Abstract. Over the last two decades there has been a worldwide increase in the morbidity and mortality associated with asthma, a chronic inflammatory disease of the airways. There is a growing body of evidence that suggests there is an association between upper respiratory viral infections, particularly rhinovirus infections, and asthma exacerbations. Virally induced airways hyperreactivity has been associated with elevated numbers of inflammatory cells in the bronchial mucosa. Upon virus infection, respiratory epithelial cells produce proinflammatory cytokines, including IL-6, IL-8, RANTES, and GM-CSF, which could contribute to the increased inflammatory cell recruitment noted in the airways. Whether or not a viral infection triggers an asthma attack may depend upon many factors, including the types of inflammatory cells recruited to the airways, the viral load, and variations in the host antiviral response. There is evidence to support the idea that eosinophils from asthmatic and symptomatic atopic subjects may be primed to respond to chemotactic cytokines produced by infected epithelial cells. Rhinovirus infections may therefore enhance airway eosinophilia in asthmatics, leading to airway hyperresponsiveness and impaired pulmonary function. Nitric oxide is a potent inhibitor of both rhinovirus-induced cytokine production and viral replication and may play an important role in the host response to viral infections. Based upon these observations, we speculate that nitric oxide donors may represent a novel therapeutic approach for the treatment of rhinovirus infections and viral exacerbations of asthma.

Asthma is a chronic inflammatory and obstructive disease of the airways that affects almost 15 million people, including 4 million children, in the United States (1). The annual medical costs in 1990 for patients with asthma were estimated to be about $6 billion, with almost half of these costs due to hospitalizations for acute exacerbations (1, 2). For unknown reasons, over the last two decades there has been a worldwide increase in the morbidity and mortality associated with asthma (3, 4). Despite recent advances in the development of new therapeutic interventions (5, 6), asthma remains a major cause of hospital admissions for people of all ages (7). A better understanding of the factors that cause asthma, and those that trigger asthma attacks, would have a significant impact upon health care delivery and associated costs.

Viral Exacerbations of Asthma

The symptoms of asthma include wheeze, cough, and breathlessness, and are thought to be related to the release of inflammatory mediators in the airways (8). Evidence is accumulating that viral infections may play an important role in both the development of asthma and the initiation of acute asthma attacks (9). Upper respiratory viral infections have been reported to cause wheezing in patients of all ages (10–12). For infants and children under the age of 2 years, respiratory syncytial and parainfluenza viruses have frequently been associated with wheezing episodes leading to hospitalization (9, 13). Of those individuals who experience virally induced wheezing in infancy, a subset develop per-
sistent asthma, suggesting that upper respiratory viral infections contribute to, but are not the sole cause of, asthma (14). Based upon current knowledge, the most significant factor implicated in the development of asthma is the genetic predisposition for an IgE-mediated response to common aeroallergens (8, 11). Interestingly, some recent data suggest that having a viral infection in early childhood may actually reduce the risk of allergen sensitization later in life (9, 15). Folkerts and colleagues have suggested that the impact of viral infection on the subsequent risk of developing asthma may depend upon the type of pathogen that infects the individual during immune development (9).

Whereas the relationship between viral infections and the development of asthma is still emerging, there is substantial data to support the concept that upper respiratory viral infections trigger asthma attacks. Among the many factors that have been linked to acute exacerbations of asthma, including exercise, exposure to drugs, allergens, and noxious gases, and gastroesophageal reflux, studies suggest that viral infections may be the most common cause (9–11, 16, 17). The association between viruses and asthma attacks was first noted during the influenza epidemic of 1957 and 1958 (18, 19). At that time it was believed that bacterial infections were also associated with exacerbations of asthma. Subsequently, numerous prospective studies confirmed a temporal relationship between asthma and viruses, but not bacteria (16, 20–25). The results of these studies indicated that 10%–50% of the acute episodes of asthma were associated with detectable viral infections, whereas the detection rate during asymptomatic periods was only 2%–3% (16, 20–22). Bacterial infections were detected in the respiratory tracts of asthmatics with equal incidence during symptomatic and asymptomatic periods (21, 24). More recent data suggest that these early studies may have underestimated the rates of viral infection, because the viruses that are frequently associated with asthma, such as rhinoviruses and coronaviruses, were elusive and difficult to culture in vitro (26). With the development of more sensitive techniques for the detection of viruses, such as the reverse transcription-polymerase chain reaction (RT-PCR), over 80% of spontaneous asthma exacerbations have been associated with upper respiratory viral infections (27, 28). In adults and children over 2 years old, the viruses most often identified in conjunction with the symptoms of asthma were rhinoviruses, which were detected during up to 60% of asthma attacks (7, 9, 13, 27, 28). By contrast, the detection rate for rhinoviruses in control, symptom-free subjects was 2% using RT-PCR (28).

Epidemiological and prospective studies have provided strong evidence that symptoms of asthma are associated with viruses, in particular rhinoviruses. The mechanisms by which upper respiratory viral infections cause airflow obstruction, the hallmark of asthma, are still uncertain. This is due partly to the fact that the relationships between viral infections and changes in lower airway physiology are complicated by many factors, including the serotype of virus, the severity of the cold, and host defense factors (9, 29). Historically, rhinovirus colds have been viewed as infections primarily limited to the upper airways. Recent studies using RT-PCR, however, have documented that rhinovirus RNA can be detected in the lower airways during infections (30). These data are consistent with observations that upper respiratory rhinovirus infections have been associated with changes in lower airway reactivity and inflammation. Increased airway reactivity has sometimes (31), but not always, been observed in normal subjects with rhinovirus infections (32–35). Virally induced changes in lower airway reactivity are much more likely to occur in allergic (12, 35) and asthmatic individuals (33, 36, 37). Increased reactivity to methacholine (36) and histamine (12, 33, 35, 37) have been documented, beginning about 2–3 days after viral exposure and, in some cases, persisting for several weeks after the infection (12, 36).

Viral induced hyperreactivity has been correlated with elevated numbers of inflammatory cells in the lower airways (33, 38), suggesting that a bronchial mucosal inflammatory response may be one mechanism by which viral infections lead to changes in airway function. Increased numbers of inflammatory cells, including lymphocytes, eosinophils, and neutrophils, have been observed in bronchoalveolar lavage fluids (39), bronchial biopsies (33, 39), and induced sputum (38), from rhinovirus-infected individuals. Interestingly, the induced sputum from infected asthmatic subjects had increased levels of the proinflammatory eosinophil product, eosinophil cationic protein (ECP), suggesting that the eosinophils in the bronchial epithelium were activated (38). Moreover, the increased ECP correlated with a decreased PC_{20} to histamine, reflective of an increase in airway reactivity. Studies using immunohistochemical techniques to quantify inflammatory cells in bronchial biopsies, showed increased numbers of eosinophils, which persisted in the epithelium of asthmatic, but not normal, subjects with a cold (33). Rhinovirus infections have also been shown to potentiate airway inflammation, especially eosinophilia, following antigen challenge in allergic subjects (40). The virally induced potentiation was accompanied by enhanced release of histamine persisting up to 48 hr after challenge (40). These data suggest that rhinovirus-enhanced airway hyperresponsiveness often occurs in conjunction with airway inflammation and more specifically, eosinophilic inflammation. Whether rhinoviruses cause changes in lower airway function directly by infecting the cells of the lower airways or indirectly via mechanisms linked to changes in the upper airways, remains to be established.

**Pathogenesis of Rhinovirus Infections**

To understand the pathophysiology of virally induced airway obstruction and asthma, the biochemical pathways involved in virus infections must be delineated. Since rhinoviruses have been identified as the type of virus most often associated with exacerbations of asthma, this review is primarily limited to those viruses. Studies to date have pro-
vided some important insights into the basic mechanisms of rhinovirus infections. Human rhinoviruses were discovered in 1960 as the principal causative agents of the most frequently experienced acute respiratory illness, the common cold (41–45). These viruses were classified as single, positive-stranded RNA viruses belonging to the family Picornaviridae (46–48). Since then, about 102 antigenically distinct serotypes of human rhinovirus have been isolated (48, 49). These cold viruses have been linked to exacerbations of several diseases, not only asthma, but also bronchitis, otitis media, and sinusitis (28, 46, 50).

The primary target of rhinovirus infections are the epithelial cells of the respiratory tract (51–53). In situ hybridization studies have detected replicating human rhinoviruses in both ciliated and nonciliated nasal epithelium (52, 53). The sites of infection were focal in nature and involved only a limited number of nasal epithelial cells. As mentioned above, recent studies have determined that rhinovirus can also be detected in cells of the lower airways (30). It is not certain how rhinovirus spreads through the airways or how many cells of the respiratory tract actually become infected. However, on a cellular level, the sequence of events in the rhinovirus infection cycle has been elucidated. The first step in the infection process involves the attachment of the virion to specific receptors in the plasma membrane of the target epithelial cell (46, 54). Human rhinoviruses have been classified into two groups, the major group that bind to cells via intercellular adhesion molecule-1 (ICAM-1), and the minor group that bind to cells via other receptors, including the low-density lipoprotein (LDL) receptor (46, 48, 49, 54–56). Following attachment, the virion undergoes a conformational change allowing delivery of the viral RNA to the cytosol of the target cell (57). Replication of the virus takes place entirely in the cytoplasm of the host cell. Using ribosomes and other protein-synthesizing molecules of the host cell, viral RNA is translated to produce a polyprotein which, following cleavage by proteinases encoded in the polyprotein, gives rise to several proteins, including a protein that initiates RNA synthesis and the necessary RNA polymerase proteins. The virus then reproduces its viral RNA, first by making complimentary negative-stranded RNA molecules that consequently serve as templates for the synthesis of many positive-stranded RNA molecules. New virions are then assembled, enclosed in coat proteins, converted from noninfectious provirions to infectious virions via cleavage of a protein, and released from the host cell. The entire replication cycle takes about 5–10 hr for most picornaviruses (47).

**Rhinovirus Infection Alters Epithelial Cell Biochemistry**

The mechanisms whereby rhinovirus infection of epithelial cells leads to changes in lower airway physiology in infected individuals are the focus of intense investigation. A number of potential mechanisms by which viral infection could contribute to airway obstruction and hyperreactivity, including changes in neural control, airway geometry, or inflammation, have been summarized in a recent review (9). Because epithelial cells are the primary hosts for rhinoviruses, it is likely that virally induced changes in the epithelial cell play an important role in the sequence of events leading to asthma exacerbations. Unlike other respiratory viruses, such as influenza, rhinoviruses are not cytotoxic to the host epithelial cell. Overt cytotoxicity has not been observed in specimens obtained from human subjects infected with rhinovirus (51, 58) or in rhinovirus-infected epithelial cell cultures (59). Infected epithelial cells may, however, contribute to airway inflammation and ultimately, the symptoms of asthma, by producing proinflammatory mediators (Table I). Studies have shown that cultures of respiratory epithelial cells infected with rhinovirus secrete elevated levels of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-11 (IL-11), granulocyte-macrophage colony-stimulating factor (GM-CSF), and RANTES (regulated upon activation, normal T-cell expressed and presumably secreted) (59–64) (and unpublished observations). Other viruses that have been associated with asthma, such as respiratory syncytial virus and influenza virus, also induce respiratory epithelial cells to produce a similar profile of cytokines, as well as monocyte chemotactic protein-1 (MCP-1), macrophage protein-1α (MIP-1α), and tumor necrosis factor α (TNFα) (61, 65–74).

Nasal secretions and induced sputum from human subjects with naturally acquired or experimentally induced colds contain increased levels of many proinflammatory cytokines, and the increased levels of cytokines frequently correlate with the severity of symptoms (Table I). For instance, IL-8, a potent chemoattractant and activator for neutrophils, was elevated in asthmatic adults and children with rhinovirus infections (37, 38, 75, 76). In children with virally induced asthma, increased levels of IL-8 in nasal aspirates correlate with increased levels of neutrophil myeloperoxidase, an enzyme that produces the cytotoxic species hypohalous acid, and, in turn, with the severity of respiratory symptoms (75). Interleukin-1 (IL-1), a cytokine that enhances adhesion molecule expression and activates T lymphocytes, was increased within 24 hr after infection and

**TABLE I. Virus-Induced Cytokine Production**

| Cytokine | Epithelial cells in vitro | Airway secretions in vivo |
|----------|--------------------------|--------------------------|
| Interleukin-1 | R/RSV | R/V |
| Interleukin-6 | R/RSV/I | R/V |
| Interleukin-8 | R/RSV/I | R/V |
| Interleukin-11 | R/RSV/I | R/RSV/V |
| GMCSF | R/RSV | |
| RANTES | R/RSV/I | RSV/I |
| TNF-α | RSV/I | R/V |
| MCP-1 | RSV |
| MIP-1α | RSV |

*Abbreviations: R = Rhinovirus, RSV = Respiratory Syncytial Virus, I = Influenza Virus, V = Undefined upper respiratory virus.*
recruited to the airways in atopic versus nonatopic individu-
als. Nonatopic subjects or seasonal atopics out of season, when infected with rhinovirus, have increased numbers of neutrophils and lymphocytes in the nasal mucosa and nasal secretions (81–84) and decreased numbers of peripheral blood lymphocytes (82). Asthmatic subjects with rhinovirus infections have not only increased submucosal lymphocytes, but also increased epithelial eosinophils in their lower airways (33). In addition, as discussed previously, rhinovi-
us infections significantly potentiate the recruitment of eo-
sinophils to the sites of allergen challenge in allergic sub-
jects (40). Although many inflammatory cells are involved in the pathogenesis of asthma, eosinophilia has been con-
sidered a cardinal feature of asthma for decades (85–87). Increased numbers of eosinophils in peripheral blood and bronchoalveolar lavage have been reported to correlate with the degree of bronchial hyperresponsiveness (88–92). In fact, autopsy studies have suggested that eosinophilic infiltrates in the airways are frequently characteristic of end-
stage asthma (89). Therefore, it follows that rhinovirus in-
factions, by virtue of their ability to enhance airway eosino-
philia, could lead to exacerbations of asthma.

Several of the cytokines produced by virally infected epithelial cells are capable of recruiting and activating eo-
sinophils. RANTES, for example, is a member of the C-C chemokine family and one of the most potent che moat-
tractants for eosinophils (65, 67, 70, 93, 94). GM-CSF has mul-
tiple effects on eosinophils, and can induce adhesion molecule expression, promote eosinophil survival, and act as a cofactor for eosinophil superoxide production and de-
granulation (59, 66, 95, 96). Recent studies from our labo-
ratory (unpublished observation) have shown that in addi-
tion to RANTES and GM-CSF, rhinovirus induces human epithelial cell gene expression for eotaxin, one of the most poten t and specific eosinophil chemotactic factors identified to date (97, 98). Importantly, elevated levels of these cyto-
kines have been detected in nasal and bronchoalveolar la-
vage fluids and have been implicated in the pathogenesis of asthma and allergy (74, 99–103). As a result, it is very possible that virally infected epithelial cells secrete cyto-
kines and chemokines that recruit and activate eosinophils and thereby contribute to enhanced airway eosinophilia and exacerbations of asthma.

Among the many questions that remain to be answered, is why do viral infections increase eosinophils in the air-
ways of asthmatics and allergic subjects, but not normal sub-
jects (33). Viral infections may differentially induce cy-
tokine production from respiratory epithelial cells, such that cells from atopics produce cytokines that effect eosinophil function, whereas normals do not. However, this is unlikely as epithelial cells from many sources produce a similar pro-
file of cytokines when infected with rhinoviruses. The con-
verse, however, is possible. Eosinophils from atopics and asthmatics may differ in responsiveness compared to eo-
inophils from normals. The idea that cells such as eosino-
phils can be ‘primed’ in vivo by exposure to circulating cytokines such as interleukin-5 (IL-5), interleukin-3 (IL-3),

**Virally Induced Cytokines Recruit Eosinophils**

Whether or not a viral infection triggers an asthma attack may depend upon the types of inflammatory cells recruited to the airways in atopic versus nonatopic individu-
or GM-CSF, in asthmatics, but not normals, has been suggested as a plausible explanation for the differential recruitment. Studies have shown that eosinophils from patients with asthma have increased chemotactic and chemokinetic responses compared to eosinophils from nonasthmatic subjects, suggesting that eosinophils from asthmatics are primed to respond to chemotactic stimuli (104–106). In vitro studies have demonstrated that cytokines such as IL-5 and GM-CSF activate eosinophils by enhancing adhesion molecule expression and increasing transendothelial and transepithelial migration (107–109). Moreover, studies from our laboratory have shown that eosinophils from nonatopic donors undergo transepithelial migration in response to chemotactic stimuli such as RANTES, only if first exposed to IL-5 (Polito and Proud, unpublished observation). It remains to be demonstrated whether these mechanisms are operating in virally induced asthma, but it is of interest that activated eosinophils have been detected in the airways of wheezing children in the emergency room (13).

Antiviral Effects of Nitric Oxide

Clearly, significant associations exist between virally induced inflammation and asthma. However, viruses do not always induce asthma attacks in every asthmatic individual. There are several explanations for this, including variations in viral load and variations in the host antiviral and immune responses. Recent studies suggest that one important component of the host antiviral response may be the ability of respiratory epithelial cells to produce nitric oxide (NO) (62). NO is a free radical molecule that mediates a broad range of important physiological processes and may act as a vasodilator, neurotransmitter, antimicrobial, or immune regulator (110–115). There are three isoforms of the enzyme nitric oxide synthase (NOS) that produce NO: Type I or neuronal NOS (nNOS), type II or inducible NOS (iNOS), and type III or constitutive NOS (cNOS) (116, 117). It has been reported that all three types are produced by epithelial cells, although evidence from immunohistochemical studies of airway and lung tissues suggest that iNOS expression usually predominates in vivo (118–121). High concentrations of nitric oxide have been detected in air derived from the nasal airways (122). In fact, there is a continuous production of NO in these airways and it has been suggested that the purpose is to act as an antimicrobial agent and maintain sterility in the human sinuses (122, 123).

The role of NO in asthma is currently unclear. Interestingly, transbronchial biopsies from asthmatic patients have shown elevated iNOS expression compared to nonasthmatic controls (124). Not only are there elevated levels of NOS immunoreactive protein in the airways, there are also significantly higher levels of NO detected in exhaled air of asthmatics compared to normals (125, 126). The increased levels of expired NO in asthmatics can be lowered by steroid therapy (126–129) and NOS inhibitors (130, 131). In some asthmatics, NO can be increased beyond the already elevated basal levels in response to allergen (132).

The increased nitric oxide measured in expired breath from asthmatics has been shown to be derived from the lower airways (133, 134) and not the nasal sinuses. The cellular source of this nitric oxide has not yet been unequivocally identified, although studies suggest that the NO originates mainly from cells in the airway surface, such as epithelial cells, rather than the cells of the pulmonary circulation (120, 135, 136).

Due to its multiplicity of actions, NO in the lower airways has been described as both beneficial and harmful. For example, elevated levels of NO have been proposed to play a beneficial role due to the bronchodilating effects of NO (137). Conversely, NO has been reported to alter the balance between TH1 and TH2 cell types, leading to the proliferation of TH2 lymphocytes, which produce a cytokine profile that has been associated with exacerbations of asthma (138–140). There is convincing evidence in animal models and in vitro studies, that NO may be beneficial due to its potent antiviral properties. A wide range of viruses, including both DNA and RNA viruses, have been inhibited by the addition of chemical donors of NO or by the induction of NOS (141–147). In vivo, inhibitors of NOS have been shown to increase viral load and decrease survival in virus-infected mice (148–152).

Recently, we have shown for the first time that NO can inhibit both rhinovirus replication and rhinovirus-induced production of cytokines in human respiratory epithelial cells (62). Because epithelial cells lose the expression of NOS when placed in culture (119), we have used the nitric oxide donor 3-(2-hydroxy-2-nitroso-1-propylhydrazino)-1-propanamine (NONOate) that releases NO in a time-dependent manner at physiological pH. In our studies, NONOate inhibited virally induced production of several proinflammatory cytokines, including IL-8, IL-6, RANTES, and GMCSF, in a dose-dependent fashion (62) (also unpublished observation). Possibly of even more significance was the ability of NONOate to inhibit rhinovirus replication. The inhibitory effects of NONOate, which has a half life of 76 min, were more pronounced at 4 hr than at 24 hr, suggesting that both viral replication and cytokine production resume as the compound decays. Additional evidence for the key role of NO released from NONOate was obtained from experiments showing that inactivated NONOate had no effect on viral titers or cytokine generation.

An exciting question that remains to be answered, is how does NO inhibit the virally induced cytokine production and viral replication in epithelial cells. Studies to date have shown that inhibition of cytokine production was observed when NONOate was added only during the viral exposure period, or only after viral exposure, suggesting that NO does not kill the virus directly or prevent the virus from entering the target cells (62). Furthermore, the inhibition of cytokine production occurred without reducing the levels of cytokine mRNA, suggesting that the inhibition was not due to the ability of NO to interfere with transcriptional regulation. The ability of NO to inhibit cytokines that are
generated late in the viral replication cycle may be secondary to the ability of NO to inhibit viral replication, but for cytokines, such as IL-8 and IL-6, that are induced rapidly after infection, the inhibitory effects of NO are not likely to be secondary to the inhibition of viral replication. Precedent exists in other cell types for NO to inhibit protein synthesis (143, 153), possibly by interfering with enzymes involved in energy metabolism (110, 154). Recent studies using [35S]-methionine to label the proteins in epithelial cells have shown that NO-mediated inhibition of virally induced cytokine generation is a selective process and is not a result of nonspecific inhibition of protein synthesis (unpublished observation). Additional studies are underway to identify how NO is inhibiting cytokine protein synthesis in rhinovirus-infected epithelial cells. Further studies are also ongoing to delineate the mechanisms by which NONOate inhibits rhinovirus replication. We have hypothesized that the effects may be due to the ability of NO to activate antiviral pathways such as the double-stranded RNA-dependent protein kinase (155) or RNase L (156), or to inhibit one or more of the cysteine-containing proteases that are critical for viral protein production (47, 157–160).

Summary

Virally induced hyperreactivity has been correlated with elevated numbers of inflammatory cells in the lower airways and the production of proinflammatory cytokines. It has been hypothesized, and supported by in vitro data, that respiratory epithelial cells, the primary target of rhinovirus infections, are an important source of these proinflammatory cytokines. The key to whether or not these virally induced cytokines trigger an asthma attack may depend upon the type of inflammatory cells recruited to the airways. Evidence suggests that eosinophils from asthmatic and symptomatic atopic subjects versus normal subjects differ in responsiveness to chemotactic cytokines such as RANTES and eotaxin that may be produced by infected epithelial cells. Since eosinophils are known to play a key role in asthma, it follows that rhinovirus infections by virtue of their ability to enhance airway eosinophilia in asthmatics could lead to exacerbations of symptoms.

The relationship between viruses and asthma attacks is complex and depends upon multiple factors including viral load, type of infecting virus, and variations in the host antiviral response. Our data demonstrate that NO is a potent inhibitor of both rhinovirus-induced cytokine production and viral replication (62). Furthermore, we have recently demonstrated that rhinovirus infection can induce the expression of iNOS mRNA in primary human epithelial cells (unpublished observation). Poliovirus, another picornavirus, has also been reported to induce iNOS in human cells (161). Inductions of iNOS and the production of NO in vivo may represent an important host response to viral infections (162). Knowing that there is a strong relationship between upper respiratory viral infection, and particularly rhinovirus infections, and asthma exacerbations, we speculate that NO plays an important anti-inflammatory and antiviral role in rhinovirus infections and that nitric oxide donors may represent a novel therapeutic approach for the treatment of colds and their related complications.

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