisations and indeed many medical treatments, as well as a diet consisting mainly of fermented vegetables, have a very different gut flora and a much lower prevalence of IgE-mediated allergic disease (Alm et al. 1999). It is, however, imperative to point out that there is no evidence that the conventional immunisation programme in any way contributes to the increasing burden of allergic disease. Indeed, in children with a high vaccine coverage there is transiently a better protection against the development of allergy in the first years of life. This is a most important public health message (Grüber et al. 2003).

Other proposed interventions to negate the effect of ‘hygiene’ have included the use of potent Th1 immune stimulators using Mycobacterium vaccae vaccines or bacterial CpG oligonucleotides, which have proved highly effective as Th1 selective adjuvants in experimental murine allergies (Kline et al. 1998). Trials with these interventions are awaited, but safety will need to be established before proceeding in humans. An additional interest in epidemiological studies has been exposure to endotoxin or lipopolysaccharide in early life and the subsequent diminished risk of IgE-mediated disease. This may be the key factor to which pregnant women and infants are exposed on farms (Oberle et al. 2003, Gern et al. 2004). It may also explain the fact the children exposed to dogs and/or cats in early infancy have less subsequent allergy. It may also explain why children with more siblings, and those who are enrolled early into day nurseries, have fewer allergies (Ball et al. 2000).

It is pertinent to note in relation to the ‘hygiene hypothesis’ that the mechanism by which microbes provide a Th1 stimulation is through pattern-recognition molecules. CD14 is one such molecule which specifically recognises lipopolysaccharide. Recent studies have identified a polymorphism in the CD14 gene manifesting as diminished levels of the soluble component of CD14 and associated increased intensity of allergy expression in American, British and Japanese but not German populations (Baldini et al. 1999, Gao et al. 1999, Sengler et al. 2003). CD14 is poorly expressed by fetuses and neonates but is present in a soluble form in amniotic fluid and at very high levels in human breast milk. A reduced supply of this molecule from the mother either in amniotic or breast milk is associated with a higher probability of early-onset atopic eczema (Jones et al. 2002b). Another pattern-recognition molecule known as toll-like receptor 4 (TLR4) is also important in binding endotoxin when complexed with CD14 to initiate a cytoplasmic signal leading to IL12 production. A study in Swedish schoolchildren has shown a polymorphism of the TLR4 gene to be associated with a fourfold higher prevalence of asthma (Bottcher et al. 2004). To what extent these polymorphisms can be overcome by relevant microbial exposure remains to be established. There has been a suggestion that the levels of soluble CD14 in breast milk might be correlated with levels of n-3 polyunsaturated fatty acids (Dunstan et al. 2004). Thus supplementation could conceivably raise levels, though this has not been shown as yet by intervention studies.

Exposure to early infection may be a two-edged sword. Some infections particularly associated with bacteria are also associated with less subsequent allergy (Williams et al. 2004), but it is important to note that viral respiratory tract infection may have a positive association with subsequent asthma (Resh et al. 2004). Whether this is a consequence of the underlying immunological aberration increasing the risk of infection, and also of allergy and asthma, remains to be established. Thus infants infected with respiratory syncitial virus (RSV) are more likely to develop bronchiolitis if they come from atopic families and are themselves atopic (Sigurs et al. 2000). Furthermore the immune response to RSV in those who develop bronchiolitis is Th2-biased compared with those who have an upper respiratory tract infection alone, who are more likely to generate IFNγ in response to the infection (Legg et al. 2003). Thus the same abnormality of immune responsiveness...
that leads to bronchiolitis also leads to allergy and may provide an explanation for the link between the two conditions. A severe infection-induced wheeze in infancy increases the risk of persistent asthma. It is possible that the inflammation caused by the infection adjuvantises a Th2-mediated allergic response. The more severe infection may in turn have been a consequence of an impaired maturation of Th1 responsiveness. There are, however, genetic predispositions to bronchiolitis and post-bronchiolitis wheezing independent of allergy involving the I1–8 promotor region (Goetghebuer et al. 2004).

Infant feeding allergy in asthma

Many studies have shown a reduced prevalence of allergy and allergic diseases with the use of human compared to cow’s milk in infancy. The predominant effect has been associated with food allergy and atopic eczema in infancy rather than asthma, although the data are conflicting (Peat et al. 1999). There appear to be differential effects depending on whether the mother has evidence of asthma herself or not. Thus, while large birth cohorts show that breast feeding can reduce asthma rates at least in early to mid-childhood, the effect would appear to be stronger in children born into families without any first-degree relative with allergy (Kull et al. 2004). Indeed, if the mother herself has asthma one study showed a significantly increased risk of asthma, particularly if the child was breast-fed for more than four months compared with being breastfed for less than four months or never being breast-fed (Wright et al. 2001). Furthermore, in the studies of children born on farms there would appear to have been a protective effect from the administration of cow’s milk to infants in reducing allergy, asthma, and rhinitis risk. However, this may have been a consequence of using unpasteurised milk with a different microbial load (Von Ehrenstein et al. 2000). It remains to be established whether the use of so-called hypoallergenic milk formulae will be protective in the long run. Extensively hydrolysed caseine formulae do reduce the prevalence of atopic eczema up to 1 year of age, particularly in those who have a family history of atopic disease (von Berg et al. 2003).

Speed of weaning has also been considered to have an impact on outcomes. Old studies suggested that the early introduction of solids at less than 4 months of age had no effect on asthma at 4 years of age (Fergusson et al. 1990), but a more recent study suggested early introduction of diverse solids in preterm infants increased the risk of eczema at 1 year of age (Morgan et al. 2004). In contrast to this, delayed introduction of egg was associated with more eczema at 5 years of age in another study (Zutavern et al. 2004), and the use of full-cream milk at 2 years of age was associated with less asthma at 3 years of age (Wiig et al. 2003). To what extent these dietary practices were influenced by the family history of allergy or were representative of some other pattern of environmental exposures is not fully investigated. Overall, therefore, the concept of reducing allergen exposure, whether dietary or inhalant, in order to reduce allergic sensitisation and disease is fraught with problems and should probably not be attempted. There are some positive benefits to be achieved by the use of human breast milk, probably because of immune adjuvantising effects rather than anything to do with avoidance of dietary allergen. The relative benefits of human milk, however, will be appreciably affected by the health and nutrition of the mother, and particularly by her allergic status (Schoetzau et al. 2002).

Airway remodelling

Airway remodelling is a constellation of histopathological features characteristic of asthma, including the hypertrophy of airway smooth muscle and the deposition of collagen within the lamina propria and also below the true epithelium basement membrane in the lamina reticularis. This abnormality occurs in parallel with the characteristic eosinophilic inflammation associated with allergy (Pohunek et al. 2005). Hitherto it has been considered that the inflammation damages the epithelium and thereby induces a repair process which leads to remodelling.
However, remodelling can occur independently of eosinophilic inflammation and has for instance been described in elite cross-country skiers who have no allergy and primarily neutrophilic rather than eosinophilic airway inflammation (Karjalainen et al. 2000). It is interesting to note that many asthmatics with more severe disease both in infancy and adulthood have a predominance of neutrophils in their airways (Marguet et al. 1999). Neutrophils, par excellence, generate a specific matrix metalloproteinase 9 (MMP9) which disrupts the collagen matrix. This in turn will release mediators within the matrix, of which TGFβ is particularly important (Cundall et al. 2003). This acts as a direct trigger of fibroblast activity to lay down matrix and also to induce a fibroblast transformation to a myofibroblast, which may in turn contribute to the smooth muscle hypertrophy (Blobe et al. 2000). This implicates neutrophils in the pathogenesis of the remodelling process rather more than eosinophils.

Bronchial hyper-responsiveness (BHR) is the one non-invasive measure that has been associated with the pathological changes of remodelling. It is notable that the presence of increased BHR at 4 weeks of age is associated with asthma at 6 years of age independent of atopy (Palmer et al. 2001). From the same cohort, reduced airway function at 4 weeks was also associated with persistent wheeze at 11 years, and this was independent of both atopy and increased BHR (Turner et al. 2004). Furthermore, increased specific airway resistance and allergic sensitisation at 3 years of age in children who had wheezed before 3 years were independent predictors of persistent wheeze at 5 years (Lowe et al. 2005). One potential interpretation of these observations is that the remodelling process is present long before the onset of disease or any evidence of allergic inflammation. Indeed it is even possible that the changes had occurred antenatally. TGFβ is a key factor involved in the regulation of lung branching morphogenesis during the pseudo-glandular stage of lung development. There is a variable spatial expression of the isoforms of TGFβ during lung development. TGFβ1 colocalises to the branch clefts with collagen 1 and 3 and fibronectin. TGF2 and TGF3 are expressed in epithelial cells at the tips of the growing lung buds (Gatherer et al. 1990). This has led to the proposal that the asthmatic state results from a reactivation of fetal airway modelling processes to generate an inappropriate remodelling response postnataally (Davies et al. 2003). The other possibility is that the modelling process in utero has already been adversely affected by the intrauterine environment interacting with genetic factors, thereby setting the scene for later asthma.

Genetic polymorphisms and airway structure and function

The first candidate gene that has been associated purely with structure and function rather than allergic inflammation has been that coding for the beta-2 adrenoceptor, where polymorphisms have been associated with susceptibility to subsensitisation on continuous beta-agonist usage and a probability of BHR and increased disease severity (D’Amato et al. 1998). More recently a wide range of novel genes associated with airway epithelium and fibroblasts rather than with inflammatory cells have been identified. There is strong linkage between markers on the short arm of chromosome 11 (11p13) and BHR with asthma. Research has focused on two related proteins, designated ESE2 and ESE3, which are members of a transcription factor family whose basal expression is restricted to epithelial cells with a secretory capacity such as those in the airway. ESE3 is thought to be involved in epithelial cell differentiation towards a mucus-secreting phenotype. It has also been shown to be up-regulated in fibroblasts and smooth muscle when they are exposed to proinflammatory cytokines (Zamel et al. 1996, Silverman et al. 2002). In addition to the cytokine gene cluster on 5q31–34, many other genes likely to be of importance to asthma are also located in the same region, including the beta-2 adrenoceptor and corticosteroid receptor. Recently, however, a gene encoding a serine protease inhibitor, Kazal type 5 (SPINK5) has also been identified. Several mutations of this gene have been associated with a condition known as
Netherton syndrome, which is characterised in part by eczema (Chavanas et al. 2000). SNPs in the same gene have been strongly associated with asthma and eczema (Walley et al. 2001). SPINK5 expression is in epithelial cells, and it has a protective function against proteases such as those produced by mast cells and those that are present in some allergen extracts such as those of house dust mite which might otherwise cause cell desquamation (Komatsu et al. 2002).

Another very important development has been the identification of a disintegrin and metallopeptinase 33 (ADAM33), as a major candidate gene for asthma and BHR found on chromosome 20 (20p13). The strength of associations of polymorphisms in this gene is increased when BHR is included in the definition of asthma and is weakest if the total IgE is raised or if there are specific IgE antibodies (Van Eerdewegh et al. 2002). Expression of the gene product is restricted to mesenchymal cells such as fibroblasts and smooth muscle, where its likely function is to influence smooth-muscle hypertrophy and airway wall remodelling, and it thereby manifests as BHR. The mouse equivalent of polymorphisms in this gene have been linked to BHR (Yoshinaka et al. 2002). This gene is also preferentially expressed during branching morphogenesis in mouse lung development, thereby linking concepts of airway modelling during embryonic development with the remodelling associated with asthma. Furthermore, polymorphisms of the ADAM33 gene are associated with more rapid decline in lung function in adult asthmatics (Jongepier et al. 2004).

One other gene selectively expressed in epithelium is mucin 8 (MUC8). This is found in similar families to those where ADAM33 was first identified. Finally a novel candidate gene linked to asthma whose product is also expressed on epithelial cells is DPP4 (CD26). It is expressed on epithelial cells where it functions as an adhesion receptor for collagen and fibronectin-associated epithelial repair. These data suggest that the range of polymorphisms may be associated with susceptibility to epithelial damage, or may be involved with repair processes and/or the processes associated with modelling of the airways in embryonic life. It is interesting to note that cells expressing ADAM33 are those whose maturation is directed by growth factors such as TGFβ. Indeed, ADAM33 is up-regulated during TGFβ-induced myofibroblast differentiation (Holloway and Holgate 2004).

Another genetic polymorphism associated with BHR in asthmatic children is in the Clara cell protein 16 (CC16) gene on 11q12–13. Polymorphisms have been associated with lower serum levels of CC16 and with asthma (Laing et al. 1998). The protein has sometimes been known as uteroglobulin or CC10, and it is expressed on bronchiolar epithelium. It has several immunomodulatory and antiinflammatory functions, and lower levels have been found in patients with chronic obstructive pulmonary disease.

Finally, on investigating gene–environment interactions there have been interesting associations between polymorphisms in the glutathione S-transferase M and T genes, GSTM1 and GSTT1. The null genotype for these has been associated with greater reductions in lung function in asthmatic children, and supplementation of a child’s diet with the antioxidants vitamin C and vitamin E may protect against an interaction between the null gene and ozone exposure (Romieu et al. 2004). While the null genotype may lead to reduced protection of the airway against postnatal oxidative stress, there is a suggestion that there may also be an impact on the developing lung, with an interaction between antenatal exposure to environmental tobacco smoke and wheezing in childhood (Kabesch et al. 2004).

Environment and airway structure

Maternal smoking severely compromises fetal health and has a clear impact on postnatal lung function. Significant differences in lung function are found in 4-week-old infants born to smoking mothers compared with non-smoking mothers (Dezateux et al. 1999). This in turn is associated with a significantly higher risk of wheezing illnesses in early life. It remains to be seen whether this antenatal compromise, in association with particular genetic polymorphisms, will be associated with more persistent
ongoing wheezing illnesses such as asthma. Hitherto the effect of maternal smoking during pregnancy has only been associated with early rather than later wheezing, and with non-allergic rather than atopic asthma (Strachan and Cook 1998). However, maternal pregnancy smoking has been associated with modification of fetal immune responses, which might be extrapolated to an increased risk of allergy with higher cord-blood mononuclear cell release of IL13 on stimulation with allergens (Noakes et al. 2003). It remains to be established whether exposure to other pollutants such as ozone might also have an interactive effect, promoting susceptibility to asthma.

In a murine model, mild maternal vitamin A deficiency has been associated with reduced expression of lung surfactant and delayed maturation of lung function (Chailley-Heu et al. 1999). A host of other dietary factors have been associated with an increased risk either of allergy or of asthma. These include the antioxidants, selenium, vitamin E, sodium and lipids (Fogarty and Britton 2000, Devereux et al. 2002, Calder 2003).

There has been an interest in the association between obesity and asthma, with a number of studies reporting a strong relationship between body mass index and asthma risk in children and adults (Schachter et al. 2003, Tantisira et al. 2003). One study has shown improved asthma control following weight reduction in obese asthmatic patients (Stenius-Aarniala et al. 2000). However, there is also a possibility that the relationship has its origins in fetal life. A study has shown that smaller size at birth, even within the normal range of birthweight, was associated with lower lung function represented by airway calibre at 6–13 weeks of age. At all levels of birthweight, higher early weight gain was also associated with reduced lung function (Lucas et al. 2004). This latter phenomenon may well represent catch-up growth as a consequence of late gestational faltering of growth. Rapid postnatal weight gain following lower birthweight may well translate into later obesity and explain the relationship between obesity and asthma.

**Summary**

There are two components of asthma, both of which are under genetic influence (Fig. 26.7). Some genetic
polymorphisms increase the risk of allergic sensitisation in association with environmental factors such as concentration of allergen exposure and variations in nutrition of the mother during pregnancy and lactation. Postnatally, further exposure to allergens in the absence of any infection polarises immune responses towards an allergic phenotype, while in the presence of infection there is a reduced risk of subsequent allergic diseases such as eczema or rhinitis. There are additional gene–environment interactions that affect the airway modelling, which in association with allergy will lead to asthma. Thus a different set of genes, in association with environmental factors such as maternal nutrition and smoking during pregnancy, modifies the fetal airway. Postnatally infections and pollutants promote an inflammatory immune response associated with the neutrophil, which induces further abnormalities in airway structure and therefore bronchial hyperresponsiveness. This, together with atopy, leads to ongoing asthma. In the absence of atopy it is associated with transient infant wheeze.

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