Development of Atopy and Asthma: Candidate Environmental Influences and Important Periods of Exposure

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Atopy is a major risk factor for the development of asthma. Immune processes that lead to the development of antigen-specific IgE are essential to the development of atopy. This review examines the immune processes that are candidate targets for modulation by environmental agents; environmental and lifestyle factors that have been suggested as modulators of the development of atopy; and the impact of known environmental agents on atopic processes in the airway. The most important periods of immune development with regard to expression of atopy are likely during gestation and early childhood. A better understanding of which environmental agents are important, as well as the period of life during which these agents may exert an important effect, is essential to devising rational environmental avoidance strategies for at-risk populations. *Key words*: asthma, atopy, immunoglobulin E (IgE), immunoglobulin G (IgG), T-helper cell type 1 (Th1), T-helper cell type 2 (Th2). — *Environ Health Perspect* 108(suppl 3):475–482 (2000).
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Asthma is one of the most common diseases in both adults and children in the United States. A major risk factor for the development of asthma is atopy. Atopy generally refers to the development of immune responses to foreign antigens that are characterized by the production of antigen-specific IgE. Development of atopic responses has been linked to several genes and gene products. Thus, atopic diseases represent a complex gene–environment interaction in which environmental antigens interact with the immune system, producing atopic (IgE) responses. Furthermore, other genes are likely important for full expression of asthma in individuals. In addition to interaction between antigens and the host immune system, other environmental and lifestyle factors may impact immune responsiveness such that IgE-type immune responses are favored over nonallergic responses.

The processes that mediate immune responses to antigens are reviewed in this paper, along with examples of how such processes are altered by genetic defects and environmental and lifestyle influences that may support the development of atopic responses. Of special interest are environmental and lifestyle factors that may act before conception, during gestation, or during early childhood such that an atopic or asthmatic phenotype is found within a given individual. Identification of environmental factors present during these critical windows of exposure may allow for intervention from both a personal and a public health perspective. Avoidance of these agents during important periods in immune development could result in decreased expression of atopic/asthmatic phenotypes in genetically susceptible individuals.

Asthma and Atopy

Asthma is a complex multifactorial disease that is characterized by reversible airway obstruction, airway hyperresponsiveness, and eosinophilic airway inflammation (*1*). Atopy is the most significant risk factor for asthma development, with approximately 85% of children who develop asthma and 40–50% of adults with new-onset asthma having an allergic response to aeroallergens (*1–4*). Several studies reveal a link between IgE and asthma (*2,4–8*). Even in nonatopic asthma, the presence of eosinophilic airway inflammation suggests that similar atopic-like immune processes are important in the development of this disease (*1,9*).

However, atopy alone does not account for asthma: many persons are atopic but not asthmatic. Given the multiple processes involved in asthma pathogenesis, it seems likely that several genes play a role in asthma development. One example is airway hyperresponsiveness, an important defining feature of asthma. A polymorphism of the gene that codes for the β_2 receptor is associated with airway hyperresponsiveness and may mediate this phenomenon in asthma (*10*). The location of this gene is of interest—it is located near a cluster of genes on chromosome 5q, which plays a key role in mediating atopic inflammation. Although it has been thought that atopic inflammation causes airway hyperresponsiveness, the clustering of these genes provides an alternate explanation for the link between atopy and airway hyperreponsiveness in asthma.

Environmental influences are also an important determinant in the development of an atopic or asthmatic phenotype. Persons prone for development of atopy may only develop such responses if living in environments that induce expression of proatopy genes. Prime examples of such environments are those rich in airborne (house dust mite and pollen) and orally encountered antigens (*11*). Likewise, with decreased exposure to such environmental factors, there is often a decrease in the severity of atopic disease in affected individuals (*3,12*). The effects of air pollutants, lifestyle factors, and urbanization on the development of atopy and asthma are more controversial.

A logical target for examination of the effect of environment and lifestyle on development of asthma is the effect of environmental influences on the immune response to antigens, with emphasis on development of atopy.

Antigen Presentation

Development of immune responses to specific antigens is a complex process, and it has been reviewed elsewhere (*13,14*). Initially, antigens are taken up by a number of cells that can act as antigen-presenting cells, which, as their name implies, process antigens and then present them to either CD8$^+$ or CD4$^+$ T lymphocytes. These lymphocytes then direct specific immune responses against processed specific antigens. CD8$^+$ lymphocytes direct responses against derived antigens produced within host cells, whereas CD4$^+$ lymphocytes direct responses against antigens encountered outside of the host cell (*13,14*).

Antigen presentation to CD8$^+$ cytotoxic cells requires major histocompatibility complex (MHC) class I molecule expression on the surface of the cell that is presenting the antigen. All body cells express MHC class I molecules and the antigens typically presented to CD8$^+$ lymphocytes are neoantigens produced within the host cell itself. These antigens usually include tumor antigens or viral proteins generated during the viral life cycle in which viral DNA or RNA uses the protein-generating capability of the host cell.

Antigenic proteins are processed in the host cell cytoplasm by proteosomes, which results in the production of derivative antigenic peptides. These peptides are then transported to the endoplasmic reticulum in the Golgi apparatus. MHC proteins and β_2 macroglobulin are produced in the Golgi apparatus and the antigenic peptide is complexed with these molecules and transported to the host cell membrane. This complex interacts with the CD8+ lymphocyte via the T-cell receptor, the CD8 molecule, and the CD3 receptor that are clustered on the surface on the CD8 cell. These activated CD8+ cells then attack the host cell bearing the antigenic peptides, destroying that cell and, when effective, the source of the antigenic peptides (13,14).

Immune responses directed against proteins produced outside of host cells are directed by CD4+ T lymphocytes. These antigens, which usually derive from bacteria, extracellular viral particles, uni- or multicellular parasites, or molecules produced by plants or animals, are processed by specialized or professional antigen-presenting cells (APCs). A number of cells can act as APCs, including dendritic cells in the skin, alveolar macrophages, B lymphocytes, and venular endothelial cells. The unique antigen presentation function of these cells is mediated by expression of MHC class II molecules (13,14).

APCs take up antigen via phagocytosis and digest these extracellularly derived molecules in a lysosome or endosome. Independently, MHC II molecules are generated in the Golgi apparatus. Vesicles containing MHC II molecules then fuse with the endosomes containing digested foreign antigens and the antigen is bound to the MHC II molecule. This complex is then transported to the surface of the APC and interacts with clustered receptors on the surface of the CD4+ T lymphocyte.

These cell molecules include the CD4+ receptor, the T-cell receptor, and the CD3 molecule. After the MHC II–antigen complex interacts with the surface molecules of the CD4+ T cell, the T cell is activated. The resulting immune response ultimately results in production of immunoglobulins directed against specific antigens (or antibodies) as well as secretion of a number of cytokines that promote growth and differentiation of effector cells in bone marrow and other tissues which complete the immune response directed against the specific antigen (13,14).

A number of inherited immune defects demonstrate how essential these processes are in directing immune responses (15). Defects in expression of CD3 and the T-cell receptor by lymphocytes are a cause of one variant of severe combined immune deficiency (SCID). Defects in tyrosine kinase activity, such as a lack of ZAP-70, also result in SCID. Lymphocytes are also susceptible to build-up of products of purine metabolism. If they are lacking in enzymes that allow metabolism of purines to nontoxic products, lymphocyte cytotoxicity occurs, resulting in other variants of SCID. These syndromes exemplify how alterations in APC/T-cell interaction or T-cell physiology alter immune responses. Although these cited alterations are due to genetic defects, environmental agents that target these processes could also impact the ultimate nature of the immune response to inhaled antigens.

Atopic T Helper (Th)-2 Versus Nonatopic Th1 Immune Responses

There is no formal or standardized definition for atopy. Persons are thought to be atopic if they have one of a number of typical allergic clinical syndromes, including atopic dermatitis, food allergy, allergic rhinitis, and at least certain forms of asthma, such as that which typically exists in early childhood. Atopic responses are often seen in response to a number of allergens (such as animal, plant, or fungal antigens), which may be encountered by oral or inhalant routes or venoms from stinging insects. One difficulty in defining atopy is that many persons may develop several allergic diseases or they may only manifest with one disease. Likewise, persons who are otherwise thought to be nonatopic may mount an atopic response to an isolated antigen, such as a venom from a stinging insect, or a protective eosinophilic response to multicellular parasites. Nonetheless, a key feature in any functional definition of atopy is the development of IgE antibodies directed against specific antigens, which is often associated with eosinophil and mast cell activity (13,14).

Conversely, CD4+-mediated responses that result in IgG production and neutrophilic inflammation are thought to be nonatopic. Nonatopic responses are typically elicited by bacteria and viruses that are encountered as the result of the infection of tissues. Atopic and nonatopic immune responses occur in persons who have an atopic disease. However, it is interesting to note that many immune deficiency diseases with blunted nonallergic responses are associated with phenotypic features of atopy, such as eczema and increased IgE production (13,14).

CD4+ lymphocytes direct B cells to produce immunoglobulin directed against specific antigens. B cells require antigen-binding and T-cell help to proceed with the initial formation of IgM. Activation of CD40 by CD40 ligand [a member of the tumor necrosis factor (TNF)-α family] is necessary for this response. This activation is expressed on T lymphocytes. IgM is the initial immunoglobulin response to any antigen by B cells, and increases in circulating IgM correlate with initial exposure to a particular antigen. After producing IgM, B lymphocytes eventually mature into plasma cells that secrete IgA, IgG, or IgE. The process of switching from IgM to production of one of the final immunoglobulin classes also requires activation of CD40 (13,14).

IgA is produced in the mucosa and is secreted by mucosal cells onto the lumen of the airway and gastrointestinal tracts. IgA is also secreted into breast milk, where it is passively consumed by infants. IgA exists as a dimer and binds to antigens, keeping them from adhering to mucosal surfaces and gaining entry into the body proper. IgG exists in the bloodstream as monomers; four subclasses of IgG have been identified. IgG1 and IgG3 are thought to bind protein antigens and IgG2 and IgG4 recognize polysaccharides. Receptors for IgG ($Fc_\gamma RI$, -II, and -III) are found on a number of effector cells, including polymorphonuclear neutrophils (PMNs). IgG binds to bacteria and can also activate complement (via domains on the CH3 region of the molecule). IgG can confer protection against foreign antigens by facilitating phagocytosis of antigens by PMNs via $Fc_\gamma Rs$ or by activation of complement, which directly causes foreign cell lysis or allows for interaction of the foreign antigen with phagocytes via complement receptors. Interestingly, IgG4 is associated with IgE and the true function of IgG4 is not clearly understood.

IgE is primarily produced in local tissues (such as the gut or airway) and, after being secreted by IgE-producing plasma cells, binds to cells that bear either high-affinity (FcERI) or low-affinity (FcERII or CD23) receptors. FcERI receptors are expressed by mast cells and basophils and cross-linking of IgE on these cells leads to the release of histamine and production of a number of cytokines. CD23-bearing cells include eosinophils and platelets. In general, IgA impedes antigen binding to mucosal surfaces, IgG facilitates neutralization or phagocytosis of foreign antigens, and IgE induces histamine release and other phenomena associated with atopy or response to parasites (13,14).

Cytokine Mediation of Th1 and Th2 Responses by CD4+ Lymphocytes

There is a difference in cytokine secretion profiles of CD4+ T cells recovered from atopic and nonatopic subjects (16,17). These cytokines mediate specific responses in immune cells and other tissues that result in the Th1 and Th2 characteristics outlined in "Atopic T Helper (Th)-2 Versus Nonatopic Th1 Immune Responses" (11). A summary of these cytokine actions follows [reviewed by Blumenthal (3) and Borish and Rosenwasser (16)].

Cytokines associated with Th1 responses include interleukin (IL)-2, interferon-γ, and IL-12. IL-2 is secreted by Th1 cells and stimulates clonal expansion of antigen-specific T cells, CD8 cell maturation, and is a switch factor for B lymphocytes, inducing them to mature from IgM-secreting cells to IgG1-secreting cells. IL-2 is thought of as a Th1 cytokine, and is essential for all immune function defects in the IL-2 receptor in lymphocytes. IL-2 receptor defects also account for one of the most common forms of SCID. Interferon-γ induces expression of FcγRs, MHC class I, and MHC class II molecules on the surface of macrophages (facilitating their actions as antigen-presenting cells), promotes B cells to switch from secretion of IgM to IgG2, inhibits B cell switch to IgE secretion, and induces other T cells to express a Th1 rather than Th2 cytokine phenotype. IL-12 is primarily secreted by macrophages (an antigen-presenting cell) and acts on T lymphocytes to induce secretion of a Th1 rather than Th2 cytokine profile.

Cytokines associated with Th2 responses include IL-4, IL-5, IL-10, and IL-13 (Table 1). IL-4 acts on B lymphocytes to induce the switch from IgM secretion to IgE and IgG4 secretion and also contributes to the expression of VCAM-1 on endothelial cells in post-capillary venules. VCAM-1 is a ligand for VLA-4, a molecule expressed on eosinophil membranes. Interaction of these molecules is essential for migration of eosinophils from the bloodstream to end-organ tissues. IL-5 promotes eosinophil maturation and survival. IL-10 acts on macrophages to inhibit expression of MHC class II molecules and inhibits Th1 responses by blunting production of interferon-γ. This cytokine also promotes T cells to exhibit a Th2 phenotype by enhancing

the action of IL-4. IL-13 is homologous with IL-4 and, like IL-4, induces B cell switching from IgM to IgE.

Many other cytokines promote both Th1 and Th2 responses, including IL-1, IL-3, IL-8, granulocyte macrophage colony-forming unit (GM-CSF), and TNF-α. IL-1 is important in general T-cell activation. IL-3 is an essential growth factor for hematopoeitic cells, and is essential for mast cell and eosinophil proliferation. IL-8 is primarily known as a chemotactic and priming agent for PMNs, but also has actions on basophils and eosinophils. GM-CSF is an important growth factor for neutrophils, eosinophils, and macrophages. TNF-α has a broad spectrum of action, including upregulation of MHC class I and II molecules and activation of virtually every cell in the immune system.

Of interest are general observations examining the Th1 versus Th2 response in humans (15). All healthy humans, whether thought to be atopic or not, have a robust Th1 response. Defects in Th1 responsiveness are associated with significant morbidities linked to decreased immune function (increased infection and increased incidence of tumors). Conversely, the majority of humans do not manifest significant signs of Th2 immune activation (atopic diseases) and an apparent lack of Th2 responses is not routinely linked to poor health. Many congenital immunodeficiency states, including several varieties of SCID, hyper IgE syndrome, and Wiskott-Aldrich syndrome, are typified by diminished Th1 and exaggerated Th2 responses. Likewise, exaggeration of atopic characteristics has been reported in some acquired immunodeficiency virus patients. There appears to be a relationship between decreased Th1 function and increased Th2 function. Uncovering the basis for Th1 and Th2 balance may uncover potential targets for study of the effect of environmental exposures on the development of atopic diseases.

Genes Associated with Atopy and Asthma

Approximately 10% of the U.S. population has an atopic disorder (with reports of as high as 30% having at least one positive skin test response). An estimated 5–7% of the U.S. population has asthma. A common feature of atopic disease is that it develops in susceptible individuals who experience exposure to significant environmental or lifestyle-related stimuli. Susceptibility for development of atopic disease appears to have familial associations and genetic components.

Evidence for a genetic component for asthma is found in studies of disease phenotypes on twin pairs (3,18,19). There is significantly greater concordance among monozygotic (MZ) twins than dizygotic

(DZ) twins with regard to asthma. MZ twins exhibited a 19.8% concordance for asthma versus 4.8% in DZ twins in one study. A second study involving 2,902 twin pairs revealed 30 versus 12% concordance in MZ versus DZ twins [reviewed by Blumenthal (3) and Edfors-Dubs (19)]. With atopy rather than asthma as an end point, 50–60% concordance has been reported in twin pairs. These studies indicate that there is a genetic component for asthma and/or atopy. However, it is clear that not all atopic persons have the same diseases. Even monozygotic twins, who have identical genomes, do not have 100% concordance with regard to the development of asthma or atopic diseases. The failure to observe 100% concordance (or even levels approaching 100%) strongly suggests that environmental as well as genetic factors influence atopic or asthma phenotype expression.

The technique of mapping genes that code for mediators important in asthma and atopy (or at least phenotypes or disease characteristics important in asthma or atopy) has been used to explore the genetic basis of asthma and atopy (the candidate gene approach). Such studies carried out in a number of laboratories suggest that chromosome 5q31-33 may be important in asthma and atopy, with genes for IL-3, IL-4, IL-5, IL-13, and GM-CSF clustered on the 5q locus (3,20–22). This technique also indicates that the β subunit of the high affinity IgE receptor is located on chromosome 11q. All-in-all, candidate gene studies have suggested linkages of potentially important genes with regions 5q (β2 AR and those listed above), 6p (HLA-DR), 11q, 12q (interferon-γ), 13q, and 14q (TCR).

Positional cloning techniques (3) allow for examination of the genome for loci associations with phenotypic features of asthmatic and atopic subjects without a priori knowledge of the inflammatory or immune function of any subsequently identified DNA sequences. Such studies suggest linkages of asthma or atopy with regions 2q, 5p, 11p, 17p, 19q, and 21q (18–22).

Development of Atopy and Asthma

Prenatal and Preconceptional Influences (Parental Influences and Exposures)

Aside from having a genetic predisposition for atopy, no specific preconceptional influences that are strongly linked to development of atopy have been identified in humans. Th2 responses can clearly occur in fetal life, as demonstrated by antigen-specific IgE and the presence of allergen-responsive lymphocyte and mononuclear cells in cord blood (5,7,11,23–27). Furthermore, infants can have positive skin tests to food allergens,

Table 1. Summary of some key cytokines in atopy.

Cytokine	Description
GM-CSF	An important growth factor for neutrophils, eosinophils, and macrophages
IL-1	An important T-lymphocyte activator
IL-3	A hematopoeitic cell growth factor important in mast cell and eosinophil growth
IL-4	Mediates immunoglobulin class switching of B lymphocytes from IgM to IgE; contributes to the expression of VCAM-1, which allows for eosinophil adhesion to endothelial cells
IL-5	An important growth factor for eosinophils
IL-8	A potent neutrophil chemoattractant, also primes eosinophil responses
IL-10	Acts as an anti-inflammatory cytokine; blunts expression of MHC class II molecules on antigen-presenting cells; blunts secretion of interferon-γ by T lymphocytes
IL-13	Mediates immunoglobulin class switching of B lymphocytes from IgM to IgE; shares some homology with IL-4

presumably due to maternal ingestion of food allergens. However, attempts at decreasing maternal exposure to food allergens have not been shown to decrease fetal levels of IgE or the likelihood of the child having atopic disease (*11*). Nonetheless, several studies have shown that maternal factors outweigh paternal factors in the development of atopy or asthma in children (*11,28*). These observations support the hypothesis that maternal influences, whether genetic, transplacental, or environmental, may play a role in the development of atopy or asthma.

Recent studies have focused on immune function of neonates as it relates to atopy development. Several studies showed that mononuclear cells recovered from cord blood have robust proliferative responses to stimulation with allergens [including house dust mite antigen, rye grass pollen extract, *Fel d* I (cat allergen), ovalbumin, and β-lactoglobulin]. Similarly, stimulated cord blood mononuclear cells secreted Th2 cytokines, including IL-4, IL-5, IL-9, IL-10 and IL-13. It is noteworthy that the Th2-type cord blood mononuclear cell (CBMC) responses occur both in infants who were thought to be at low risk for development of atopy (based on family history) as well as those thought to be at higher risk for atopy (*11,24,29–35*). Interferon-γ also appears to be important in the development of atopy at a young age (*30,31,36–38*). Interferon-γ is associated with Th1 responses, antagonizes the action of IL-4, and blunts production of IL-4 (*16*). Studies in many laboratories demonstrate that CBMCs or peripheral blood mononuclear cells from neonates have diminished ability to produce interferon-γ compared to cells from normal adults after stimulation with mitogens. This blunting generally resolves by 5 years of age. However, there appears to be a trend in which interferon-γ responses in cells obtained from children who develop atopic disease are even more blunted than those from nonatopic children. Some have argued that production of adult levels of interferon-γ by mononuclear cells occurs later in atopic children than in nonatopic children. Thus, blunting of Th1 responses may be important in maintaining Th2 responses.

These results, and others, have led to the argument that a Th2 phenotype is relatively universal in all neonates and that subsequent postnatal development of atopy may be due to the failure of Th1 responses to adequately develop. This notion is supported by animal observations that dendritic cell function is dampened in neonatal rodents. This prevents robust Th1 responses from occurring during fetal life. It has been speculated that dampening Th1 responses is important for fetal survival and that all infants are predisposed for

atopy until Th1 stresses (and response to those stresses) occur (*24,25,31,37*).

Postnatal Influences and the Development of Atopy and Atopy in Children

Early exposure to allergens in susceptible individuals has been postulated as an important factor in development of an atopic phenotype (eczema, rhinitis, or asthma) during childhood. Correlations between early exposure to seasonal airborne allergens and development of atopic responses to those allergens have been reported. Specifically, seasonal allergens prevalent during the first month of life seem to predict eventual development of atopic airway disease related to that allergen in later childhood. Similar arguments have been made regarding house dust mite exposure, with children living in environments with increased levels of mite allergen in collected house dust during the first year of life being more likely to develop asthma. Studies that examined efforts to reduce the incidence of atopic airway disease by decreasing allergen exposure during the first year of life are in the very early stages. One such study reported that the combination of decreased airway and oral allergen exposure seems to decrease incidence of atopic diseases at 2 and 4 years of age (*5,11,31*).

Perhaps better understood is the relationship between food allergen exposure and the development of atopic responses [reviewed by the Early Treatment of the Atopic Child study group (*5*), Bjorksten et al. (*11*), and Platts-Mills et al. (*37*)]. Positive allergy skin tests have been reported within the first 3 months of life in nearly 30% of children born to atopic parents. The disease state most commonly associated with food allergy in infants is eczema, and 80–90% of infants with eczema will develop a positive skin test to airborne allergens. It has been argued that both maternal and neonatal exposure to food allergens contributes to the development of specific allergen responses. A milk and egg allergy is perhaps the most common of the food allergy states in infants (*5,11*).

Infants who are exclusively breast-fed appear to have decreased risk for the development of food allergy than infants who are not breast-fed or who are have other food exposures. It has also been suggested that food allergens that may be secreted into breast milk may pose a risk for sensitization in those exposed infants. Although decreased foreign food exposure is one mechanism by which breast-feeding of infants may be protective, the presence of maternal IgA in breast milk may also be important. Although data can be cited on either side of the breast-feeding argument regarding its role in protection against food allergies, the weight of evidence indicates

that food allergen avoidance in at-risk infants is protective against the development of atopy (*11*).

In addition to allergen exposure, maternal tobacco smoking has also been linked to increased rates of wheezing and asthma in exposed children, increased bronchial reactivity, and increased total and antigen-specific IgE (*11,39–41*). Exposure to environmental tobacco smoke (ETS) may enhance atopy by a number of mechanisms. These include increased airway mucosal permeability or direct effect on immune function. The link between ETS and asthma appears clear with regard to exacerbation of preexisting disease. Although there is still debate on the effect of ETS in the development of asthma or atopy, the preponderance of the evidence supports the hypothesis that ETS enhances atopy development in susceptible individuals.

Environmental Influences in the Development of Atopy and Asthma

I have outlined "normal" factors that may influence the development of asthma and atopy in fetal life and in early and later childhood. These factors primarily dealt with allergen exposure, maternal dietary exposure, and patterns of immune expression in fetal and early life. I now expand on the initial review and examine a number of potential lifestyle and environmental influences that have been proposed as modulating factors in the development of atopy. These include living in urban versus rural settings, dietary factors, exercise patterns, having experienced infections, or the use of antibiotics (which might influence deviation of the immune system away from Th2 responses and toward Th1 responses).

Additionally, there are some data examining the role of specific environmental influences (including ETS, diesel exhaust, endotoxin exposure, and criteria air pollutants) having an effect on developing certain patterns of immune expression. I review what is known or commonly hypothesized about the role of these influences on the development of atopy and asthma and highlight the known influence of specific pollutants on immune responses.

Lifestyle Influences on the Development of Atopy

A number of epidemiological studies have pointed to the potential role of lifestyle as a factor that modulates the expression of atopy in susceptible individuals [reviewed by Platts-Mills et al. (*37*)]. One of the most intriguing examples of the effect of lifestyle is the examination of the prevalence of asthma and atopy in children from eastern and western Germany

at times after the political reunification of that state. Shortly after reunification in 1990, studies of the prevalence of atopy and asthma in children from East and West Germany revealed that children from the east, albeit more likely to be diagnosed with bronchitis, were less likely to have atopy, had fewer positive skin tests, and were less likely to have asthma than their western counterparts (42). Although it was unclear which lifestyle factors were influencing atopy development, there were some candidate influences. Children in the east were more likely to be placed in day care than those in the west. Also, potential differences in diet, especially fat intake, were suggested as possible influences. In the early 1990s, particulate pollution was higher in the east, whereas private automobile use and ozone exposure were more common in the west. Allergen exposure was not thought to be substantially different in the east than in the west.

It is now a few years later, and rates of atopy in eastern Germany have increased and are approaching those found in western Germany. This has been associated with the development of a more westernized lifestyle in the east, including decreased use of coal in industry, increased automobile use, and increased availability of high-fat foods. Decreased exercise and changes in architectural style have also been associated with the development of atopy and asthma. A comparison of heating styles in rural versus urban western Germany shows decreased asthma and atopy in the rural setting, in which wood- and coal-burning furnaces are used to heat homes. It was thought that bedroom and indoor temperatures were less in these homes than in urban homes, which might contribute to decreased expression of atopy. Dampness and water damage have also been associated with increased expression of allergic disease.

Similar observations have been made between other previously Eastern Bloc and western countries, as well as comparisons of asthma in rural versus urban Africa, Europe, and the Pacific (New Zealand and Australia). This effect of more recent westernization of lifestyle in these locations mirrors the development of asthma in previously westernized countries. Likewise, the problem of asthma in inner-city minority populations suggests a role for urbanization in the expression of atopy (37).

All in all, it seems quite likely that urbanization is a key feature in the development of asthma and atopy. This likely represents alterations in the environment, which allow for the expression of important genes that result in an atopic phenotype. Delineation of the specific features of urban lifestyle that allow the atopic phenotype to be expressed is incomplete.

Infections and Antibiotics: Effect on Th1 versus Th2 Cytokine Expression

There is evidence to suggest that fetal immune function is primarily of the Th2 type (23–25, 30,31,33,34,37,43). It has been further suggested that environmental stresses which suppress Th1 responses of the infant may allow for the persistence of Th2 immune function, thus increasing the potential for development of atopic diseases such as asthma (31,37,44). Among the most frequently reported immunological features manifesting in children with atopic diseases is decreased ability of circulating mononuclear cells to produce interferon-γ after in vitro stimulation with either mitogens or specific allergens (24,30,36,31,45). Interferon-γ plays a key role in expression of a Th1 phenotype and antagonizes the action of IL-4, which allows for the development of IgE. Against this backdrop, the role of interventional modifiers of Th1 responsiveness (vaccines, infections, and antibiotics) on atopy will be reviewed.

Perhaps one of the most interesting and controversial observations on the role of Th1 stimuli on Th2 expression is found when examining the effect of the antituberculosis vaccine Bacillus Calmette-Guerin (BCG) on the development of atopy. Japanese schoolchildren, who routinely undergo BCG vaccination, had a significant inverse relationship between delayed hypersensitivity to Mycobacterium tuberculosis and incidence of asthma and elevation of IgE (46). As with many studies of environmental influences on atopic disease, there are confounding data. Studies in Britain fail to show a relationship between response to BCG vaccination and atopy (46). Furthermore, whether the observations by Shirakawa et al. (45) result from the effect of the vaccine itself or the innate ability of that child to respond to that vaccine is open to debate (46). However, experiments in mice, which demonstrate that immunization with BCG blunts development of allergen-specific IgE and eosinophilic responses to allergen after allergen challenge, support the idea that BCG vaccine is a potent Th1 stimulus (47). Taken together, these observations support the notion that BCG stimulates increased Th1 function and is associated with decreased Th2 immune responsiveness.

It has also been argued that decreased incidence of infection and frequent use of antibiotics may also be contributing to the development of atopy. The hypothesis is that by decreasing exposure to Th1 stimuli (either by active infection or by alteration of bacterial colonization, which may stimulate Th1 immune responses), the Th2 immune responsiveness expressed by the fetus has a better chance of being maintained, thus allowing for expression of the immune phenotype. The use of antibiotics in the early years of life

correlates with the subsequent development of atopy. Similarly, in societies in which antibiotic use is decreased and natural infections are more frequent, atopy occurs less frequently. However, despite these data, it seems unlikely that children at very high risk for asthma (inner-city African Americans) have increased exposure to antibiotics compared to more affluent populations at lesser risk (31,37).

In addition to antigen-specific immune responses, it has been suggested that accessory molecules expressed by bacteria (classic Th1 stimuli) may contribute to immune maturation such that Th1 responses are emphasized (31,48). Lipopolysaccharide (LPS) is a molecule expressed on all gram-negative bacteria that interacts with antigen-presenting cells and other immune effector cells via the CD14 receptor. Treatment of APCs with LPS results in secretion of IL-12, which in turn blunts Th2 responses and stimulates interferon-γ secretion. It has been argued that mucosal colonization with bacteria, including LPS-bearing organisms, allows for nonspecific deviation toward the Th1 phenotype. Recently, it was found that the gene for CD14 colocalizes with genes for IL-3, IL-4, and GM-CSF on the chromosome region 5q31.1. Furthermore, a specific polymorphism has been identified (a C-to-T transition at base pair -159) in which those children homozygous for the T allele have significantly higher levels of soluble CD14 than do heterozygotes or those who are homozygous for the C allele (48). In turn, serum levels of CD14 (which could mediate LPS interaction with APCs) have a significant positive correlation with interferon-γ and a negative correlation with IL-4 (48). This observation supports the hypothesis that an imbalance between Th1 and Th2 influences the development of atopic disease.

Specific Pollutants and Their Effects on Th2 Inflammation

A number of ambient air pollutants are thought to contribute to the exacerbation of asthma. Indeed, criteria pollutants such as NO_2 and O_3 have been associated with asthma exacerbation in epidemiological studies and, in challenge and animal studies, can enhance immediate and late-phase responses to inhaled allergens in already sensitized individuals (49–51). However, with perhaps some animal data to the contrary, these pollutants do not appear to play a significant role in the actual development of the Th2 phenotype. In contrast, diesel exhaust particles (DEPs) shift the immune phenotype toward a Th2 pattern (51).

DEPs. Numerous animal (murine) and in vitro studies have demonstrated that DEPs enhance allergen-induced immune responses, including increasing IgE production and

enhancing cytokines involved in eosinophilic or allergic inflammation, especially IL-4, IL-5, and GM-CSF, as well as airway hyperresponsiveness (*51–56*). DEPs induce B-lymphocyte immunoglobulin isotype switching to IgE (*57,58*). Polyaromatic hydrocarbon residues on DEPs may be responsible for this effect on allergic inflammation (*56*).

Diaz-Sanchez et al. (*57*) used nasal challenge studies in humans and reported that challenge of volunteers (four atopic and seven nonatopic) to DEP increased nasal IgE production 4 days after DEP challenge without any effect on IgG, IgA, or IgM. They also noted shifts in the ratio of the five isoforms of IgE with the challenge. This effect was very dose specific; only a 0.3-mg dose of DEP caused this result.

Diaz-Sanchez et al. (*57*) also found that DEP challenge of the nasal mucosa causes increased cytokine production by cells recovered in lavage fluid. Subjects underwent lavage pre- and postchallenge with 0.3 mg DEP. Cells recovered in the prechallenge lavage had detectable mRNA levels for interferon-γ, IL-2, and IL-13, whereas those recovered postchallenge were associated with detectable levels of IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and interferon-γ in recovered cells. IL-4 protein was also measured in postchallenge lavage [reviewed by Peden (*50*)]. Although it is unclear which type of cells were present in lavage fluid before or after challenge, it was not thought to be due to increased lymphocyte number. When coupled with challenge with a specific allergen (ragweed), DEP yielded an enhanced ragweed-specific IgE and IgG response to ragweed allergen compared to ragweed alone. This effect included increased expression of IL-4, IL-5, IL-6, IL-10, and IL-13, decreased expression of interferon-γ and IL-2, and no effect on total IgE and IgG.

Compared to other pollutants such as ozone, DEP appears to be unique in its effect on IgE production. The *in vivo* effect of DEP on IgE isotype switch can be replicated *in vitro* with extracts from DEP containing the polyaromatic hydrocarbon (PAH) fraction from these particles, as well as the specific PAH compounds phenanthrene and 2,3,7,8-tetracholorodibenzo-*p*-dioxin (*56*). Thus, PAHs, by their action on B cells, appear to play a central role in the effect of diesel exhaust on allergic inflammation. Additionally, DEPs can also promote CD80 (an important molecule for MHC class II antigen presentation) expression in macrophages as well as in enhanced LPS-induced IL-10 responses. These effects on macrophages could alter their ability to present antigen in such a way as to promote Th2 responses to those antigens.

ETS. There is extensive literature indicating that ETS is a significant exacerbating factor for a number of respiratory tract diseases, including asthma, which has been extensively reviewed (*39–41*). Asthma-specific end points increased by ETS include the risk of hospitalization, medication use, airway hyperresponsiveness and, rarely, atopy or increased IgE (*39–41*). ETS or maternal smoking during pregnancy appears to be an especially important risk factor for the development of asthma in the first year of life. However, despite strong epidemiological evidence that ETS poses a significant risk for asthma exacerbation, candidate mechanisms of action remain undefined (*40*).

Recently, tobacco smoke extracts were shown to alter monocyte function in mice (*59*). Among those functions suppressed are those that are commonly mediated by interferon-γ, including phagocytosis of opsonized antigens, MHC class II molecule expression, oxidative burst, and NO synthesis. Activities not suppressed, including TNF-α production, are not induced by interferon-γ. Similarly, PAHs, the same species of molecules that likely mediate the Th2-promoting actions of DEPs, are also found in ETS.

Endotoxin. An inability to respond to LPS (mediated by CD14 receptor polymorphisms) may enhance initial expression of a Th2-immune phenotype (*47*). However, in persons already sensitized, it seems likely that LPS augments the expression of Th2 inflammation. There is evidence that levels of LPS in house dust are more predictive of asthma severity in mite-sensitive asthmatics than mite allergen levels in the same samples (*60*). Furthermore, asthmatics have increased nonspecific airway responsiveness after exposure to LPS [reviewed by Peden (*50*)]. Atopic subjects yield eosinophilic responses to LPS and LPS pretreatment enhances response to inhaled allergens (*50,61*). Likewise, allergen challenge yields increased levels of CD14 in bronchoalveolar lavage fluid and enhances PMN and eosinophil responses to LPS in the nasal airways (*50,62*). However, for the most part, the ability of LPS to induce asthma and atopic responses has only been observed in subjects already found to have atopy.

Ozone and other criteria pollutants. As with LPS, ozone clearly has adverse effects on persons already diagnosed as atopic or asthmatic (*48–51*). This pollutant is linked to increased medication use, increased hospitalization, and increased emergency room visits. Likewise, in both animal and human models, this gas can enhance both immediate and late-phase inflammation associated with allergen exposure and can directly induce eosinophil responses in atopic subjects. However, there is no clear indication that this pollutant, SO₂, or NO₂ play a role in asthma

pathogenesis or the induction of atopy. There are several complete reviews outlining the effects of ozone and other criteria air pollutants in asthma and atopy (*48–51*).

Summary and Gaps in Knowledge

The development of atopy is a complex immune process. I have outlined some of the processes involved in immune responsiveness, including antigen presentation, action of either CD8⁺ or CD4⁺ lymphocytes, and expression of a Th1 or Th2 phenotype for CD4⁺-mediated responses. I also reviewed a number of defects that exist in the immune system that serve as experiments of nature, which demonstrates the multitude of potential targets for environmental modification of immune responsiveness.

These findings suggest that fetal immune responsiveness mediated by CD4⁺ lymphocytes is skewed toward Th2 expression. In turn, subsequent development of atopic disease during postnatal life may actually be persistence of the fetal atopic state. It has been additionally suggested that the relative failure of Th1 influences to deviate the immune response away from a Th2 expression is important in the development of clinically significant atopy. Some of this may be due to certain genetic influences that support Th2 responses (genes supporting the production of IL-4) as well as genetic predisposition against full development of Th1 responses (i.e., genes which might blunt production of interferon-γ). However flawed this hypothesis may prove to be, it does provide a construct on which we might begin to examine the role of environmental influences on the expression of atopic disease in postnatal life.

Superimposed on any genetic predisposition that may exist is the role of environmental influences that might induce either Th1 responses (certain immunizations such as BCG, bacterial infections and stimuli, and colonization with bacteria) or Th2 responses (early exposure to allergens, especially food allergens and indoor aeroallergens). Other influences may be important as well, including toxicological stimuli that may shift the immune response toward a Th2 response (such as PAH moities from diesel exhaust and tobacco smoke).

Another important question is if the timing of exposure to potential environmental stresses is important in subsequent postnatal expression of an atopic/asthmatic phenotype (Table 2). Other than carrying genes that predispose an individual to developing atopic responses, preconceptional exposure of parents to environmental factors is unlikely to be an important window of exposure for the development of atopy in subsequent offspring.

Table 2. Probable importance of timing of exposure to environmental agents in development of postnatal asthma or atopy.

Exposure period	Maternal exposure	Paternal exposure	Environmental exposure of child
Preconception	Very little to none	Very little to none	Not applicable
Prenatal	Moderate	Very little to none	Not applicable
First 2 years of life	Small	Very little	Very important
After 2 years of age	Small	Very little	Moderately important

With regard to prenatal exposures, it seems reasonable to focus on maternal exposures. This emphasis is justified by the observations that maternal smoking is more likely to be associated with asthma and the fact that there is at least some potential for transplacental influences from the mother to her fetus. However, there is little support that the severity of allergic disease in the mother influences expression of atopy in her offspring. It is reasonable to hypothesize that transplacental transfer of Th2 cytokines, transplacental exposure to allergens, or fetal exposure to toxic immunomodulators (such as PAHs) could influence the development of atopy. Thus, the effect of environmental influences during pregnancy are likely important.

However, it is likely that an even more important window of exposure for the impact of environmental agents is during the first year of life. This could include allergen exposure (food and airborne), natural or vaccine stimulation of Th1 responses, and the potential for agents such as PAH to modify Th1 or Th2 responses during this period. Several studies indicate that the risk of developing atopic disease and maintaining it throughout life is greatest if it occurs during the first year of life.

Many atopic infants lose a significant degree of atopic expression from 2 to 5 years of age and it is a well-recognized phenomenon that children may grow out of asthma by puberty. It is interesting that one of the hallmark features of Th1 immune maturation is an ability to mount an adequate IgG response to polysaccharide antigens, usually occurring by 2 years of age. This suggests that avoidance of pro-Th2 environmental stressors (allergen avoidance and the avoidance of environmental adjuvants of Th2 responses such as diesel exhaust and ETS) during the toddler years is important. Also, it suggests that potentially inappropriate removal of Th1 stimuli (such as gut flora), perhaps by the inappropriate use of antibiotics, might also promote persistence of atopy.

Although unique and very strong environmental exposures might induce allergy during adulthood, this is relatively rare. The best examples of such adult-onset allergies are occupational diseases, some of which are "typical" Th2-based IgE responses to naturally presented antigens, whereas others are unique sensitizing agents that cause immunologically

unique disorders. Taken together, it seems that the exposures to environmental stressors during the perinatal, neonatal, and toddler years are of the most importance. Likewise, maternal rather than paternal influences during perinatal life could be significant. Environmental stresses that support Th2 inflammation are obviously important. However, the impact of stresses that decrease Th1 responses (including the production of interferon-γ and IL-12) cannot be ignored.

Finally, better understanding of the molecular basis for the development of atopy, including production of relevant cytokines and their receptors, antigen presentation, T-cell influence on B-cell function and direct alteration of B-cell function, are essential to appropriately identify immune processes that may be targets for the action of known and as yet unappreciated environmental agents which influence the development of atopy and asthma in susceptible people.

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