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AIRWAY HYPERRESPONSIVENESS IN ASTHMA

Asthma is described as the presence of wheezing, cough, dyspnea, and chest tightness and by variable airway narrowing and airway hyperresponsiveness to inhaled bronchoconstrictor stimuli. Reversible airway narrowing is the *sine qua non* of asthma. Airway hyperresponsiveness is an increased sensitivity of the airways to constrictor agonists, as indicated by a smaller concentration of the agonist needed to initiate the bronchoconstrictor response; a greater reactivity of the airways, as indicated by an increased slope of the concentration-response curve; and a greater maximal response to the agonist (Woolcock *et al.*, 1984) (Fig. 81.1). Airway hyperresponsiveness is present in virtually all asthmatics with current symptoms (Crockcroft *et al.*, 1977); however, it can be found in patients with other airway diseases (Ramsdale *et al.*, 1985).

Airway hyperresponsiveness in asthma has been the focus of extensive research over the past 30 years. This research has examined a variety of methods of measuring airway responsiveness, the clinical significance and the effects of antiasthma medications on these measurements, and the pathophysiology and pathogenesis of airway hyperresponsiveness in asthmatic patients. As a result of this research, the methods of measuring airway responsiveness have been standardized and become widely accepted. Also, there is some agreement about the clinical significance and effects of treatment on airway hyperresponsiveness.

Airway hyperresponsiveness is measured by subjects inhaling increasing concentrations of a stimulus ranging from bronchoconstrictor mediators, such as histamine (Crockcroft *et al.*, 1977) and methacholine (Juniper *et al.*, 1992), to physical stimuli, such as cold air (O’Byrne *et al.*, 1982), or hypertonic or hypotonic solutions (Anderson *et al.*, 1983). Airway hyperresponsiveness is nonspecific, in that asthmatics who are hyperresponsive to one bronchoconstrictor stimulus will be hyperresponsive to others. The bronchoconstrictor mediators most often used in clinical studies are histamine or the cholinergic agonist, methacholine. The degree of airway responsiveness correlates with the severity of asthma (Crockcroft *et al.*, 1977) and the treatment required to control symptoms (Juniper *et al.*, 1982).

TRANSIENT AIRWAY HYPERRESPONSIVENESS AND INFLAMMATION

The identification of stimuli that can cause asthma has proven to be important in studies of the pathogenesis of airway hyperresponsiveness in asthma. These stimuli include environmental allergens (O’Byrne, 1988), occupational sensitizing agents (Chan-Yeung and Malo, 1995), certain viruses (Lemanske *et al.*, 1989), and the atmospheric pollutant ozone (Holtzman *et al.*, 1979). Each of these stimuli is known to induce airway hyperresponsiveness in experimental models. Studies of the mechanisms of airway hyperresponsiveness in animal models have identified temporal associations between the presence of inflammatory cells in the airways and airway hyperresponsiveness (Holtzman *et al.*, 1983a; Abraham *et al.*, 1988). These studies have led to the hypothesis that activated inflammatory cells, and mediators released from these cells, are responsible for the development of airway hyperresponsiveness after inhalation of various stimuli in human subjects. Subsequently, this hypothesis was extended to suggest that persisting airway hyperresponsiveness in asthmatics, not obviously related to exposure to a specific stimulus such as allergen, is a result of structural
changes in the airways caused by long-standing airway inflammation.

**ALLERGEN-INDUCED AIRWAY RESPONSES**

Environmental allergens are among the most important stimuli known to cause airway inflammation and airway hyperresponsiveness. The pathogenesis of allergic asthma has been greatly clarified by studying the effects of inhaled allergens in mild, allergic asthmatic subjects. Inhalation of allergen by a sensitized subject in the laboratory results in an early asthmatic response; a late asthmatic response occurs in 50% to 60% of adults and 70% to 80% of children who develop early responses. A further consequence of inhaling allergen is the development of airway hyperresponsiveness, the magnitude and duration of which appear to be related to the occurrence of late asthmatic responses (Bhalla et al., 1992).

In human subjects, allergen-induced airway responses are associated with airway inflammation, and with increases in activated airway eosinophils (Gauvreau et al., 1999) and mast cells and basophils (Gauvreau et al., 2000). Some of the mediators that cause allergen-induced bronchoconstrictor responses also have been identified. The cysteinyll leukotrienes (Taylor et al., 1991), thromboxane (Manning et al., 1991), and histamine (Roquet et al., 1997) are involved in early responses, whereas the leukotrienes (Hamilton et al., 1997) and histamine (Roquet et al., 1997) are mainly responsible for bronchoconstriction during the late response.

The identification of allergen as an important cause of asthma has resulted in the development of a variety of animal models of allergen-induced early and late responses, airway hyperresponsiveness, and airway inflammation. The species used include dogs, sheep, guinea pigs, rabbits, rats, mice, and primates.

**Dogs** have been used since the early 1980s to examine the mechanisms of allergen-induced airway inflammation. They are often naturally sensitized to the parasite, *Ascaris suum* (Chung et al., 1984), possibly by cross-reactivity to epitopes present on the parasite *Toxocara canis*. In addition, dogs have been sensitized at birth to ragweed (Becker et al., 1989). Sensitized dogs develop acute bronchoconstriction within 15 minutes after inhaling allergen, and have airway inflammation and airway hyperresponsiveness 12–24 hours after allergen (Chung et al., 1984). The cellular infiltrate consists of eosinophils (Woolley et al., 1995) and/or neutrophils (Chung et al., 1985). Interestingly, despite the development of allergen-induced airway inflammation, dogs do not develop allergen-induced late responses unless the endogenous production of glucocorticosteroids is blocked by metyrapone (Sasaki et al., 1987).

The origin of the inflammatory cells recruited during allergen-induced airway inflammation and the factors that regulate this have been evaluated in dogs. *A. suum*-induced neutrophilic airway inflammation is associated with an increase in the progenitors for neutrophils (granulocyte-macrophage progenitors) in the bone marrow (Woolley et al., 1994), in response to the release of a hematopoietic factor into the bloodstream (Inman et al., 1996). The presence of the serum factor, rather than the bone marrow’s response to the factor, is what determines the subsequent increase in progenitors. Newly formed cells from the bone marrow are subsequently recruited into the airways after allergen inhalation (Wood et al., 1998). Therefore, the development of allergen inflammation is presumably determined by both the airway’s ability to produce a factor to stimulate the bone marrow and the marrow’s increased production of progenitors.

Advantages of a canine model of airway hyperresponsiveness include the size of the animal, which allows physiologic measurements to be made easily and reproducibly, and repeated access to the airways and other sites, such as the bone marrow, to evaluate inflammatory events following allergen inhalation. A major limitation of a canine model is the lack of specific immunologic reagents, although occasionally cross-reactivity with monoclonal antibodies can occur, which allows for studies in dogs with antibodies developed against human epitopes (Li et al., 1992).

**Sheep** are also naturally sensitized to *A. suum* and develop early and late responses (Delehunt et al., 1984) and airway hyperresponsiveness (Lanes et al., 1986) after inhalation of that allergen. The time course of these physiologic changes is similar to that of responses to allergen inhalation in humans. The changes are also associated with increases in airway eosinophils (Abraham et al., 1988). This model has the same advantages and suffers from many of the same limitations of dog models. It has, however, often been used to evaluate the antiallergic activity of drugs thought to be useful in asthma.
(Abraham et al., 1986; Soler et al., 1990; Tomioka et al., 1989).

**Primates** are the third large-animal species used to evaluate the pathophysiology of allergen-induced airway responses. However, because of the high cost of acquisition and maintenance, their use has largely become restricted to the pharmaceutical industry. Monkeys also are sensitized to A. suum antigen and develop early and late responses (Gundel et al., 1992), airway inflammation (Gundel et al., 1991), and airway hyperresponsiveness (Wegner et al., 1990). The airway inflammatory response consists of increases in both eosinophils and neutrophils.

**Rabbits** can be sensitized to ragweed (Murphy et al., 1986) or alternaria (Shampain et al., 1982) antigens shortly after birth, and when challenged develop allergen-induced early and late responses (Shampain et al., 1982), airway inflammation, and airway hyperresponsiveness (Murphy et al., 1986). Sensitization during the neonatal period is essential for the development of increases in specific IgE to the allergens (Shampain et al., 1982). The inflammatory infiltrate consists of eosinophils and neutrophils, and ablation of the airway inflammatory response prevents the development of the physiologic changes (Murphy et al., 1986).

**Guinea pigs** are widely used for studying allergen-induced airway responses because of the ease of sensitization, mainly to ovalbumin (Hutson et al., 1988), and the relative ease of making physiologic measurements. The Hartley strain is the most widely used. Allergen inhalation causes early and late responses, airway inflammation mainly consisting of increases in eosinophils and neutrophils (Hutson et al., 1990), and airway hyperresponsiveness. However, efforts to measure the physiologic responses in awake guinea pigs have been confounded by the large component of upper airway responses in the measurements (Johns et al., 1990). In addition, guinea pigs have an airway eosinophilia, even in a baseline state before allergen inhalation, which can make the evaluation of changes after allergen inhalation difficult to interpret, in the absence of appropriate controls.

**Rats** can be sensitized to ovalbumin and, when challenged, develop early and late airway responses (Eidelman et al., 1988), airway inflammation (Richards et al., 1996), and airway hyperresponsiveness (Elwood et al., 1991). Several strains of rats have been used, but Norwegian rats are the best characterized and develop the most consistent responses (Wang et al., 1996).

**Mice** have become a widely used species to investigate the immunologic mechanisms of allergen-induced airway inflammation. The success of this model is a reflection of the large number of reagents for use in mice and the ability to selectively manipulate immunologic responses by using knockouts, genetic variants, or mice that overexpress a molecule of interest. Although a variety of strains have been used to evaluate allergen-induced airway inflammation, BALB/c are the most often used because these mice develop sensitization to allergens, such as ovalbumin; increases in allergen-specific IgE; and allergen-induced airway eosinophilia (Oshiba et al., 1996).

Development of airway hyperresponsiveness associated with airway eosinophilia and increases in TH2-type cytokines is a consistent finding following allergen challenge in sensitized mice (Inman et al., 1999; Foster et al., 1996). Typically, both the inflammation and functional changes are transient, returning to baseline within 2 weeks of the brief exposure to allergen. There is a report of early and late bronchoconstrictor responses to allergen (Cieslewicz et al., 1999); however, direct measurements of airway or pulmonary resistance were not made. Several investigators have used brief allergen exposure to investigate the mechanisms of the ensuing airway hyperresponsiveness. Clearly, the development of both inflammatory responses and airway hyperresponsiveness is dependent on T cells (Hogan et al., 1998a) and, more specifically, CD40 ligand-mediated responses (Mehlhop et al., 2000). Sorting out which of the T-cell cytokines are involved in the response has proven difficult. Evidence has been presented that many of the TH2-type cytokines, including IL-4 (Corry et al., 1996), IL-5 (Foster et al., 1996), and IL-13 (Grunig et al., 1998), are involved to some extent in establishing allergen-induced airway hyperresponsiveness. However, there is also strong evidence that airway hyperresponsiveness can develop in the absence of IL-4, IL-5, and airway eosinophilia (Hogan et al., 1998b). These models have also been used to demonstrate that several mediators, including interferon gamma (Yoshida et al., 2002), IL-6 (Wang et al., 2000), IL-10 (On et al., 2002), IL-12 (Kips et al., 1996), and IL-18 (Walter et al., 2001), act to prevent or reverse allergen-induced airway hyperresponsiveness in mice. Further studies have demonstrated that conventional and experimental antiasthma drugs including corticosteroids (Leign et al., 2002), antileukotriene agents (Blain and Sirois, 2000), and phosphodiesterase-4 inhibitors (Kung et al., 2000) can prevent allergic inflammation and airway hyperresponsiveness in these models.

More recently, investigators have begun to develop models of chronic allergen challenge in mice. These studies have to some extent been successful in their aim to include aspects of airway wall remodeling, including subepithelial fibrosis, goblet cell hyperplasia, and increased smooth muscle mass, in the pathologic changes induced as a result of allergen exposure (Temelkovski et al., 1998; Tanaka et al., 2001), which are similar to the changes considered to indicate airway remodeling in asthma. It is believed that inclusion of these aspects of asthmatic-type airway pathology will make these models more relevant for studying the mechanisms of airway dysfunction. These models all have demonstrated airway hyperresponsiveness in association with both acute cellular inflammation and some features of airway wall remodeling. More recently, it has further been demonstrated in a similar model of chronic allergen exposure that airway hyperresponsiveness can persist well beyond the resolution of cellular inflammation, suggesting a fundamentally different mechanism than found in models of brief allergen exposure. Such chronic models have been used to begin to elucidate the mechanisms underlying the development of airway wall remodeling, demonstrating a role for IL-13.
Blease et al., 2001), but not IL-4 or IL-5 (Foster et al., 2000) (Fig. 81.2). Further studies will be required to explore the role of these cytokines in the development of airway hyperresponsiveness, which appears to continue beyond the resolution of airway inflammation. Interestingly, it has been demonstrated that treatment with an antileukotriene agent throughout the period of chronic allergen exposure can prevent several aspects of airway wall remodeling but not the associated airway hyperresponsiveness. Whether any intervention can prevent, or more importantly reverse, the sustained airway hyperresponsiveness should be a primary aim of ongoing research with these models.

VIRAL INFECTIONS AND AIRWAY HYPERRESPONSIVENESS

Viral airway infections can cause airway hyperresponsiveness in normal individuals and can exacerbate asthma (Busse et al., 1997; Johnston et al., 1995). It is estimated that viral infections are associated with as many as 50% of wheezing illnesses and asthma exacerbations in children and as many as 20% of those in adults (Johnston et al., 1995; Pattemore et al., 1992). Mild viral respiratory tract infections seldom cause wheezing in normal individuals, but they frequently exacerbate symptoms in asthmatics, and severe infections can cause life-threatening asthma attacks (Ferreira et al., 2002).

Several different viruses, including rhinoviruses, coronaviruses, adenovirus, influenza B, respiratory syncytial virus, and parainfluenza virus, can evoke asthmatic symptoms (Pattemore et al., 1992; Casale et al., 1997; Gern and Busse, 1995). Furthermore, exposure to influenza virus or rhinovirus can cause airway hyperresponsiveness (Empey et al., 1976; Grunberg and Sterk, 1999). Rhinovirus infections can also increase the likelihood of late allergic reactions to antigen (Lemanske et al., 1989). Respiratory viruses damage the airway epithelium and evoke the release of cytokines and inflammatory mediators that can stimulate mast cells, increase vascular permeability, attract inflammatory cells, and initiate an immune response that may have lasting consequences (Busse, 1995). The released mediators and cellular changes can lead to airway hyperresponsiveness, edema, allergic responses, and airflow obstruction (Busse, 1995; Hegele, 1997).

Some viral respiratory infections have long-lasting consequences. After respiratory syncytial virus (RSV) infection, a common cause of bronchiolitis in children, lymphocytes can acquire sensitivity to specific food or mite antigens, thereby predisposing to the onset of allergic disease (Noma et al., 1996). Furthermore, latent or persistent viral infections may be associated with long-lasting airway hyperresponsiveness and chronic inflammation. Portions of the genome of adenovirus, RSV, or Epstein-Barr virus have been detected in the lungs of some patients with chronic obstructive lung disease (Hegele, 1997; Hogg, 2001).

Airway hyperresponsiveness is a well-documented consequence of viral respiratory infection in rats and guinea pigs. After inoculation with type 1 parainfluenza virus (Sendai virus), adult rats become abnormally sensitive to intravenous methacholine (Sorkness and Lemanske, 1996).

This abnormality lasts about 4 weeks, and thus outlasts the 2-week duration of the acute inflammatory response to the infection. The hyperresponsiveness is dependent upon intact vagus nerves. When neonatal rats are infected, the methacholine hyperresponsiveness can last as long as 16 weeks (Kumar et al., 1995). Pathologic changes in the airways of young rats include thickening, fibrosis, and recruitment of macrophages, mast cells, lymphocytes, and eosinophils (Uhl et al., 1996). These changes are more severe in Brown-Norway rats than in F344 rats and are associated with increased expression of transforming growth factor-beta (TGF-β) in mucosal macrophages (Uhl et al., 1996). Rat-adapted influenza virus infection in Brown-Norway rats can increase serologic titers of allergen-specific IgE and inhibit tolerance to repeated exposure to aerosolized allergen (Lebrec et al., 1996).

One mechanism by which viral infections may lead to airway hyperresponsiveness is through their effect on M2 muscarinic receptors on airways. For example, type 3 parainfluenza virus infection in guinea pigs decreases the function of M2 receptors (Jacoby and Fryer, 1999). However, M3 muscarinic receptors on airway smooth muscle are unchanged by the infection. Viral infection may decrease M2 receptor function by damaging the receptors as a result of viral neuraminidase-induced cleavage of sialic acid residues or by inducing inflammation, as suggested by the leukocyte dependency of the effect (Fryer et al., 1997).

Respiratory syncytial virus (RSV) infection in mice results in eosinophilic inflammation and increased airway responsiveness to inhaled methacholine (Schwarze et al., 1997). Both the influx of eosinophils and the hyperresponsiveness
can be blocked by an antibody to IL-5 (Schwarze et al., 1999).

Another mechanism by which viral infections may lead to airway hyperresponsiveness is through an effect on lung mast cells. Neonatal rats inoculated with Sendai virus have more than 100 times as many mast cells in their lungs as their age-matched controls (Castleman et al., 1989). Mast cells become particularly concentrated in bronchiolar walls. The increased number of mast cells is first detectable at 30 days after infection and is still present at 90 days (Castleman et al., 1990). During this period the rats have methacholine hyperresponsiveness (Castleman et al., 1990). After Sendai virus infection, Brown-Norway rats have higher viral titers, less efficient viral clearance, larger increases in bronchiolar mast cells, more persistent inflammatory responses, and greater airway responsiveness than do F344 rats (Sorden and Castleman, 1995b). The increased number of mast cells results from the proliferation of mast cells, as shown by bromodeoxyuridine labeling, and from the recruitment of mast cell precursors from blood (Sorden and Castleman, 1995b).

Respiratory infections can exaggerate neurogenic inflammation in the airway mucosa (McDonald et al., 1991). For example, Sendai virus infection in rats increases the amount of plasma leakage that occurs in the airway mucosa after an injection of capsaicin (Piedimonte et al., 1990a). This augmented response coincides with the epithelial damage and influx of inflammatory cells that peak 4–6 days after inoculation (Piedimonte et al., 1990a) and can be prevented by pretreatment with dexamethasone (Piedimonte et al., 1990b).

Sendai virus infection in guinea pigs increases the bronchoconstrictor response to substance P and capsaicin (Dusser et al., 1989). The mechanism of this change may involve a decrease in substance P-degrading neutral endopeptidase (NEP) in the airway epithelium, because after infection the NEP inhibitor phosphoramidon no longer potentiates the response to substance P or capsaicin. Bronchomotor responses to acetylcholine are unaffected by the infection (Dusser et al., 1989). Similar accentuated smooth muscle contractile responses to substance P have been reported in ferret tracheas infected in vitro with human influenza virus A (Jacoby et al., 1988). Here, the activity of NEP is decreased by 50% (Jacoby et al., 1988).

The question of how viral airway infections can produce long-lasting changes, resembling the persistent nature of asthma and other chronic airway diseases, is beginning to be addressed in animal models. One approach is through experiments on virally mediated lymphocyte activation (Gern et al., 1996). Another approach focuses on latent viral infections. After intranasal inoculation with adenovirus 5, guinea pigs develop bronchiolitis that can persist for more than 6 weeks (Vitalis et al., 1996). Viral DNA can be detected by polymerase chain reaction at this time in most of the animals, and viral protein can be detected by immunohistochemistry in some (Vitalis et al., 1996). Additional research will be needed to determine the mechanism underlying the prolonged inflammatory response.

### Mycoplasmal Infection and Airway Hyperresponsiveness

Respiratory infection caused by Mycoplasma pneumoniae is considered an etiologic or precipitating factor in some cases of asthma (Berkovich et al., 1970; Mok et al., 1979). Mycoplasma pulmonis infections cause a chronic respiratory disease in rats and mice (Lindsey et al., 1971; Lindsey and Cassell, 1973). The severity of M. pulmonis disease ranges from subclinical to lethal, with genetic and environmental factors playing important roles in the outcome. The organisms are prokaryotic extracellular parasites with no cell wall that attach to the luminal plasma membrane of airway epithelial cells. The initial acute inflammatory response, in which neutrophils predominate, evolves into chronic inflammation characterized by the accumulation of mucosal lymphoid tissue, peribronchial lymphoid follicles, and enlarged hilar, mediastinal, and cervical lymph nodes. Dendritic cells, T and B lymphocytes, and macrophages accumulate in the airway mucosa (Davis et al., 1982). Lymphoid tissue, which is rare or absent in the tracheal mucosa of pathogen-free rats and mice, can occupy 75% of the mucosa after M. pulmonis infection (McDonald et al., 1991). Other pathologic changes include hyperplasia of epithelial ciliated cells and goblet cells, mucous gland hypertrophy, angiogenesis, and fibrosis (McDonald et al., 1991; Schoeb et al., 1985; McIntosh et al., 1992; Cartner et al., 1995; Huang et al., 1989). These changes can result in a several-fold increase in the thickness of the airway mucosa (Fig. 81.3) (McDonald et al., 1991). Lifelong inflammatory airway disease, with chronic tracheobronchitis, bronchiectasis, airway wall thickening and fibrosis, and lung consolidation, can eventually develop (McIntosh et al., 1992).

Although it has not yet been determined whether the airways of M. pulmonis-infected animals are hyperresponsive to methacholine, other forms of hyperresponsiveness do occur. For example, the newly formed microvasculature is abnormal, one manifestation of which is hyperresponsiveness to certain irritants and predisposition to plasma leakage (Fig. 81.3) (Kwan et al., 2001; McDonald, 2001). Also, airway mucous secretion is exaggerated (Huang et al., 1989).

### Ozone-Induced Airway Responses

Ozone is a powerful oxidizing agent that is classified as a "secondary air pollutant." Secondary air pollutants are not emitted into the atmosphere but formed from subsequent atmospheric chemical reactions of primary pollutants (nitrogen dioxide, sulfur dioxide, particles, carbon monoxide, and lead). Ozone is used in a variety of animal models to induce airway hyperresponsiveness and airway inflammation.

**Dogs:** Airway hyperresponsiveness develops in dogs after ozone inhalation (Lee et al., 1977). The airway hyperresponsiveness is most marked at 1 hour after ozone inhalation, is still present 1 day later, and is back to baseline levels by 1 week (Holtzman et al., 1983b). Ozone-induced airway
Fig. 81.3. Remodeling of airway mucosa after *Mycoplasma pulmonis* infection. (See page 5 of the color plates.) A, B. Histologic sections of rat tracheas stained with toluidine blue showing the thin mucosa of a pathogen-free rat (A) and the much thicker mucosa of a rat infected with *M. pulmonis* for 4 weeks (B). Arrows mark the outer and inner limits of the mucosa. C, D. Whole mounts of rat tracheas showing leaky mucosal blood vessels (arrows, blue) after an intravenous injection of substance P to mimic neurogenic inflammation. The amount of neurogenic inflammation, as reflected by the number of leaky blood vessels, is much smaller in the pathogen-free rat (C) than in the rat infected with *M. pulmonis* for 4 weeks (D). E, F. Whole mounts of rat tracheas showing the amount and architecture of the mucosal vasculature after staining by perfusion of biotinylated *Lycopersicon esculentum* lectin. Relatively sparse, straight capillaries (arrows) in a pathogen-free rat (E) contrast with the abundant, tortuous angiogenic blood vessels in a rat infected with *M. pulmonis* for 4 weeks (F). Some of the angiogenic blood vessels form focal networks (arrows). G, H. Whole mounts of rat tracheas stained immunohistochemically with antibody ED2 to show tissue macrophages. Tissue macrophages are irregularly shaped and scattered in the mucosa of the pathogen-free rat (G), but in the rat infected with *M. pulmonis* for 4 weeks they are rounded and concentrated in focal clusters around tortuous networks of angiogenic blood vessels (H). Scale bar 150 μm in A, B, G, H; 300 μm in C–F.
hyperresponsiveness in dogs is associated with a marked reversible neutrophil influx in the epithelium and bronchoalveolar lavage (Holtzman et al., 1983a) (Fig. 81.4). The dogs responsive to ozone have an increased number of epithelial cells in the lavage 1 hour after ozone inhalation (Fabbri et al., 1984). There is a close correlation between the degree of airway hyperresponsiveness and the number of neutrophils in the epithelium (Holtzman et al., 1983a). Ozone-induced airway hyperresponsiveness is prevented by depleting circulating neutrophils (O’Byrne et al., 1984). These studies suggest a causal relationship between the onset of ozone-induced hyperresponsiveness and neutrophil influx into the airways. However, other investigators have been unable to attenuate the ozone-induced airway hyperresponsiveness in dogs with cyclophosphamide-induced neutrophil depletion (Imai et al., 1990). Further evidence that the ozone-induced neutrophilia is not necessarily related to the ozone-induced airway hyperresponsiveness comes from the study of Li et al. (1992), who blocked the ozone-induced leukocyte migration with antileukocyte adhesion molecule (anti-Mo1) without inhibiting ozone-induced airway hyperresponsiveness.

**Sheep:** Airway hyperresponsiveness to inhaled carbacol develops in sheep 24 hours after ozone inhalation, with no change in tracheal mucous velocity (Phipps et al., 1986). Ozone also increases in airway responsiveness to inhaled radioactively labeled histamine. The airway hyperresponsiveness parallels ozone-induced increases in epithelial permeability, which was estimated from the rate of appearance of the labeled histamine in the blood plasma (Abraham et al., 1984). Tracheal mucous velocity significantly decreases after ozone inhalation (Allegra et al., 1991). Ozone inhalation also causes a dose-dependent increase in the bronchial artery blood flow in sheep, as a reflection of vasodilatation of the bronchial vasculature (Schelegle et al., 1990).

**Primates:** Airway responsiveness to methacholine increases 2.5-fold in rhesus monkeys exposed weekly to ozone for 19 weeks (Menzel, 1996). After a single ozone inhalation, reinfused labeled neutrophils appear in lung tissue and bronchoalveolar lavage fluid, and this effect is maximal at 12 hours and returns to baseline by 24 hours after ozone inhalation (Hyde et al., 1992). Epithelial necrosis is seen in the trachea and bronchioles of rhesus monkeys at 1 hour and 12 hours after inhaled ozone. The epithelial necrosis and repair is associated with the presence of granulocytes in the epithelium and interstitium (Hyde et al., 1992). This is seen as early as 4 hours after inhaled ozone, and is maximal between 12 and 24 hours (Castleman et al., 1980). Eosinophils are maximally increased in the bronchial mucosa at 24 hours when epithelial necrosis and lavageable protein are also maximally increased (Hyde et al., 1992).

**Rabbits:** Exposure of rabbits to 2ppm ozone, 6 hours daily for 3 days, results in increased pulmonary resistance, epithelial damage, increased submucosal edema in the large airways and in terminal and respiratory bronchioles, and thickened alveolar walls in the proximal alveolar ducts (Yokoyama et al., 1989). Ozone inhalation also results in a large increase in PGE₂ and PGF₂α₄ and small increases in TxB₂ and 6-keto-prostaglandin F₁α. This effect decreases and eventually disappears as the animals grow toward adulthood. Acute ozone inhalation experiments have not been carried out in rabbits. In vitro ozone exposure of lung macrophages results in elaboration of PGE₂ and PGF₂α₄.

**Guinea pigs:** In guinea pigs, ozone inhalation causes airway hyperresponsiveness without neutrophil influx into the tracheal mucosa (Murlas and Roum, 1985). Guinea pigs exposed to ozone for 2 hours also develop airway hyperresponsiveness to subcutaneous histamine (Gordon and Amdur, 1980). Methacholine airway hyperresponsiveness occurs after ozone inhalation of 1ppm for 1.5 hours, but not after inhaling 1ppm for 0.5 hours, suggesting that the ozone-induced airway hyperresponsiveness in guinea pigs is significantly correlated with the ozone dose (the product of time and concentration). Ozone inhalation (3ppm for 1 hour) increases neutrophils in bronchoalveolar lavage in ovalbumin-sensitized and nonsensitized male guinea pigs (Campus et al., 1992). Furthermore, these investigators found that ozone inhalation increases the in vivo bronchoconstrictor response to both histamine and allergen. Neutrophils rapidly accumulated in the guinea pig lung interstitium after ozone inhalation and returned to near control values within 24 hours (Schultheis and Bassett, 1991). By contrast, neutrophils recovered in bronchoalveolar lavage peak at 3–6 hours and remain elevated for 3 days. Leukocyte depletion with cyclophosphamide in steroid-treated guinea pigs does not prevent acetylcholine airway hyperresponsiveness at 2 or 6 hours after ozone inhalation (Murlas and Ram, 1985), suggesting that airway neutrophil infiltration is a consequence of ozone-induced mucosal injury, but is not the cause of airway hyperresponsiveness.

![Fig. 81.4](image-url) Increases in neutrophils in the epithelium and subepithelium of bronchial mucosal biopsies from dogs with ozone-induced airway hyperresponsiveness. No increases were demonstrated in dogs that did not develop ozone-induced airway hyperresponsiveness, or in circulating neutrophil numbers in either group. (Reprinted with permission from Holtzman et al., 1983b).
**Rats:** Exposure of rats to high ozone concentrations causes airway hyperresponsiveness, epithelial damage, and in most studies, airway neutrophil influx, but not increased vascular permeability. Rats developed acetylcysteine airway hyperresponsiveness immediately after ozone inhalation, and the airway responsiveness returned to control levels by 24 hours (Evans et al., 1988). The ozone-induced acetylcysteine airway hyperresponsiveness has been suggested to result from a marked inhibition of acetycholinesterase (Gordon et al., 1981). Inhalation of lower concentration of ozone also causes airway hyperresponsiveness, but without neutrophil influx into the tracheal mucosa or increases in vascular permeability (Mustafa et al., 1983). However, a similar study, from the same laboratory, reports that the number of neutrophils in bronchoalveolar lavage is significantly increased after the same concentration of ozone (Hotchkiss et al., 1989). To investigate the importance of neutrophils in ozone-induced airway hyperresponsiveness, Joad et al. (1993) perfused rat lungs with buffer with or without neutrophils, and with or without exposure to ozone. They concluded that perfusion of neutrophils in the absence of ozone decreased pulmonary compliance and increased pulmonary resistance, total bronchoalveolar lavage protein, lung weight, and pulmonary arterial pressure, probably as a result of increased vascular leakage. However, neutrophil perfusion alone neither changed airway responsiveness to methacholine nor damaged the airway epithelium. Yet, neutrophil perfusion did have an additive effect on the ozone-induced impairment of pulmonary function and a synergistic effect on ozone-induced airway epithelial injury.

These studies suggest that neutrophils play a role in modulating the repair processes after ozone-induced injury and that plasma extravasation from alveolar capillaries does not occur following ozone inhalation.

**Mice:** Some investigators have suggested that the magnitude of the inflammatory response of the lungs to inhaled ozone in animals is, at least in part, under genetic control. In support of this is an 11-fold difference in inflammatory responses demonstrated in an acute ozone-induced pulmonary inflammation-susceptible strain of mice to inhaled ozone as compared with ozone-exposed control mice (Kleeberger et al., 1990). Ozone-induced airway hyperresponsiveness and an associated neutrophilic airway inflammation has been described in mice (Shore et al., 2001). The airway hyperresponsiveness, but not the neutrophilic inflammation, is mediated by tumor necrosis factor (TNF) (Shore et al., 2001).

**INTERACTIONS OF OZONE- AND ALLERGEN-INDUCED AIRWAY RESPONSES**

The additive or synergistic effects of ozone and allergen inhalation are potentially important clinically because high concentrations of atmospheric ozone often coincide with peak levels of airborne allergens. It also has been suggested that epithelial damage induced by ozone could exaggerate allergic reactions to inhaled foreign proteins (Osebold et al., 1980). In atopic subjects, inhaling ozone at concentrations selected to produce no detectable effects on baseline lung function increases airway responsiveness to inhaled allergen in atopic asthmatics (Molfino et al., 1991). Guinea pigs challenged with ozone also have been shown to be more sensitive to inhaled allergens (Matsumura, 1970). Ozone inhalation increases acetylcysteine airway responsiveness in dogs nonallergic and allergic to *A. suum* (Yanai et al., 1990). Immediately after ozone inhalation, the *Ascaris*-sensitive dogs were 4.7-fold more responsive to the inhaled *Ascaris* allergen and to acetylcysteine. Two weeks later, these dogs were still significantly hyperresponsive to *A. suum* inhalation (1.6-fold increase), but no longer to acetylcysteine.

**CONCLUSIONS**

Inhaled allergens, ozone, and some viruses can cause airway inflammation and airway hyperresponsiveness in humans and in a variety of laboratory animals. Studies using these stimuli have contributed significantly to the understanding of the pathophysiology of airway inflammation and airway hyperresponsiveness. Indeed, a causal link between airway inflammation and airway hyperresponsiveness was initially demonstrated in dogs exposed to ozone. The importance of inflammatory cells and various mediators in airway hyperresponsiveness remains controversial. Differences among species may stem from differences in the experimental models and types of measurements, as well as biologic differences. Overall, much more needs to be done to fully characterize the pathophysiology of allergen-, pathogen-, and ozone-induced airway hyperresponsiveness.

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