11 Asthma, Infection, and Environment

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Contents

Key Points

- The immune response of neonates has a natural bias towards a T helper cell type 2 (Th2) cytokine profile.
- Th2 cytokines facilitate development of allergic sensitization.
- Exposure to infectious agents during early childhood is thought to modulate development of allergic sensitization and asthma.
- Respiratory syncytial virus, influenza virus, rhinovirus, and parainfluenza viruses have been associated with wheezing during early childhood and have been implicated in induction of asthma.
- Respiratory syncytial virus has been shown to induce an IgE response, particularly in atopic children.
- Infection with non-viral agents such as *Mycoplasma pneumoniae* and *Chylamydia pneumoniae* is associated with asthma in adults.

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- Results from studies with a murine model show that exposure to environmental tobacco smoke facilitates allergic sensitization to inhaled allergens through production of T helper cell type 2 cytokines.
- Results from studies with a murine model show that exposure to ozone facilitates allergic sensitization to inhaled allergen.
- Studies in humans and mice show that diesel exhaust particles increase local IgE production in response to allergen.
- Ozone and environmental tobacco smoke exposure stimulate airway hyperresponsiveness in a guinea pig model.

Introduction

The incidence of asthma has been increasing during the past 20 yr, especially in industrialized nations. Epidemiological evidence has linked exposure to both indoor and outdoor air pollution with development and exacerbation of allergic asthma. Moreover, the link between infection with certain respiratory viruses, early in life and development of chronic allergic asthma, in adulthood, is well documented. Also, during the past 20 yr, studies using animal models have demonstrated that environmental tobacco smoke, ozone, and diesel exhaust particulates can increase allergic sensitization to inhaled allergens. Other animal model studies have demonstrated a link between infection with respiratory syncytial virus (RSV) and allergic sensitization. It is apparent from both epidemiological and laboratory studies that environmental conditions contribute to the increased incidence of asthma in the population.

Complex genetic factors that govern both immunoglobulin E (IgE) responsiveness and the development of airway hyperreactivity have been recognized as important to the etiology of asthma. "Atopy" is a term used to describe an inherited tendency to develop allergy. It is widely known than individuals with one or more asthmatic parent are more likely to develop asthma than are those individuals with a nonasthmatic family history. Allergy and asthma are closely related diseases that are brought about by a complex interaction of genetics and environment. Recent studies have begun to unravel the genetics of asthma. Using both candidate gene studies and genome-wide screens, several potential genetic factors for susceptibility to asthma have been identified *(1)*. Among the genes that are thought to be involved are the cytokine gene cluster on chromosome 5 (coding for interleukin-3 [IL-3], -4, -5, -9, and -13), the gene coding for the β-chain of the IgE high-affinity receptor on chromosome 11, the gene coding for the IL-4 receptor on chromosome 16, and Stat 6 on chromosome 12 *(2)*. Other genes, such as major histocompatibility complex class II genes, T-cell receptor genes, and genes coding for enzymes involved in mediator leukotriene synthesis, may also be involved in the development of allergic asthma. In individuals with the appropriate genotype, the development of asthma is influenced by contact during infancy or early childhood with appropriate pathogens and/or indoor and outdoor sources of air pollution. It is highly likely that the increase in exposure to these adverse external conditions is

an important factor influencing the increase in the incidence of asthma in the human population.

Modulation of Allergic Response by Infectious Diseases

A basic feature of the immune response is the cooperation that occurs between T- and B-lymphocytes after exposure to antigen. In 1989, Mossman and Coffman *(3)* described different patterns of cytokine secretion that lead to different patterns of immune responsiveness. It is now well recognized that $CD4^+$ T-cells have two different profiles of cytokine production: T helper 1 (Th1) and T helper 2 (Th2). Cell-mediated immunity is stimulated by the Th1 cytokines; humoral immunity is stimulated by Th2 cytokines *(4)*. Th1 and Th2 responses are balanced in a normal immune system. Th1 cytokines (γ-interferon [IFN-γ], IL-2, IL-12) can downregulate Th2 cytokine (IL-4, IL-5, IL-10, IL-13) production. A very strong Th2 response facilitates IgE production and consequent development of allergy. as shown in Fig. 1. Factors that are important in determining the balance of Th1 and Th2 cytokines include antigen dose, antigen presenting cells, genetic background, and local tissue environment (including co-stimulatory factors).

It has been speculated that a decrease in certain infectious diseases, and resultant changes in vaccination practices, have modulated the immune response of children toward the allergic phenotype. In a normal pregnancy there is a bias toward a Th2 immunity, and consequently the neonate is born with the same Th2 bias *(5)*. Thus, early influences on the cytokine milieu could play a significant role in immune regulation. More specifically, the idea that environmental pollutants or viral infection might influence allergic sensitization of the neonate to inhaled allergen is based on these recent findings *(6)*, which suggest there is a natural bias of the neonatal immune system toward a Th 2 cytokine profile.

The difference between the incidence of atopic disease in children living in industrialized countries, compared with that in areas of Europe that have less industrialized lifestyles, is cited as evidence for the theory that environmental factors have influenced the development of allergy in children *(7)*. According to this theory, exposure to inhaled allergen during the first year of a child's life will cause a permanent predominance of Th2 cytokines during future subsequent exposures to the allergens that were encountered during early life. Recent data has shown that some fetuses can be sensitized to antigen while still *in utero (8)*, as demonstrated by in vitro experiments using cord blood cells. The ability of T-cells to secrete IFN-γ is diminished in all neonates. However, in neonates from atopic families, the reduction is even greater. Borres et al. *(9)* found that, although levels of IL-4 were not detectable in stimulated cord blood cell culture, IL-4 levels in serum of children less than 18 mo of age, who later became atopic, were notably greater than nonatopic age-matched controls. Children raised in rural farming communities have lower incidence of asthma, presumably because there is a preferential stimulation of the Th1 cytokine response by exposure to bacteria and their products, such as endotoxin *(10,11)*. This idea has been termed the "hygiene hypothesis."

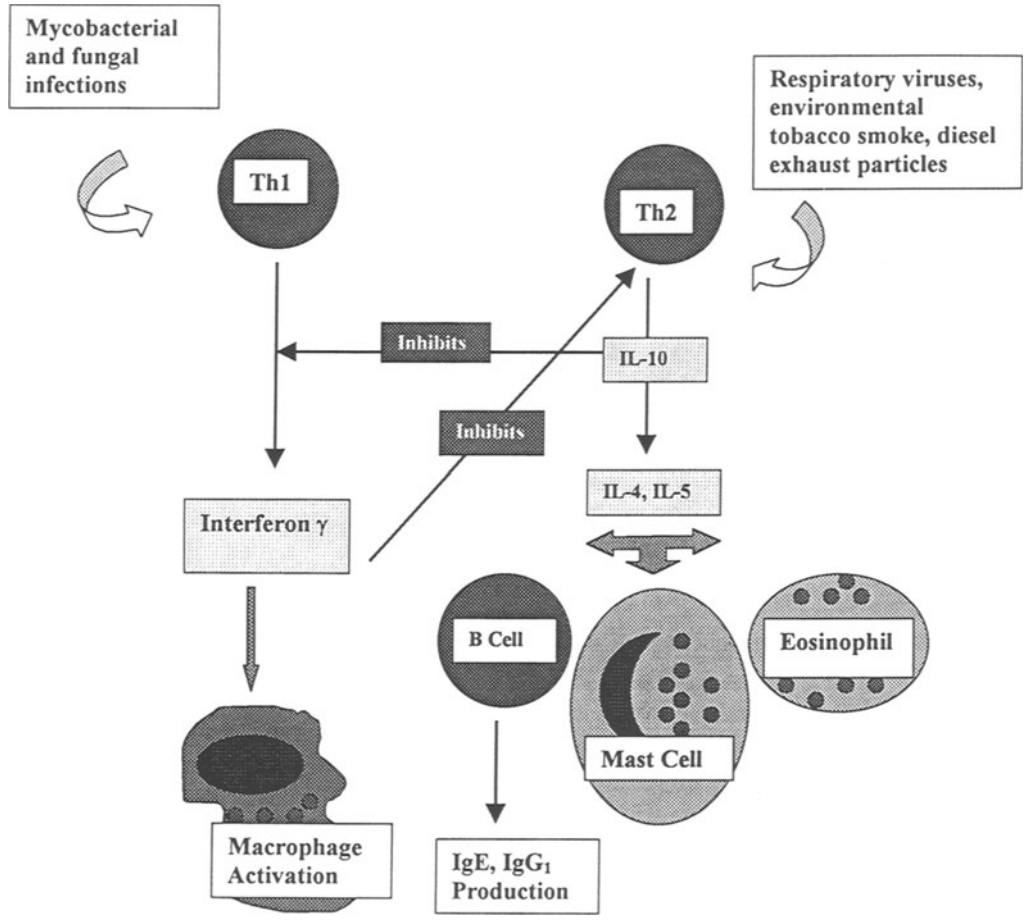

Fig. 1. A proposed mechanism for immune regulation by viruses and pollutants involves selective stimulation of T helper type 2 lymphocytes with subsequent IgE production. The opposite response is evolved by certain microbial antigens.

Further evidence for the importance of microbial exposure to development of a balanced immune response is provided by the work of Lewis and Britton *(12)*, who addressed the possibility that the decrease in natural measles resulting from use of vaccination, has removed a natural Th1 stimulus and helped to facilitate the Th2 skewed response necessary for development of allergy. In contrast, the IgE-promoting effects of *Bordetella pertussis* are well known, and it has been suggested that vaccination for whooping cough may stimulate development of allergy. However, Bjorksten et al. *(13)* have performed a large prospective study on the subject, and have essentially concluded that *B* pertussis vaccination is not a likely cause of increases in the incidence of allergy in the human population.

A recent hypothesis suggests that the allergic phenotype may be prevented by infection with an organism capable of inducing an opposing type of immune response. Shirakawa *(14)* addressed this hypothesis in a study of Japanese school children who had received immunization against tuberculosis with the Bacille-

Calmette-Guérin (BCG) vaccine. Results indicated that there was a strong inverse association between delayed-type hypersensitivity to *Mycobacterium tuberculosis* and the presence of type I immediate hypersensitivity. Thus, students who had positive TB skin tests had lower serum IgE and a lower incidence of allergy. Moreover, the cytokine profile of these children showed a bias toward Th1 cytokines. It is well recognized that mycobacterial antigens stimulate a strong Th1 response, causing production of cytokines such as IFN-γ. These Th1 cytokines downregulate the Th2 response, and thus diminish the allergic response. The recognition that the BCG vaccinated children had significantly less allergy than non-BCG vaccinated children demonstrated that immune regulation during early life is very likely an important factor influencing development of allergic respiratory disease in future years.

A subsequent study of the effect of BCG vaccination on the development of allergy was performed in Sweden *(15)*. In this study, 216 children with an atopic family history were vaccinated with BCG when they were less than 6 mo of age. Another 358 age-matched children were not vaccinated with BCG. The family risk factors for developing allergy were similar in both groups. Results of the study showed that 36% of the BCG group and 41% of the control group developed clinical signs of atopy. Neither results of serum levels of allergen-specific IgE nor skin-prick tests were significantly different. The conclusion reached in that study was that, in children with a family history of atopy, early vaccination with BCG did not seem to affect the development of atopic disease in early childhood.

Animal models have recently been used to determine the validity of these observations on effects of BCG vaccination on development of allergic asthma. In one study by Herz et al. *(16)*, Balb/c mice were sensitized with BCG, ovalbumin (OA), or both. Mice that were sensitized with OA developed the expected Th2 cytokine response, IgE production, airway hyperresponsiveness, and eosinophilia. Those mice that received BCG prior to OA immunization showed decreased IgE production, normalized cytokine production, and lacked airway hyperresponsiveness and eosinophilia. In a study by Tukenmez et al. *(17)*, newborn mice were immunized with either *Mycobacterium bovis* or *Mycobacterium vaccae*, phosphate-buffered saline solution (PBSS), or were not injected. At adulthood, mice were immunized with a series of intraperitoneal injections of OA, followed by an aerosol challenge. Mice immunized with *M bovis* and *M vaccae* had significantly lower IgE levels than those mice in the PBSS groups. The effect of a potent Th1 stimulus (*M vaccae*) on an already primed animal was examined in another study, which used mice previously sensitized to OA. Want et al. *(18)* found that a single injection of *M vaccae* was able to cause a decrease in serum IgE, and two injections inhibited IL-5 production, as well. Based on all of these findings, the potential for using a Th1 modulator, such as *M vaccae*, for treatment of atopy has been suggested.

The importance of microflora in the intestinal tract for modulating development of the immune response is reviewed by Bjorksten *(19)*. The immune responses to microbes and food proteins presented by the oral route differ, so that tolerance is the expected response to foods, but active antibody (Ab) production accompanies immune stimulation with microbial pathogens. Studies on infants in Estonia and in Sweden have demonstrated differences in intestinal microflora of atopic and nonatopic children.

Postnatal colonization with lactobacilli was greater in the Estonian infants *(20)*. Shida et al. *(21)* demonstrated that *Lactobacillus casei* inhibits Ag-induced IgE secretion through cytokine release, probably IL-12. Thus, lack of the appropriate lactobacilli may facilitate a further Th2 cell response in the infant.

Viral Infections and Asthma

Recurrent wheezing in infants and young children has recently been reviewed by Hopp *(22)*. This review summarizes the various terms used to describe recurrent wheezing: "reactive airway disease," "recurrent bronchiolitis," "chronic bronchiolitis," "wheezy bronchitis," "chronic bronchitis," and "asthmatic bronchitis." Some of the children in all of these categories are truly asthmatic. An infection with RSV that causes bronchiolitis is often followed by episodes of recurrent wheezing. Hopp describes three types of episodic wheezing in young children: transient early wheezers, late wheezers, and persistent wheezers *(22)*. The determination that episodes of wheezing, with subsequent viral infection, are likely to evolve into clinical asthma is affected by factors such as a family history of atopy, the presence of eosinophilia and high IgE, and exposure to tobacco smoke. Generally, wheezing after the age of 6 yr is caused by asthma. Prior to the age of 6 yr, frequent viral infections with agents such as influenza virus, RSV, and rhinovirus (RV), are important causes of wheezing episodes.

An association between production of the pleiotropic cytokine, IL-11, in the lung and induction of airway hyperresponsiveness has been shown by Einarsson et al. *(23)*. After infection with RSV, RV, and parainfluenza virus, production of IL-11 by stromal cells was increased in the lung. In this same study, IL-11 production was also documented in nasal aspirates of virus-infected children with wheezing. This same group has found that viruses implicated as inducers of wheezing (RSV, RV, parainfluenza virus) are all inducers of IL-11, but that those pathogens that fail to induce wheezing (cytomegalovirus, herpes simplex virus, pyogneic bacteria) do not induce IL-11 production.

Respiratory Syncytial Virus

Often, the initial recognition that a child will become an asthmatic is the advent of wheezing with respiratory infections. One virus in particular, RSV is most frequently associated with development of lower respiratory tract infection characterized by wheezing (Fig. 2). RSV has been recognized as an important cause of bronchiolitis in infants and young children since 1957 *(24)*. The majority of all children are infected by 2 yr of age. Subsequent reinfection occurs during childhood, but re-infection often consists of simply upper respiratory symptoms described as a "cold," except in some children who continue to wheeze.

Welliver et al. *(25)* studied infants and children infected with RSV, and grouped them into those with only upper respiratory tract disease, those with lower respiratory tract disease, and those with lower respiratory tract disease who showed

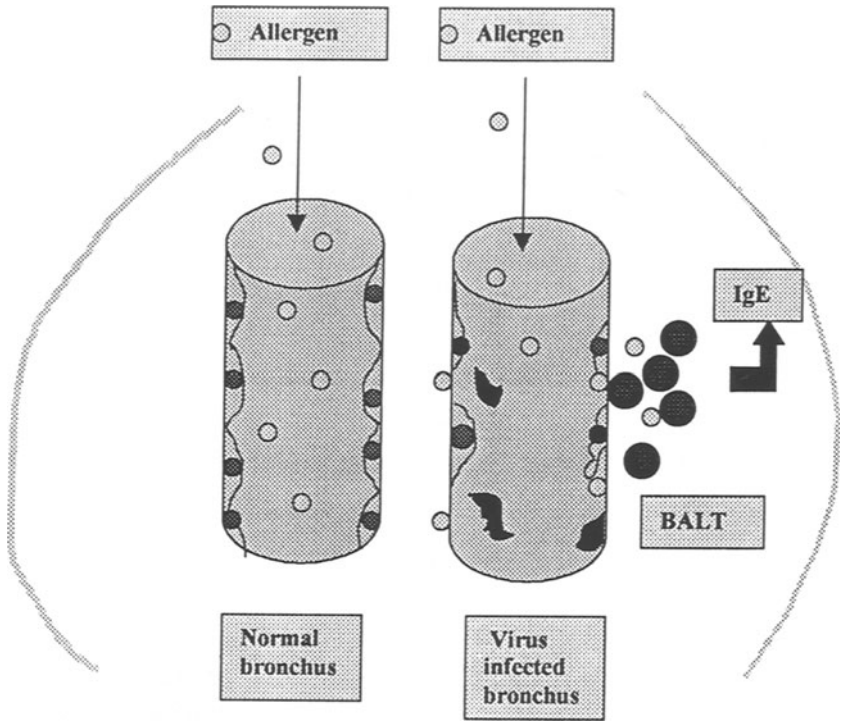

Fig. 2. A proposed mechanism for virus-enhanced allgergic sensitization would involve inceased access of allergen to bronchus-associated lymphoid tissue and antigen-presenting dendritic cells.

evidence of wheezing. They reportedly found that RSV-specific IgE and nasal levels of histamine were increased in the group that wheezed. In a prospective Brazilian study on children from birth to age 13 yr *(26)*, virus isolation, pulmonary function, frequency of wheeze, IgE concentrations, and skin test reactions were evaluated. A strong association between RSV infection and increased risk of wheezing was found in children up to the age of 6 yr. There was no such association by age 13 yr. The study failed to demonstrate an association between RSV infection and increased risk of allergic sensitization.

The limitations of epidemiological data have led to performance of in vitro assays, using specimens from human patients, as well as the establishment of several animal models for examination of the potential augmentation of sensitization to inhaled allergens that appears to occur during or as a sequel to RSV infection. In one study by Noma et al. *(27)*, the relationship between the onset of recurrent wheezing and antigen-specific IL-2 responsiveness was analyzed. IL-2 is the cytokine responsible for proliferation of antigen-specific T-lymphocytes. The data indicated that RSV infection of infants induced responsiveness to the respiratory allergen, *Dermatophygoides farinae* (housedust mite) and also to ovalbumin, a food antigen. A RSV-infected OA-sensitized mouse model has been used to study the response to inhaled ovalbumin during infection. In one such study *(28)*, RSV was found to enhance allergic airway sensitization, with resultant eosinophilia and airway

hyperresponsiveness. In that study, the role of the T-lymphocyte was examined using adoptive transfer of lymphocytes from peribronchial lymph nodes. Transfer of T-cells from RSV-infected mice was shown to result in development of airway hyperresponsiveness, eosinophilic pulmonary infiltrates, and increased IL-5 production. When experiments were performed in which either CD4 or CD8 T-cells were depleted before transfer, the most important cell in mediating these responses was the CD8 T-cell, although the CD4 T-cell was also important. Infection with RSV is usually an acute event that may have sequelae, but the initial virus infection has not generally been considered a persistent infection. In contrast to these beliefs, experiments with guinea pigs *(29)* have shown that viral protein and genome can be detected for at least 125 d after RSV infection of juvenile animals. Riedel el al. *(30)* found that RSV protein was still present in guinea pig lung after 60 d, and that airway hyperresponsiveness was still present 6 wk after RSV inoculation.

Other Respiratory Viruses Affecting Asthma

Viruses, such as influenza, RV, and parainfluenza, have also been studied for their asthma-enhancing effects. The incidence of respiratory tract infection in adults requiring hospitalization for asthma was examined in a study involving 79 patients and 54 controls over a period of 12 mo *(31)*. The most common viral agents in these adult patients were influenza A and rhinovirus. In another study *(32)*, of 169 children, a total of 256 attacks of acute asthma were followed during a 2-yr period. Of these 256 attacks, 29% were associated with a diagnosis of viral respiratory infection. Results indicated that RV caused 45% of the virus infections and RSV caused 19%. In a recent study using polymerase chain reaction (PCR) to detect seven common respiratory viruses *(33)*, the prevalence of viruses was determined using nasal swabs obtained from 21 clinically stable asthmatic children, 16 children not diagnosed by a physician as asthmatic, but having symptoms of asthma during exercise (exercise-induced asthma), and 33 nonasthmatic controls. The multivirus PCR panel detected adenovirus, coronavirus 229E, coronavirus OC43, influenza A, parainfluenza virus, RV, and RSV. The mean age of the children in the three groups varied from 11.4 to 11.6 yr. There was no significant difference in the numbers of viruses detected in the three groups. More than two viruses were detected in 30% of controls and 43% of asthmatics. No virus was detected in another 39% of controls and 38% of asthmatics. That study supports the hypothesis that wheezing with viral infections is of greatest significance to the under 6-yr-old population.

A variety of animal models has been developed to study the complex interaction of respiratory tract infections with viruses and the development of allergic sensitization and asthma. Several of these models have been reviewed by Hegele *(34)*. Using a rat model infected with the parainfluenza type 1 (Sendi) virus, Castleman *(34)* demonstrated that numbers of mast cells in the bronchiole walls were increased in virus-infected rats 4–10× that in control rats. In addition, airway hyperresponsiveness was present in these rats for 3 mo after infection *(35)*.

Additional studies with animal models of asthma have been performed using influenza virus. In one study using the OA mouse model *(36)*, infection with influenza A virus increased IgE production and airway responsiveness. Mice were infected with virus by the intranasal route, and received OA aerosol on d 3 of infection. After 2 wk, the mice received OA aerosol challenge, and were tested for airway hyperresponsiveness with methacholine inhalation. Levels of OA-specific IgE were found to be increased in mice infected with virus and sensitized to OA; those sensitized with OA, and not infected with influenza virus, did not show increases in IgE. Moreover, airway responsiveness was also increased in the virus-infected group, but not in the uninfected group. The bronchoalveolar lavage fluid (BALF) of these mice showed increases in CD8 T-lymphocytes in the infected-sensitized mice. Eosinophilia was not observed. Thus, like RSV, influenza virus infection enhances allergic sensitization and airway hyperresponsiveness in a mouse model of asthma.

The important role of the eosinophil in the pathogenesis of asthma has recently been deduced. In one study using a guinea pig model sensitized with OA and infected with parainfluenza virus *(37)*, depletion of eosinophils with antibody to IL-5 blocked viral-induced airway hyperresponsiveness. The eosinophil is also important in the rhinoviral-induced exacerbation of asthma. In virus-induced exacerbation of asthma increased numbers of eosinophils are often found in respiratory secretions. RVs use intracellular-adhesion molecule-1 (ICAM-1), an adhesion molecule on bronchial epithelial cells, as a receptor *(38)*. In one study using chimpanzees, Huguenel et al. *(39)* found that soluble ICAM-1 was effective in inhibiting RV infection. Exposure to inhaled allergen has been shown to induce ICAM-1 expression on epithelial cells on conjunctiva of allergic patients *(40)*. In vitro experiments using RV serotype 16, and eosinophils pretreated with granulocyte-macrophage colony stimulating factor (GM-CSF); there was significant binding of the RV to eosinophils *(38)*, which was inhibited by monoclonal antibodies against ICAM-1. RV was shown to activate peripheral blood mononuclear cells through an ICAM-1-mediated mechanism. Furthermore, supernatant from $CD3^{+}CD69^{+}$ cells, which had been activated by RV, showed activity in vitro that promoted eosinophil survival *(41)*. Other in vitro studies performed by Schroth et al. *(42)* showed that RV (serotypes 16 and 49) induced secretion of RANTES, IL-8, and GM-CSF from human bronchial epithelial cells. Stimulation of eosinophil accumulation in airway interstitium is an anticipated result of secretion of these cytokines/chemokines. In a recent study performed by inoculation of 11 atopic asthmatics and 10 nonatopic controls with RV serotype 16 symptoms *(43)*, lung function, nasal lavage, and sputum were evaluated. The experimental infection was not sufficiently severe to cause exacerbation of asthma in the asthmatics. Although there were increases in levels of proinflammatory cytokines IL-6, IL-8, and neutrophils in nasal lavage, there were no differences detected between the asthmatics and the nonasthmatics. Factors to be considered when comparing these results with some of the other studies include the roles of viral load and the difference in virulence between lab-propagated virus used in experimental infection and naturally acquired field virus.

Multiple mechanisms have been suggested *(44)* for enhancement of asthma by respiratory viruses. Effects of virus infection on the atopic infant or child may be primarily to promote sensitization to allergen and development of the allergic phenotype. Allergic sensitization and elicitation of asthmatic reactions may be facilitated by increased permeability of the respiratory epithelium to protein antigens. It has been well demonstrated that respiratory viruses (such as RSV) infect and kill bronchial epithelial cells. Increased access of inhaled allergen to bronchus-associated lymphoid tissue could increase opportunities for antigen presentation and subsequent stimulation of T- and B-lymphocytes. Recruitment of dendritic cells, important for antigen presentation, into the airway epithelium, during the inflammatory response to virus and bacterial pathogens, has been implicated, by McWilliams et al. *(45)* as a factor favoring allergic sensitization. In their studies, rats were challenged with live virus and/or bacterial pathogens, and their lungs were analyzed for cell recruitment. A variety of C-C chemokines were implicated in recruitment of the dendritic cells to the site of the inflammation. Increased access to mast cells in the pulmonary interstitium may also facilitate degranulation, leading to release and/or synthesis of bronchoconstrictors and chemokines. Direct effects of virus on developing airways, including innervation, may also be involved in the development of airway hyperresponsiveness during and after a viral infection. In contrast, the major effects of RV may be brought about by eliciting the asthmatic symptoms in the older asthmatic by enhancing eosinophil infiltration, production of inflammatory mediators, and the airway hyperresponsiveness that generally accompanies eosinophilia.

Nonviral Infectious Agents and Asthma

Infection with *M pneumoniae* is associated with exacerbation of asthma in adults. Recent studies have implicated *M pneumoniae* in the pathogenesis of chronic asthma. Kraft *(46)* attempted to substantiate these observations by following 18 stable chronic asthmatics and 11 nonasthmatic controls. Multiple infectious agents were tested for, and, in 10/18 (compared with 1/11 controls), *M pneumoniae* was detected by PCR. No significant differences were found for any of the other pathogens. However, neither enzyme immunoassay, culture, nor serology indicated *M pneumoniae* infection. The more sensitive technique of PCR was not performed for viral detection. The increased presence of *M pneumoniae* in the airways of these chronic asthmatics lends support to the hypothesis that the infection may have a role in chronic asthma.

Kifuji et al. *(47)* found that the release of histamine from human peripheral blood leukocytes, after challenge with *M pneumoniae* was found to be similar between subjects with positive and negative *M pneumoniae* antibody titers. Even though histamine release occurred as a result of the challenge, it was unrelated to the presence of IgE or other antibody isotypes.

Another nonviral agent that has recently been associated with asthma is *Chlamydia pneumoniae*. In a study by Allegra et al. *(48)*, to examine the potential role of

this organism in asthma, serum from 74 adult asthmatics was tested for antibody titers to several respiratory viruses, *M pneumoniae*, and *C pneumoniae*. Isolation of *C pneumoniae* from pharyngeal swabs was also attempted. Twenty percent of the patients showed seroconversion to at least one of the pathogens. Of the 15 patients who seroconverted, six were infected with *C pneumoniae* as the single agent.

Hahn *(49)* examined the association between acute *C. pneumoniae* infection and asthmatic bronchitis in adults. In that prospective study, serology, bacteriology, and clinical assessment was evaluated in 365 patients with signs of respiratory disease, with and without serologic titers positive for *C pneumoniae*. Forty seven percent of patients who had acute *C pneumoniae* infection developed bronchospasm Of these patients 96% failed to show evidence of co-infection with any other respiratory pathogens. There was a significant association between antibody titer (after, but not before, infection) to *C pneumoniae* and development of asthmatic bronchitis. The study concluded that repeated or prolonged exposure to *C pneumoniae* may be a cause of wheezing, asthmatic bronchitis, and adult-onset asthma.

A subsequent study by the same group *(50)* attempted to further investigate the association of *C pneumoniae* infection with adult reactive airway disease. In that study, not only serology and pharyngeal cultures were used to determine infection status, but lung function was also monitored by peak flow measurements in patients with wheezing and dyspnea. The conclusion was that seroreactivity to *C pneumoniae* was indeed associated with both chronic asthma and acute asthmatic bronchitis. Recently, evidence for implication of *C pneumoniae* in asthma, as well as in chronic obstructive pulmonary disease, has been reviewed *(51)*. It is notable that 15/18 controlled epidemiologic studies reviewed showed a significant association between *C pneumoniae* infection and asthma The disappearance of asthma symptoms, after long-term antibiotic therapy, was noted in several studies.

Childhood asthma has also been associated with *C pneumoniae* infection. In a study on 32 infants and 43 children hospitalized for severe asthma, multiple conventional and molecular techniques were used to detect pathogens in nasal aspirates *(52)*. Both *C pneumoniae* and *M pneumoniae* were detected in asthmatic children, although RV and RSV were the most frequently detected pathogens.

Recent advances in understanding of the pathogenesis of asthma, increased epidemiological data providing evidence for the involvement of infectious agents in asthma, and focused animal model experimentation have proven that infection and asthma are closely linked in both childhood and adult-onset asthma. The relative roles of the nonviral agents (mycoplasma and chlamydia) and viruses remain to be fully elucidated. Finally, the ability of certain infections to induce Th1- or Th2-type immune predisposition is an important observation that may provide the potential for development of new preventive or therapeutic intervention strategies.

Environmental Tobacco Smoke and Asthma

The adverse effects of smoking have been well elucidated in recent years. Cancer and emphysema are common sequelae of a lifetime of smoking. The effect of

secondhand or environmental tobacco smoke (ETS) which contaminates the environment of the smoker, and is inhaled by the nonsmoker inhabiting the same space, is less well recognized. Asthmatics have known, for more years that any documentation in the literature suggests, that their symptoms of asthma are readily initiated by inhabitation of smoky environments. Yet it has only recently been realized that, aside from the irritant effect ETS has on hyperirritable airways, there is a subtler role for ETS in initiation of allergic lung sensitization, particularly in children. Data compiled from epidemiological studies performed in several countries provides evidence that supports a link between early exposure to ETS and the development of allergic asthma. For example, a study performed on 11,534 children from 24 communities, between 1988 and 1990 *(53)*, showed that children exposed in the home to ETS had a relative odds for wheezing of 1.42, compared to 1.0 for children never exposed to ETS ($p < 0.01$). The relative odds increased to 1.70 ($p < 0.01$) when there were three smokers in the home, compared to 0. The compounding effect of ETS and respiratory infection was demonstrated by the relative odds of wheezing with colds of 1.65 for children currently exposed to smoke, compared to 1.0 for those never exposed to smoke ($p < 0.001$).

Effects of maternal smoking during pregnancy often compound the effect of ETS inhalation on development of asthma in children. In one study, performed by Ehrlich et al. *(54)*, maternal smoking and current exposure to secondhand smoke were found to be independent contributing factors to asthma and wheezing in young children. Survey questionnaires, urinary cotinine levels, and parental interviews were used to determine the relative influence of household smoke on asthma/wheeze in schoolchildren ages 7–9 yr. Household smoking was found to be an important risk factor. In another study *(55*, maternal smoking during pregnancy was found to be a significant risk factor for asthma in inner-city children. Data from the 1981 National Health Interview Survey was analyzed by Weitzman et al. *(56)* to generate data on 4331 children between the ages of 0 and 5 yr old. The focus of that study was to determine the association between maternal smoking and a variety of factors relating to asthma, including prevalence and age of onset. The odds ratio for development of asthma in children of mothers who smoked 0.5 packs/d was 2.1, compared with 1.0 for children of nonsmokers. The odds ratio for development of asthma during the first year of life of these children was 2.6. These data further support the hypothesis that exposure to ETS enhances development of asthma in the infant and young child.

In an effort to demonstrate an irrefutable link between enhancement of allergic sensitization and inhalation of ETS, and to determine the mechanism by which this may occur, animal models have again been employed. Using a mouse model system and an ETS-generation and exposure system, the author's group has shown that, not only does ETS exposure enhance IgE production, but it also increases IL-4 production by pulmonary T cells, thereby proving that ETS enhances a Th2-type response *(57)*. In one experiment to understand how inhalation of ETS effects the response of previously sensitized mice to an inhaled allergen, Balb/c mice were sensitized by the intraperitoneal route with OA precipitated in aluminum hydroxide. For the next 17 d, mice were housed in chambers that containing ETS,

produced by a generator system, or were housed in similar chambers containing filtered ambient air (controls). On d 17 after the priming OA injection, mice were exposed to aerosolized OA for 60 min. Smoke or control-chamber exposures continued until d 43. T-lymphocytes from homogenized lung were stimulated in vitro, and supernatants from cultures were analyzed for cytokine content. IL-4 production was significantly greater from cells in the lung of the OA-sensitized mice exposed to ETS than in those exposed to filtered ambient air. IFN-γ production was below level of detection. Initial experiments performed with adult mice were repeated with neonatal mice, and the enhancement effect of the ETS was found to be even greater if exposure to ETS commenced during the first few days of life *(58)*. In another study, by the author's group *(59)*, both airway hyperreactivity and eosinopilia were enhanced in *Aspergillus-fumigatus*-allergen sensitized mice, compared with mice breathing ambient air. Thus, animal models support environmental evidence that inhalation of ETS increases both sensitization to allergen and elicitation of airway hyperreactivity after respiratory challenge with allergen.

Outdoor Air Pollution and Asthma

The external environment is increasingly contaminated with substances that result from industrialization. Agents such as diesel fuel exhaust particles (DEP) have recently been shown *(60)* to induce IgE responses and allergic airway hyperresponsiveness in animal models. Ozone concentrations, in areas of the country that have high ambient levels of photochemical smog, are excessive. During times of particularly high ambient air ozone concentrations, emergency rooms report that they have increased numbers of asthma patients presenting with severe attacks of dyspnea. Levels of nitrogen dioxide have also been linked to severe asthma *(62)*. Epidemiological studies support these observations.

A recent study on air pollution and asthma was based on questionnaires from 3676 children in grades 4, 7, and 10, from 12 communities in southern California *(61)*. A positive correlation between air pollution and the presence of bronchitis and phlegm was found only in the asthmatic children, compared with other children, who were either normal or had a history of wheeze without diagnosis of asthma. The risk for development of bronchitis correlated most strongly with the amount of particulate matter in the air.

Ozone and Allergic Sensitization

Twenty yr ago, several animal model studies were performed that demonstrated the enhancement effect of inhaled ozone on allergic sensitization. Exposure of mice to cycles of ozone (0.8 and 0.5 ppm) for 4 d, immediately preceding aerosolization with OA, caused increases in numbers of IgE-producing cells in lungs of duel-treated mice, compared with mice receiving only ozone or ambient air and OA *(63)*. In addition, ozone- and OA-exposed mice were more likely to die from anaphylactic shock after intravenous injection of OA than ambient air controls *(64)*.

Diesel Exhaust Particles and Respiratory Allergy

Another potential factor influencing the incidence of allergic airway disease, in recent years, is the increase in DEPs in the air of industrialized countries. Studies on the effects of DEPs on respiratory allergy in humans *(65–67* demonstrate that aerosolized DEPs increase local production of IgE in the upper respiratory tract, particularly in association with allergen. Studies using the mouse model *(68)* have also demonstrated that DEPs produce an adjuvant effect on IgE production. It seems clear that, in both species, DEPs can enhance allergic sensitization. Moreover, studies in both species *(69,70)* strongly suggest that the IgE-enhancing effects of the chemicals present in DEPs are caused by direct effects on B-lymphocytes. Diaz-Sanchez et al. *(66* examined the effect of DEPs on cytokine production by cells in the human nasal mucosa. Participants in the study were healthy nonsmoking volunteers who received intranasal saline solution (control) or DEPs. After 18 h, the levels of mRNA for cytokines in nasal lavage cells was assessed. Compared with levels of cytokines from prechallenge lavage cells, postchallenge cytokines showed enhanced levels of IFN-γ, IL-2, and IL-13 (present at low levels before challenge with DEPs), as well as IL4, IL-5, IL-6, and IL-10, which are cytokines not present in baseline samples. IL-4 protein was present in postchallenge lavage fluid as well. Levels of IgE were also elevated on d 4 after challenge. That study further emphasizes the potential for induction of a Th 2 response by DEPs, thereby facilitating allergic sensitization to inhaled allergens.

Mechanisms of Enhancement of Airway Hyperreactivity

Constriction of bronchial smooth muscle, with resultant wheezing and dyspnea, can be stimulated by more than one mechanism. The increased sensitization, with subsequent reactivity of IgE with mast cells and mediator liberation, influx of eosinophils, and synthesis of late-phase reactants, such as leukotrienes, is involved in mediation of the bronchoconstriction that occurs in allergic asthma. However, some environmental agents that cause exacerbation of asthma act via neurological pathways as well.

The autonomic nervous system regulates smooth muscle tone and secretion of mucous glands, permeability, and blood flow in the bronchial circulation *(71)*. The β-adrenergic receptors that are present on smooth muscle in bronchi are activated by catecholamines, and are responsible for smooth muscle relaxation. A decrease in this β-adrenergic response as a cause of airway hyperreactivity has been shown *(72)*. In contrast, the cholinergic nervous system controls constriction: Stimulation of parasympathetic nerves causes constriction of airway smooth muscle. The cholinergic and adrenergic nervous systems act together to regulate homeostasis in the airways *(73,74)*. A third nervous system is the nonadrenergic, noncholinergic, autonomic nervous system, which functions with neuropeptides as transmitters *(75)*. Nerve fibers containing the neuropeptide, vasoactive intestinal peptide (VIP), a substance that causes relaxation of smooth muscle, have been

found in airway smooth muscle *(76)*. However, in one study *(77)*, immunoperoxidase techniques failed to find any VIP in airways of five asthmatic patients. Since the airway epithelium is directly in contact with inhaled irritants, such as pollutants, and is the target of infection for some viruses (RSV), theories have been proposed suggesting that excitation of afferent receptors in the epithelium initiates reflexes that mediate constriction of bronchial smooth muscle. It is well established that there are intraepithelial nerves in human bronchi *(78)*. Stimulation of sensory nerves in the respiratory tract can cause neurogenic inflammation as a result of the release of neuropeptides, such as substance P *(79)*. The resultant increased capillary permeability, vasodilation, and smooth muscle contraction resembles the physiological effects of mediators, such as histamine. Activation of C-fibers, causing neurogenic inflammation, has been proposed *(80)* as one mechanism for development of airway inflammation in asthma. It is possible that environmental pollutants and viral infection may act through this pathway to augment the inflammatory response in asthma.

Guinea pigs have been used by several groups to evaluate the effect of ETS on C-fiber, neurokinin-mediated airway hyperresponsiveness. In a study designed to evaluate the effect of chronic ETS exposure on lung function of developing guinea pigs *(81)*, animals were exposed to either filtered air or ETS for 6 h/d for 5 d/wk, from the age of 8 to 43 d. In an in vitro perfused lung system, lung function, in response to increasing doses of capsaicin (a C-fiber stimulant) or substance P, was evaluated. Results indicated that ETS exposure caused increased lung compliance without changing alveolar size or deposition of elastin. In addition, activity of the C-fiber system was decreased without changing responsiveness to the neurotransmitter, substance P *(81)*.

In another similar study *(82)*, ETS exposure was found to augment substance-P-evoked lung rapidly adapting receptor activity, but not substance P-evoked increases in peak tracheal pressure or arterial blood pressure. This type of reactivity, if accentuated in asthmatic humans, would be expected to promote increased airway hyperresponsiveness and thus exacerbation of asthma.

Another group *(83)* used guinea pigs to examine the effect of neonatal and *in utero* exposure to ETS on airway hyperreactivity. Guinea pigs were exposed to room air, sham exposed, ETS *in utero* and room air neonatally, or room air *in utero* and ETS neonatally. Exposures were during d 28–55 of pregnancy and d 8 and 24 of life. In a follow up experiment similar groups were treated with capsaicin to deplete substance P. The study concluded that, in the ETS/ETS group, substance P induced a significantly larger decrease in peak maximal expiratory flow than in the other groups. This finding pointed out the important influence of *in utero* ETS exposure on airway hyperreactivity. The increase was abolished by capsaicin treatment. Chronic neonatal exposure to ETS also induced an increase in bronchial response to substance P *(83)*.

Guinea pigs have also been used to evaluate the effect of another air pollutant, ozone, on airway hyperresponsiveness. It is known that ozone induces a transient bronchoconstrictive response in humans who inhale it. Tobacco smoke was combined with ozone exposure, and lung function was monitored in guinea pigs

Table 1
Potential Mechanisms for Enhancement of Asthma by Infectious Agents and Environmental Toxicants

Mechanisms for increased sensitization	Mechanisms for increased airway hyperactivity
Increased permeability for allergen	Increased pulmonary inflammation
Increased/altered Ag presentation	Changes in neurokinin secretion
Adjuvant for Th 2 cytokines	Alterations in bronchial innervation
Dampened Th 1 cytokines	Changes in mast cell mediator production
Altered chemokine gene expression	Altered chemokines causing eosinophilia
Direct effect: B-cell differentiation to IgE	Direct stimulation of smooth muscle

exposed to either the pollutants or sham. Results showed that ozone exposure induced airway hyperresponsiveness to inhaled cigarette smoke. This effect was mediated by endogenous tachykinins *(84)*.

Summary

There seems to be little doubt that oxidant air pollutants, DEPs, ETS, and certain infections early in life can contribute toward the development of allergic asthma. Mechanisms by which this occurs are less well recognized. Although the development of the IgE response to inhaled allergen and the appropriate cytokine responses have been demonstrated in several animal models; the exact triggers for how these responses are facilitated have not been elucidated. Do these agents, by virtue of their effect on airway epithelium, enhance exposure of underlying BALF to antigen? Are antigen presenting cells in the lung upregulated by virtue of exposure to pollutants and/or infectious agents? Do pollutants, such as ETS and ozone, perpetuate the chronicity of the lesion by facilitating additional cell recruitment and chronic changes in airway interstitium? Finally, is the innervation of the small airways modified to become "hyperactive" by exposure to these external influences early in life? These are all appropriate questions that will need to be addressed by researchers in the future. Figure 1 illustrates a potential mechanism by which virus infections may increase allergic sensitization. Table 1 describes suggested mechanisms by which infection and environmental constituents may act to increase allergic asthma. Some of the experimental studies discussed in this chapter have already begun to elucidate the nature of enhancement mechanisms.

References

1. Howard TD, Wiesch DG, Koppelman GH, Postma DS, Meyers DA, et al. Genetics of allergy and bronchial hyperresonsiveness. *Clin Exp Allergy* 1999; 29: 86–89.
2. Borish L. Genetics of allergy and asthma. *Ann Allergy Asthma Immunol* 1999; 82: 413–424.

3. Mossman TR, Coffman RL. Different patterns of cytokine secretion lead to different functional properties. *Ann Rev Immunol* 1989; 11: 245–268.
4. Roitt I, Brostoff J, Male D. *Immuunology*, fifth edition. 1998. Mosby International Ltd. pp. 125–127.
5. Wegmann T, Lin H, Gulbert L, Mosmann T. Bidirectional cytokine interactions in the material-fetal relationship: is a successful pregnancy a TH2 phenomenon? *Immunol Today* 1993; 14: 353–356.
6. Bjorksten B. The environment and sensitization to allergens in early childhood. *Pediatr Allergy Immunol* 1997; 8: 32–39.
7. VonMutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, et al. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; 149: 358–364.
8. Rinas U, Horneff G, Wahn V. Interferon-gamma production by cord blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993; 4: 50–64.
9. Borres M, Einarsson R, Bjorksten B. Serum levels of interleukin-4, soluble CD23, and interferon-gamma in relation to the development of allergic disease during the first 18 months of life. *Clin Exp Allergy* 1995; 25: 543–548.
10. Braun-Fahrlander
11. Holt P, Sly P, Bjorksten B. Atopic versus infectious disease in childhood: a question of balance? *Pediatr Allergy Immunol* 1997; 8: 1–5.
12. Lewis S, Britton J. Measles infection, measles vaccination and the effect of birth order on the aetiology of hay fever. *Clin Exp Allergy* 1998; 28: 1493–1500.
13. Nilsson L, Kjellman N-I, Bjorksten B. A randomized controlled trial of the effect of pertussis vaccines on atopic disease. *Arch Pediatr Adolesc Med* 1998; 152: 734–738.
14. Shirakawa T, Enomoto T, Shin-ichiro S, Hopkin J. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; 275: 77–79.
15. Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. *Lancet* 1997; 350: 400–403.
16. Herz U, Gerhold K, Gruber C, Braun A, Wahn U, et al. BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. *J Allergy Clin Immunol* 1998; 102: 867–874.
17. Tukenmez F, Bahceciler NN, Barlan IB, Basaran MM. Effect of pre-immunization by killed *Mycobacterium bovis* and *vaccae* on immunoglobulin E response in ovalbumin-sensitized newborn mice. *Pediatr Allery Immunol* 1999; 10: 107–111.
18. Wang TN, KO YC, Chao, YY, Huang CC, Lin RS. Association between indoor and outdoor air pollution and adolescent asthma from 1995 to 1996 in Taiwan. *Environ Res* 1999; 81: 239–247.
19. Bjorksten B. The intrauterine and postnatal environments. *Curr Allergy Clin Immunol* 1999; 104: 1119–1127.
20. Bjorksten B, Naaber P, Sepp E, Mikelsnar M. The intestinal microflora in allergic Estonian and Swedish 2-year old children. *Clin Exp Allergy* 1999; 29: 342–346.
21. Shida K, Makino K, Morishita A, Takamizawa K, Hachimura S, et al. Lactobacillus casei inhibits antigen-induced IgE secretion through regulation of cytokine production in murine splenocyte cultures. *Int Arch Allergy Immunol* 1998; 115: 278–287.
22. Hopp RJ. Recurrent wheezing in infants and young children: a perspective. *J Asthma* 1999; 36: 547–553.
23. Einarsson O, Geba BP, Zhu Z, et al. Interleukin 11: Stimulation in vivo and in vitro by respiratory viruses and induction of airways hyperresponsiveness. *J Clin Invest* 1996; 97: 915–924.

24. Chanock RM, Parrott RH, Vagosko AJ, Kapikian AZ, Knight V, and Johnson KM. Respiratory Syncytial Virus. *Am J Pub Health* 1962; 252: 918–924.
25. Welliver RC, Wong DT, Sun M, Middleton E, Vaughan RS. The development of respiratory syncytial virus specific IgE and the release of histamine in nasopharyngesl secretions after infection. *N Engl J Med* 1981; 305: 841–846.
26. Stein RT, Sherill D, Morgan WJ, Holberg CJ, Halonen M, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; 354: 541–545.
27. Noma T, Mori A, Yoshizawa I. Induction of allergen-specific IL-2 responsiveness of lymphocytes after respiratory syncytial virus infection and prediction of onset of recurrent wheezing and bronchial asthma. *J Allergy Clin Immunol* 1996; 98: 1–26.
28. Schwarze J, Makela M, Cieslewiez G, Dakhama A, Lahn M, et al. Transfer of the enhancing effect of respiratory syncytial virus infection on subsequent allergic airway sensitization by T lymphocytes. *J Immunol* 1999; 163: 5729–5734.
29. Hegele RG. Role of viruses in the onset of asthma and allergy: lessons from animal models. *Clin Exper Allergy* 1999; 29: 78–81.
30. Reidel F, Oberdieck B, Streckert HJ, et al. Persistence of airway hyperresponsiveness and viral antigen following respiratory syncytial virus bronchiolitis in young guinea-pigs. *Eur Respir J* 1997; 10: 639–645.
31. Teichtahl H, Buckmaster N, Pertnikovs E. The incidence of respiratory tract infection in adults requiring hospitalization for asthma. *Chest* 1997; 112: 591–596.
32. Carlson KH, Orstavik I, Leegaard J, Hoeg H. Respiratory virus infections and aeroallergens in acute cronchial asthma. *Arch Dis Child* 1984; 59: 310–315.
33. West JA, Azzeddine D, Jkan MA, Sverre V, Hegele RG. Community study using a polymerase chain reaction panel to determine the prevalence of common respiratory viruses in asthmatic and nonasthmatic children. *J Asthma* 1999; 36: 605–612.
34. Hegele RG. Role of viruses in the onset of asthma and allergy: lessons from animal models. *Clin Exper Allergy* 1999; 29: 78–81.
35. Castleman WL, Sorkness RL, Lemanske RF, McAllister PK. Viral bronchiolitis during early life induces increased numbers of bronchiolar mast cells and airway hyperresponsiveness. *Am J Pathol* 1990; 137: 821–831.
36. Suzuki LS, Suzuki Y, Yamamoto N, Matsumoto Y, Shirai A. Influenza A virus infection increases IgE production and airway responsiveness in aerosolized antigen-exposed mice. *J Allergy Clin Immunol* 1989; 102: 732–740.
37. Adamko DJ, Yost BL, Gleich GJ, Fryer AD, Jacoby DB. Ovalbumin sensitization changes the inflammatory response to subsequent parainfluenza infection. Eosinophils mediate airway hyperresonsiveness, m(2) muscarinic receptor dysfunction, and antiviral effects. *J Exp Med* 1999; 190: 1465–1478.
38. Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, et al. Eosinophils bind rhinovirus andactivate virus-specific T cells. *J Immunol* 1997; 160: 1279–1284.
39. Huguenel ED, Cohn D, Dockum DP, Greve JM, Fournel MA, et al. Prevention of rhinovirus infection in chimpanzees by soluble intercellular adhesion molecule-1. *Am J Crit Care Med* 1998; 155: 1206–1210.
40 Canonica GW, Ciprandi G, Pesce GP, Buscaglia S, Paolieri F. ICAM-1 on epithelial cells in allergic subjects: a hallmark of allergic inflammation. *Int Arch Allergy Appl Immunol* 1999; 107: 99–102.
41. Gern JE, Vrtis R, Kelly EA, Dick EC, Busse WW. Rhinovirus produces nonspecific activation of lymphocytes through a monocyte-dependent mechanism. *J Immunol* 1996; 157: 1605–1612.

42. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, et al. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am Respir Cell Mol Biol* 1999; 20: 1220–1228.
43. Fleming HE, Little FF, Schnurr D, Avila PC, Wong H, et al. Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. *Am J Respir Crit Care Med* 1999; 160: 100–108.
44. Stempel DA, Boucher RC. Respiratory infection and airway reactivity. *Med Clin North Am* 1981; 65: 1045–1053.
45. McWilliam AS, Napoli S, Marsh AM, et al. Dendritic cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J Exper Med* 1996; 184: 2429–2432.
46. Kraft M, Cassell GH, Henson JE, Watson H, Williamson J, et al. Detection of *Mycoplasma pneumoniae* in the airways of adults with chronic asthma. *Am J Resp Crit Care Med* 1998; 158: 998–1001.
47. Kifuji K. Study of the mechanisms of histamine release from human leukocytes by challenge of mycoplasma. *Arerugi* 1989; 38: 1226–1235.
48. Allegra L, Blasi F, Centanni S, Cosentini R, Denti F, Raccanelli R, Tarsia P, Valenti V. Acute exacerbations of asthma in adults: role of *Chlamydia pneumoniae* infection. *Eur Respir J* 1944; 7: 2165–2168.
49. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991; 266: 225–230.
50. Hahn DL, Golubjatnikov R. Asthma and chlamydial infection: a case study. *J Fam Pract* 1994; 38: 589–595.
51. Hahn DL. *Chlamydia pneumoniae*, asthma, and COPD: what is the evidence? *Ann Allergy Asthma Immunol* 1999; 83: 271–288.
52. Freymuth F, Vabret A, Brouard J, Toutain F, Verdon R, et al. Detection of viral, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* infections in exacerbations of asthma in children. *J Clin Virol* 1999: 131–139.
53. Cunningham J, O Conner GT, Dockery DW, Speizer FE. Environmental tobacco smoke, wheezing, and asthma in children in 24 communities. *Am J Crit Care Med* 1996; 153: 218–224.
54. Ehrlich RI, Du Toit D, Jordaan E, Zqwarenstein M, Potter P, et al. Risk factors for childhood asthma and wheezing. Importance of maternal and household smoking. *Am J Respir Crit Care Med* 1996; 154(3 Pt 1): 681–688.
55. Hu FB, Persky V, Flay BR, Zelli A, Cooksey J. Prevalence of asthma and wheezing in public schoolchildren: association with maternal smoking during pregnancy. *Ann Allergy Asthma Immunol* 1997; 79: 80–84.
56. Weitzman M, Gortmaker S, Walker DK, Sobol A. Maternal smoking and childhood asthma. *Pediatrics* 1990; 85: 505–511.
57. Seymour BWP, Pinkerton KE, Friebertshauser KE, Coffman RL, Gershwin LJ. Second-hand smoke is an adjuvant for T helper-2 responses in a murine model of allergy. *J Immunol* 1997; 159: 6169–6175.
58. Seymour B, Friebertshauser K, Coffman R, Gershwin L. Exposure of neonatal mice to environmental tobacco smoke enhances the allergic response to ovalbumin at adulthood. *Annual Investigator Meeting Tobacco-Related Disease Research Program*. 1999. Abstracts.
59. Seymour B, Pinkerton K, Friebertshauser K, Kurup V, Coffman R, Schelegle E, Gershwin L. The effects of second-hand smoke on a murine model of allergic

bronchopulmonary aspergillosis. (Annual Investigator Meeting Tobacco-Related Disease Research Program). 1998. *Program and Abstracts*. pp. 28–29.
60. Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, et al. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. *J Allergy Clin Immunol* 1986; 77: 616–623.
61. Ruznak C, Devalia JL, Davies RJ. The impact of pollution on allergic disease. *Allergy* 1994; 49(18 Suppl): 21–27.
62. McConnell R, Berhane K, Gilliland F, London SJ, Vora H, et al. Air pollution and bronchitic symptoms in southern California children with asthma. *Environ Health Perspect* 1999; 107: 757–760.
63. Gershwin LJ, Osebold JW, Zee YC. Immunoglobulin E-containing cells in mouse lung following allergen inhalation and ozone exposure. *Int Archs Allergy Appl Immun* 1980; 65: 266–277.
64. Osebold JW, Gershwin LJ, Zee YC. Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. *J Environ Path Toxicol* 1980; 3: 221–234.
65. Nel AE, Diaz-Sanchez D, Ng, D, Hiura T, Saxon A. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. *J Allergy Clin Immunol* 1998; 102: 539–554.
66. Diaz-Sanchez D. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. *Allergy* 1997; 52: 52–56.
67. Fujieda S, Diaz-Sanchez D, Saxon A. Combined nasal challenge with diesel exhaust particles and allergen induces in vivo IgE isotype switching. *Am J Respir Mol Biol* 1998; 3: 507–512.
68. Takafugi S, Suzuki S, Koizumi K, Tadokoro K, Miyamoto T, et al. Diesel-exhaust particles inoculated by the intranasal route have an adjuvant activity of IgE production in mice. *J Allergy Clin Immunol* 1987; 79: 639–643.
69. Takenaka H, Ahang K, Diaz-Sanchez D, Tsien A, Sazon A. Enhanced human IgE results from exposure to the aromatic hydrocarbons in diesel exhaust: diesel effects on B cell IgE production. *J Allergy Clin Immunol* 1995; 95: 103–115.
70. Tsien A, Diaz-Sanchez D, Saxon A. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon constituent, enhance IgE production by IgE-secreting EBV-transformed human B cells in vitro. *Toxicol Appl Pharmacol* 1997; 142: 256–263.
71. Barnes PJ. Neuropeptides and asthma. *Am Res Respir Dis* 1991; 143 (Suppl): 28–32.
72. Gamboa PM, de la Cuesta CG, Sanz ML, Garcia BE, and Oehling A. Decrease of beta-receptors after the antigen-specific bronchial provocation test in bronchial asthma. Allergol Immunopathol. 1990; 18: 115–119.
73. Doidge DM, Satchel DG. Adrenergic and non-adrenergic inhibitory nerves in mammalian airways. *J Auton Nerv Syst* 1982; 5: 83–99.
74. Laitinen A, Laitinen LA. Neuropeptides and classic innervation: neural structures in human airways, in *Clinical Allergy and Immunology, Neuropeptides in Respiratory Medicine*. 1994. (Kaliner MA, Barnes PJ, Kunkel GHH, Baraniuk JN, eds.), Marcel Dekker, New York, 1990; pp. 1–19.
75. Dey RD, Mitchell HW, Coburn RE. Organization and development of peptide-containing neurons in the airways. *Am J Respir Cell Mol Biol* 1990; 3: 187.
76. Barnes PJ. Neuropeptides in the lung: localization, function, and pathophysiologic implications. *J Allergy Clin Immunol* 1987; 79: 285–295.

77. Ollerenshaw S, Jarvis D, Woolcock A, Sullivan C, Scheibner T. Absence of immunoreactive intestinal polypeptide in tissue from the lungs of patients with asthma. *N Engl J Med* 1989; 320: 1244–1248.
78. Laitinen A. Ultrastructural organization of intra-epithelial nerves in the human airway tract. *Thorax* 1985; 40: 488–492.
79. Nadel JA. Neutral endopeptidase modulates neurogenic inflammation. *Eur Respir J* 1991; 4: 745–754.
80. Laitinen A, Laitinen LA. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis* 1993; 147: 697–704.
81. Jode JP. Sidestream smoke effects on lung morphology and C-fibers in young guinea pigs. *Toxicol Appl Pharmacol* 1995; 131: 289–296.
82. Bonham AC, Kott KS, Joad JP. Sidestream smoke exposure enhances rapidly adapting receptor responses to substance P in young guinea pigs. J Appl Physiol 1996; 81: 1715–1722.
83. Lai YL, Thacker A, Gairola CG. Sidestream cigarette smoke exposure and airway reactivity during early life. *J Appl Physiol* 1994; 77: 1868–1874.
84. Wu ZX, Morton RF, Lee LY. Role of tachykinins in ozone-induced airway hyperresponsiveness to cigarette smoke in guinea pigs. *J Appl Physiol* 1997; 83: 958–965.