

REVIEW

# Viruses as precipitants of asthma symptoms

## II. Physiology and mechanisms

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### Introduction

Clinical observation and anecdotal evidence have long indicated a relationship between the development of an upper respiratory tract viral infection (a 'cold') and exacerbations of asthma symptoms. Indeed, many patients relate the start of their disease to a particularly severe bout of 'flu' or a cold. Prospective studies have indicated that asthma attacks are associated with a viral infection in as many as 20–50% of cases [1,2], although the symptoms may sometimes be difficult to distinguish clinically from various forms of rhinitis and hay fever. Epidemiological evidence reviewed in a previous article [3] confirm a close temporal association between exacerbations of asthma and intercurrent viral infections. Unlike bacteria, viruses are associated with wheezing illnesses in populations, in individuals, and in time and are rarely cultured from the airways during asymptomatic periods. The predominant viruses causing wheezing are human rhinovirus (HRV) and respiratory syncytial virus (RSV), although the causative viral agent may vary according to age. Difficulties with the diagnosis of HRV infections are likely to have contributed to a lower reported than actual incidence of this virus and it is possible that this virus plays a more important role in the induction of asthma attacks than is currently appreciated. The mechanisms whereby upper respiratory viruses might induce or contribute to attacks of asthma are currently unknown. It is the purpose of this review to examine the evidence available and to discuss the merits of the various mechanisms so far proposed. A further article will describe some recent advances in the molecular aspects of HRV infection and HRV diagnosis and will discuss briefly some therapeutic implications for the asthmatic patient.

### Possible mechanisms of virus-induced asthma

Although acute viral respiratory illnesses account for up to 53% of acute conditions seen in the USA annually [4],

the study of the relationship between the infecting agent and pulmonary pathophysiological mechanisms is still poorly understood. Evidence has accumulated steadily that viruses may be instrumental in the origin and/or exacerbation of asthma, but investigations have been hampered both by difficulties in establishing a viral origin in patients who manifest clinically significant bronchial symptoms such as cough, wheezing or sputum production, and by difficulties in obtaining endobronchial tissue for studies of the histology and the microanatomical changes associated with viral infections. A relationship between upper airway infection and the simultaneous or subsequent development of lower airway symptoms is often observed in the clinical situation, but how this occurs and how the lower airway abnormalities are caused remain ill-defined. There are many theoretical mechanisms whereby viruses might induce or exacerbate lower airway disease. These may be conveniently divided into (i) those proposing a virus infection that involves both the upper and lower respiratory tracts, and leads directly to the pathophysiological changes observed, and (ii) those proposing a virus infection confined to the upper respiratory tracts, which by indirect or remote means results in pathophysiological changes in the lower respiratory tracts. The mechanisms operating may vary according to the type of virus causing the infection, as well as the characteristics of the host and a combination of many of the proposed mechanisms appears likely. The chief mechanisms so far proposed are summarized in Table 1 and are discussed more fully below.

### Direct effects on the lower airways

#### *Epithelial damage*

Some viral infections are known to cause severe pathological abnormalities in the lower airways and throughout the entire respiratory tract. Influenza A infection induces diffuse inflammation of the bronchi, trachea and larynx with desquamation of ciliated epithelial cells down to a one-cell-thick basal layer [5]. This is accompanied by submucosal oedema coupled to infiltration by neutrophils and mononuclear cells with complete resolution of

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**Table 1.** Proposed mechanisms whereby viral infections may lead to exacerbations of asthma*Direct effects on the lower airways*

- 1 *Epithelial damage*
  - Increased neural sensitivity
  - Increased allergen penetration
  - Reduced ciliary clearance
  - Reduced neutral endopeptidase and increased tachykinins
  - Decreased protective mediators, e.g. EDRF
- 2 *Increase in chemical mediators*
  - Histamine release
  - Arachidonic acid metabolism
  - Kinin generation
- 3 *Increase in cellular driven inflammation*
  - Increased production of cytokines
  - Chemotaxis
  - Cell activation
- 4  *$\beta$  receptor down regulation*
- 5 *Virus specific IgE production*
  - Allergic sensitization
  - Mediator release
- 6 *Antibody dependent enhancement of infection and cytotoxicity*

*Remote mechanisms*

- 1 *Mouth breathing*
  - Reduced nasal filtering of inhaled allergen and increased penetration to lower airway
  - Reduced conditioning of inspired air, low temperature/humidity, bronchospasm
  - Reduced lower airway temperature may favour viral replication
- 2 *Increased circulating mediators/cytokines*
  - Increased bone marrow production and activation of inflammatory cells
- 3 *Increased total IgE*

the epithelial necrosis taking up to 6 weeks. Further studies have demonstrated delay in tracheobronchial clearance for up to 1 month after influenza A infection [6], and ciliary defects in the nasal mucosa of children with infections with influenza types A and B, parainfluenza types 1, 2 and 3, adenovirus, RSV and herpes simplex virus, though not in the single case of rhinovirus infection studied [7]. Rhinoviruses appear to cause little or only patchy epithelial damage [8]. A study with human nasal cultured epithelium demonstrated a marked cytopathic effect followed by destruction of the cell monolayer with both adenovirus and influenza type A infections, while no cytopathic effect was observed with rhinovirus and coronavirus infections, despite active viral replication being demonstrated [9]. In addition, nasal biopsies from

subjects with HRV colds show no definitive histopathological changes, although hyperaemia and exudation of seromucinous fluid is found coupled to cytological changes such as epithelial metaplasia, and is accompanied by impaired mucociliary flow rates [10], a feature reproduced in a separate study [11]. Studies in organ cultures of bovine tracheal epithelium show marked changes in epithelial microstructure after bovine rhinovirus infection [12]. Desquamation of ciliated epithelial cells was evident leaving a smooth epithelial surface with relatively few remaining ciliated cells. This pattern of mucosal damage was also suggested by the increase in ciliated epithelial cells found in nasal secretions after experimental HRV infection in volunteers [13]. There are several mechanisms by which virus induced epithelial damage might result in deleterious effects in asthmatic patients. Exposure of rapid-adapting sensory parasympathetic fibres could cause bronchoconstriction via increased efferent vagal activity [14], a theory supported by Empey *et al.* [15] to explain increased cholinergic responses found in normal subjects after virus infection. Similarly the penetration of allergen into the epithelium would be facilitated both by epithelial stripping, and by reduced ciliary clearance, resulting in easier access of allergen to antigen presenting cells and therefore increased allergic inflammation. The epithelium is also an important source of regulatory proteins or mediators with protective roles, such as epithelium derived relaxant factor [16] or bronchodilator prostaglandins E<sub>2</sub> and I<sub>2</sub>, which may play a role in maintaining bronchial patency [17].

Neuropeptides are thought to play a role in the pathogenesis of asthma [18], and are broken down by neutral endopeptidases found in airway epithelium. Destruction of neutral endopeptidases by virus induced epithelial damage might result in increased levels of neuropeptides, and therefore increased neurogenic inflammation [19].

New evidence also suggests that sequential viral induced parenchymal lung damage may be cumulative as demonstrated after infection of Swiss mice by parainfluenza 1 virus followed by Influenza A 30 days later [20]. Repeated viral infections inducing endobronchial inflammation may conceivably lead to marked structural and functional changes in airway epithelium and submucosal tissues; abnormalities that may not be totally reversible when the infection resolves. In patients with pre-existing airway disease (bronchiectasis, asthma) who may be predisposed to repeated viral infections, this may have devastating long-term consequences.

*Increased cholinergic sensitivity*

Damage to the airway epithelium by viruses has been proposed by Empey *et al.* [15] to explain increased

cholinergic responses in normal subjects after infection because loss of epithelial cells may expose rapid-adapting sensory parasympathetic fibres which could cause bronchoconstriction via increased efferent vagal activity [14]. They demonstrated the development of bronchial hyper-responsiveness to histamine in the infected group which was prevented by pretreatment with inhaled atropine and reversed by a beta-agonist (isoproterenol), suggesting a role for vagal over-activity after viral infection. Further evaluation of this possible mechanism has not been carried out although similar observations were made after vagus nerve transection in parainfluenza-3 infected guinea-pigs [70]. The animals' airways also exhibited increased degrees of obstruction when the transected nerve was stimulated, suggesting that the efferent limb of the vagus nerve was altered along with afferent limb and contributed to the increases in BHR. There have not been any reports linking abnormalities of smooth muscle function in the airways to viral infections.

#### *Release of inflammatory mediators*

The pathological changes described after viral infections bear many similarities to those found in asthma and it is easy to envisage how pre-existing airway inflammation and obstruction could be exacerbated by a further inflammatory stimulus such as viral infection. Release of tissue-damaging substances from leucocytes and other cells may be one mechanism involved in this process.

It was originally thought that respiratory viruses may produce endobronchial inflammation via epithelial denudation, cell death and the resultant release of tissue damaging enzymes (such as lysosomal enzymes). However, recent evidence indicates that viruses may have the capacity to directly induce the release of pro-inflammatory mediators from alveolar macrophages, neutrophils, eosinophils and mast cells and may augment superoxide production.

**Histamine** Several studies have demonstrated that histamine release from leucocytes (including basophils and mast cells), following immunological and non-immunological stimuli, is increased by infection or incubation with a variety of respiratory viruses including influenza A, rhinovirus parainfluenza virus and RSV [21-31]. Recent work with parainfluenza viruses in Brown Norway rats [32] has demonstrated increased release of histamine coupled to a virus-induced increase in bronchiolar mast cells.

Although the exact role of mast cells and basophils in the pathogenesis of airway inflammation is still uncertain, increased release of inflammatory mediators in the airways from mast cells (or basophils) may have a profound

effect in already inflamed airways and contribute to exacerbation of airway obstruction. It has been suggested that basophils migrate from the peripheral circulation to the airways during an attack of asthma [33]. Lett-Brown *et al.* [34] also demonstrated that basophilic chemotactic responses are selectively enhanced after *in vivo* incubation of leucocytes with parainfluenza virus and this observation raises the possibility that viruses may not only increase mediator release but also replenish airway inflammatory cell populations.

**Interferon** An early study by Ida [22] suggested that interferon may be an important factor mediating viral enhancement of histamine release after immunological stimulus. This work was corroborated by Chonmaitree [23] and extended by Busse, who showed that T-cell depletion attenuated the enhanced histamine release from peripheral blood mononuclear cells following incubation with influenza A [24], but that interferon did not alter histamine release after non-immunological stimulation [21]. Viral infections and interferon have also both been shown to increase the biosynthesis of prostaglandins in the macrophage [35].

**Bradykinin** Bradykinin and lysylbradykinin are potent inflammatory mediators causing vasodilatation, increased vascular permeability and glandular secretion and stimulate pain production via neuronal reflexes [36]. Using HRV39 and HRV HH, Naclerio *et al.* [37] have shown that there is a close association between symptoms of a cold and rises in the levels of bradykinin and lysylbradykinin in nasal secretions and that albumin levels increase, probably reflecting increased vascular permeability. Similarly, infection of the bronchial epithelium by HRVs might release bradykinin and lysylbradykinin and explain the frequent occurrence of lower airway symptoms such as cough and wheeze and the development or amplification of BHR. Surprisingly, the levels of histamine and PGD<sub>2</sub> in nasal washes were normal implying that significant mast cell degranulation had not occurred.

**Complement** Influenza viral infections cause considerable tissue damage to the upper and lower airway as evidenced by epithelial desquamation and infiltration of leucocytes and mononuclear cells [5]. Recent investigation has implicated complement, a central mediator of inflammation, in the pathogenesis of influenza viral infections and demonstrated increases in C3a and C5a in human volunteers after infection with Influenza A virus [38]. The binding of complement components to epithelial cells had earlier been described *in vitro* [39] and *in vivo* [40] during infection with RSV, and provides an illustration of the ability of viruses to instigate and propagate inflammation

via the release of chemotactic and cell activating factors from airway cells or via transudation from the bloodstream.

**Lipoxins** Lipoxins (LX) are a newly discovered class of trihydroxy-conjugated tetraene derivatives of arachidonic acid produced in human leucocytes including eosinophils [41] as well as by alveolar macrophages [42]. Biological properties include contraction of lung parenchymal strips [43], activation of protein kinase C [44], decreased natural killer cell activity [45] and superoxide anion production by human leucocytes [46]. Kim [47] has recently demonstrated that alveolar macrophages obtained from rats inoculated with a spectrum of rodent viruses exhibited marked increases in LX production. The author proposed that LX are putative yet undocumented novel inflammatory mediators and immunomodulators associated with viral infections. Recently the presence of LXA<sub>4</sub> was reported in BAL from patients with selected pulmonary diseases including pneumonia and asthma [48].

**Other lipid mediators and superoxide** In the study of Naclerio *et al.* [37], neutrophil numbers were markedly increased in nasal lavages, in keeping with an earlier study of nasal biopsy specimens after HRV infection, showing infiltration of the mucosa with inflammatory cells including abundant neutrophils [49], and with the findings of Levandowski who also found increased numbers of neutrophils and lymphocytes in nasal lavage in the presence of HRV infection [50]. Recent *in vitro* observations have indicated that neutrophils exhibit enhanced adhesion to airway epithelial cells infected with human parainfluenza viruses [51] and recruitment of such cells could contribute further to a perpetuation of inflammation via secretion of lipid mediators, proteases and oxygen radicals. Indeed Faden *et al.* demonstrated increased superoxide production and release of thromboxane B<sub>2</sub> from neutrophils incubated with RSV antibody complexes [52].

#### *Activation of cellular immune responses*

**Antibody dependent enhancement** Viral infections do not lead to marked immunological responses from the body after infection. Although it is known that both humoral and cellular immunity is activated, it is doubtful whether humoral responses are essential for recovery from viral illness as suggested by rises in local and neutralizing antibody after resolution of the clinical illness [4]. Indeed there is evidence to suggest that in RSV infection the severity of infection may be increased by subneutralizing antibody titres, a phenomenon called antibody dependent

enhancement [53]. It is postulated that low levels of antibody insufficient to neutralize the virus will bind and facilitate viral entry into cells, such as macrophages, bearing Fc receptors. The infected macrophage can then promote the spread of the viral infection to other parts of the respiratory tract, in addition to allowing viral replication to take place. Recent studies have provided support for this theory, demonstrating by means of northern blot analysis, the replication of RSV in human alveolar macrophages [54], and showing that asthmatic subjects have higher baseline IgA and IgG, and a greater response of IgA to attenuated influenza infection, than do normal subjects [55]. The same study also postulated a role for the increased levels of antibody in asthmatic subjects in promoting inflammation via eosinophil antibody-dependent cytotoxicity.

**Systemic cellular immune responses** Uncertainty surrounds the role of systemic cellular immune responses after viral infection. Levandowski *et al.* demonstrated decreases in numbers of circulating T-lymphocytes in peripheral blood after HRV infection and proposed that this may be related to recruitment of these cells to the nasal mucosa where they may perform a local immunological function [56], they then went on to demonstrate increased numbers of lymphocytes in the nasal secretions during HRV infection [39]. Other have suggested that this decrease may be related to increased levels of interferon- $\gamma$  [57]. Cytokine production from T-lymphocytes has implied a pivotal role for these cells in the orchestration of inflammatory processes and viruses may exert a profound influence on the induction and modulation of inflammation in the airways via interference with this mechanism. In asthmatics pre-existing endobronchial inflammation may be amplified by viral-induced release of cytokines such as IL-3 and IL-5 from lymphocytes, accounting for eosinophil maturation and recruitment to the bronchial mucosa coupled to IgE increases mediated by IL-4 secretion [58]. Indeed *in vitro* evidence has recently been provided for just such a process, with increased numbers of low density eosinophils being demonstrated after incubation with influenza virus and HRV [59]. It has also recently been shown that RSV induces eosinophil degranulation and release of eosinophil cationic protein in nasopharyngeal secretions from children with bronchiolitis, but not with bacterial pneumonia [60].

In a study combining local antigen challenge and broncho-alveolar lavage (BAL) prior to, during and after HRV16 experimental infection, the viral infection alone did not induce significant inflammatory changes as assessed by BAL in either normal or allergic rhinitic subjects [61]. However, after segmental antigen broncho-provocation there was an increase in eosinophils, protein

exudation and total BAL cell count, in the allergic rhinitic subjects, suggesting that allergic airway inflammation had been virally modulated, possibly by potentiating antigen-induced release of various proinflammatory and chemotactic substances. The mechanisms entailed in these observations are at present speculative but raise the possibility that asthmatic airways may react in a unique fashion to viral infections exhibiting a greater tendency to amplification of inflammatory bronchial events when challenged with antigen.

**Cytokines** Support for this hypothesis has come from *in vitro* studies by Hsia *et al.* [62] of peripheral blood mononuclear cells after experimental human infection with an unnumbered HRV. The cellular immune response was evaluated by measuring IL-2 and IFN- $\gamma$  production after mitogen stimulation, assessment of natural killer cell activity and via specific antigen stimulation blastogenesis measured at baseline, on days 1–5 of virus infection and after resolution of infection. IL-2 production increased over baseline by a factor of 4 and was inversely related to the duration of virus shedding. Interestingly, the level of increase in IL-2 was unrelated to total symptom scores or convalescent antibody titres. IFN- $\gamma$  production was also increased but to a much smaller extent compared to baseline (factor of 2), whilst natural killer cell cytolytic activity was also amplified. [ $^3$ H]thymidine incorporation by cells was increased in the presence of HRV antigen and directly related to mucus production and days of viral shedding. The results suggest an important role for at least one cytokine (IL-2) in viral infections and further studies may extend this to other products of lymphocyte and macrophage activation. Lymphocyte activation as measured by blastogenic responses has been found in other studies [63] and may be a marker for the activation of viral specific cellular immune responses contributing to symptoms as well as inflammatory sequelae of this type of infection. An earlier study by Welliver and colleagues found increased lymphocyte activation in subjects who wheezed during the 6 months after infection with RSV, when compared to subjects with no wheeze after RSV infection [64].

#### Decreased $\beta$ -receptor function

Viruses may contribute to the development of wheezing in asthmatic patients partly as a result of a decrease in  $\beta$ -adrenergic function. Sympathetic  $\beta$ -receptors are found on airway smooth muscle as well as on circulating leucocytes and stimulation of adrenergic airway receptors opposes parasympathetic effects on the airways. An imbalance of this mechanism has been proposed as being a fundamental abnormality in asthma [65]. It has been

shown that asthmatics exhibit abnormal metabolic [66,67] and physiological [68] responses to adrenergic stimulation and it has been postulated that viruses may exacerbate this abnormality. Busse and colleagues [69] in a study of *in vitro* leucocyte responses after upper respiratory viral infection, reported decreased leucocyte  $\beta$ -adrenergic receptor function and proposed that this may apply to the identical receptors on bronchial smooth muscle. An additional effect of  $\beta$ -agonists on leucocytes is a decrease of lysosomal enzyme release and diminished  $\beta$ -adrenergic function may thus lead to inflammatory effects related to amplified liberation of tissue-damaging enzymes from dysfunctional neutrophils.

The above observations were confirmed by Buckner *et al.* [70] who used parainfluenza infected guinea-pigs to show a decrease in sensitivity to  $\beta$ -agonist medication after viral infection in relation to antigen challenge but not to histamine or carbachol. The mechanisms involved in this reduced sensitivity were investigated in mice, and found to be a result of attenuation of receptor and post receptor activation of adenylate cyclase activity [71]. The clinical relevance of such observations are still uncertain and further comprehensive studies of  $\beta$ -receptor function in the airways of normal and asthmatic subjects are required to enable extrapolation of these *in vitro* observations to human airways.

#### Synthesis of viral specific IgE

The work of Welliver *et al.* [72,73] demonstrated that RSV infections induced the production of virus specific IgE bound to exfoliated respiratory epithelial cells and that the magnitude of this response correlated with subsequent wheezing, increased histamine in nasal secretions and the degree of arterial hypoxia. Children with a genetic atopic disposition appeared to develop elevated specific IgE antibody more readily and the authors speculated that viruses may induce asthma and other allergic disease on the basis of this genetically predetermined phenomenon. Further prospective studies have strengthened this impression, showing that only 28% of subjects with undetectable IgE to RSV had subsequent wheezing, whilst 70% with elevated RSV-IgE wheezed at a later stage [74]. The same workers have also demonstrated the production of specific IgE to parainfluenza virus [75] and its relation to the severity of croup, wheezing and the levels of histamine in the nasal secretions, while other workers have demonstrated the production of high titres of specific IgE in *Mycoplasma pneumoniae* infections in asthmatic or allergic subjects [76].

Increased production of IgE induced by viral infections has significant implications in atopic diseases. Mast cell bound IgE will induce degranulation and mediator

release on cross-linking of antibody by antigen. Recent studies have suggested a pivotal role for the mast cell in inflammatory processes via the release of mediators [77] and the production of various cytokines [78], all of which may lead to an amplification of inflammation in the already diseased airways of asthmatics who develop viral infections. The development of a seemingly innocent 'cold' in a genetically predisposed individual may set in motion a sequence of events that cause the emergence of a spectrum of allergic symptoms including severe life-threatening airway obstruction. Viral-specific IgE production may be a factor linking these events.

### Remote mechanisms

#### *Nasal blockage and mouth breathing*

Nasal blockage is a major symptom of upper respiratory viral infection, and is frequently severe enough to result in prolonged periods of enforced mouth breathing, particularly so in subjects with pre-existing rhinitis. Mouth breathing allows inspired air to pass directly into the lower respiratory tract without undergoing the normal conditioning performed by the nasal mucosa, resulting in the delivery of air at lower temperatures and lower humidity. These factors are known to play a role in the genesis of bronchospasm in exercise induced asthma, and may therefore play a role in the genesis of lower airway obstruction in viral upper respiratory infections. In addition to conditioning inspired air, the nasal mucosa also acts as a filter reducing the load of inhaled particles delivered to the lower airway. The absence of this protective mechanism in upper respiratory infections may increase the penetrance of inhaled allergen to the lower airway, resulting in further bronchospasm in those sensitized to the allergen. Neither of these two mechanisms have been extensively investigated, but both may play a role in the precipitation of asthma symptoms during upper respiratory viral illness.

#### *Increase in total IgE secretion*

Studies by Frick *et al.* [79,80] have suggested an IgE immunoregulatory role for viral respiratory infections in children who are genetically prone to allergy and to increased production of IgE. They found a strong temporal association between infection with parainfluenza 3 virus and RSV and the development of symptoms and signs of allergy coupled to sharp rises in total IgE (< 5–50 U/ml within 3 months). Animal studies in dogs have also suggested that allergic manifestations such as facial dermatitis as well as IgE increases develop more frequently in dogs receiving viral vaccination compared to controls [79]. A similar study in mice showed

that allergen challenge prior to, or after recovery from influenza A infection resulted in no detectable IgE production, while similar challenge during the acute phase of the illness resulted in significant IgE production [18].

Transient modulation of the ratios of T-lymphocyte subsets has been demonstrated after booster immunization of live attenuated measles virus in humans with increases in T-helper cells as well as IgE levels [82], and it is feasible that this was mediated by increased IL-4 production from the TH<sub>2</sub> subset of the T-helper cell population [83]. An early study by Saryan *et al.* [84] suggested that a factor secreted by T cells from atopic individuals induced IgE synthesis in normal B-cells after incubation in culture. This 'inducer' was not present in cells from normal subjects and may reflect IL-4 mediated preferential IgE production resulting from an imbalance between the TH<sub>1</sub> and TH<sub>2</sub> subsets (possibly exacerbated by viral infection) in subjects with atopy.

#### *Release of pro-inflammatory mediators into the circulation*

Viral respiratory illnesses are frequently accompanied by fever and a biphasic response in the blood total leucocyte and lymphocyte counts, consisting of an initial fall and then a rise. It is postulated that these effects may be mediated by a release into the general circulation of locally produced cytokines, resulting in the systemic symptoms of viral illness such as fever, fatigue, myalgia and headache. The other well known effects of many cytokines include increasing bone marrow production, and release into the circulation with activation of many different inflammatory leucocytes, resulting in a systemic up-regulation of inflammatory responses. Patients with an inflammatory illness such as asthma may therefore suffer an exacerbation of their disease as a result of this up-regulation. However, as the levels of cytokines released into the general circulation are too small to be detected by currently available methods, there is no firm evidence to support this mechanism.

#### **Evidence for invasion of the lower respiratory tract by HRV**

It is well accepted that viruses such as Influenza, Parainfluenza, RSV and Adenovirus cause lower respiratory tract disease including pneumonia and bronchiolitis. Recent interest in respiratory viruses has centred on HRV, of which more than 100 serotypes have now been described, and which are reported to cause 40–50% of all acute colds [4]. Clinical infection is characterized by the typical symptoms of a cold which is indistinguishable from that caused by other viruses and lower respiratory symptoms are frequently present, especially cough which

was present in 86% [85], 71% [86] and 40% [87] of normal subjects with naturally occurring infection. Further proof of the importance of this group of viruses has come from experimental viral infection studies which have demonstrated the development of late asthmatic responses in a group of atopic subjects [88], in addition to epidemiological studies that have strongly implicated HRV in exacerbations of asthma and as a cause of 'wheezy bronchitis' in children [1,2,89].

There is as yet no conclusive proof of the presence of HRV in the lower airways in epithelial or alveolar cells although Halperin *et al.* [90] did culture HRV from tracheal secretions obtained bronchoscopically; they could not exclude the possibility of contamination during instrumentation of the upper airway. Busse *et al.* [61] performed bronchoalveolar lavage (BAL) in six normal and seven allergic rhinitic subjects before, during and after experimentally induced HRV16 infection. BAL fluid analysis at all three stages showed no evidence of gross epithelial damage or of other inflammatory changes, although total protein in the BAL was significantly increased after infection with HRV16. Because the findings in BAL fluid may not reflect tissue events accurately and the cellular changes in the mucosa may be very mild, further interpretation will require careful biopsy-based studies to demonstrate HRV genomic presence as well as the histological characteristics of this infection in the lower respiratory tract.

Further evidence for lower airway HRV infection has come from Horn *et al.* [2] who investigated sputum, nasal and throat swabs from 22 children during 72 episodes of wheezy bronchitis, and showed that HRVs could be isolated in 49% of all episodes and in 64% of episodes of severe illness requiring corticosteroids. Viral cultures from sputum were more often positive than either nose or throat swabs suggesting that viral replication had occurred in the lower airways. Cytopathic effects (CPE) developed more quickly in cultures from expectorated sputum as compared to upper airway samples implying that more virus was present in the lower airways (though had they compared nasal aspirate samples rather than swabs, with sputum, they may have found no differences) and that detection in sputum was not simply due to contamination by upper airway secretions. Rhinoviruses were predominant and could be cultured throughout the study, a finding that may reflect their importance as respiratory pathogens as well as technical expertise and experience in the culture of this fastidious group of viruses. However, this work only presents indirect evidence for involvement of the lower airways via direct mucosal invasion by respiratory viruses. Optimal temperatures for growth of HRV are 33°C, the temperature of the nasal mucosa, and this has been advanced as an

argument to support the possibility of infection being localized exclusively to the upper airways. Recent descriptions of air temperatures of less than 37°C in the tracheobronchial tree under certain breathing conditions would be a means for providing a suitable environment for viral replication in healthy subjects [91], and in asthmatics.

Post-mortem studies have demonstrated that HRVs are able to infect patients with compromised immunity as in a patient with myelomatosis in whom RV13 was recovered from three specimens of lung tissue [92]. Although HRV13 was cultured with demonstration of prominent CPE in human diploid cells, no specific histological changes attributable to the viral infection was observed in tissue sections. Rhinovirus 47 was cultured post-mortem from an 11-month-old infant with a history suggestive of bronchial asthma who had died suddenly at night [93]. The findings of the rest of the post-mortem examination were consistent with death due to hypoxia and the authors postulated that sudden severe airway obstruction associated with asthma may have been precipitated by the coexistent viral infection. Thus, from these reports it seems likely that HRVs as well as other viruses can invade the respiratory epithelium. Other viruses are also able to infect lower airway cells in subjects with compromised immunity as demonstrated by Krueger *et al.* [94] in subjects with AIDS. Transbronchial biopsies stained positive for human herpes virus-6 (HHV-6) with the monoclonal antibody D12 against HHV-6 P41 and this was confirmed by *in situ* hybridization. Cells predominantly affected were cuboidal epithelial cells of apparently terminal bronchioli.

The importance of the actual presence of HRV or other viral genomic material in the lower airways is obvious. Mechanisms for the induction of endobronchial inflammation and tissue damage by a direct effect of cytokine production, release of proinflammatory mediators and locally increased IgE production are all feasible if the virus is present within lower airways cells. If not, other mechanisms have to be postulated including reflex neurogenic effects and systemic immunological changes that secondarily affect the lung. Recent developments in immunohistochemistry and *in situ* hybridization will allow carefully controlled biopsy studies in different groups of pulmonary pathologies to explore these possibilities and clarify viral-lower airway interactions.

#### **Respiratory viruses may contribute to the development of bronchial hyperresponsiveness**

Non-specific bronchial hyperresponsiveness (BHR) may be defined as an increase above normal in both the ease and the magnitude of airway narrowing on exposure to a

**Table 2** Airway reactivity in studies of experimental rhinoviral respiratory tract infection

Study	Subjects	Virus	Challenge agent	Severe cold	BHR present
Bush [106]	7 Normal	RV16	M	0/7	No
Halperin [104]	9 Normal 10 Atopic	RVHH	H	0/13	3/13
Jenkins [103]	21 Normal 10 Atopic 13 Asthmatic	Wild type RV6/15	H	7/15	4/15
Halperin [90]	21 Asthmatic	RV39 RVHH	H	9/19	4/19
Lemanske [88]	10 Atopic	RV16	H Allergen	5/10	9/10
Summers [107]	16 Normal 11 Atopic	RV2 RV EL	H Bradykinin	6/20	0/20

H, histamine. M, methacholine. RV, rhinovirus.

number of non-sensitizing bronchoconstrictive stimuli [95]. The role of measurements of BHR in the diagnosis and follow-up of asthma is controversial [96,97], although it is widely accepted that it may be useful as a non-invasive means to assess bronchial inflammation and to assist in the diagnosis of occupational asthma [98]. Although the specific mechanisms responsible for exacerbations of asthma during viral upper respiratory tract infections (URTIs) have not been defined, the development or amplification of BHR may represent a pathway whereby lower respiratory tract symptoms such as wheezing and cough appear in normal subjects and increase in severity in asthmatic patients. This possibility has been examined in studies that assessed BHR in relation to viral URTIs, and although results have varied as a result of differing methodologies, evidence indicates that viral URTIs can induce BHR in normal people and aggravate it in those in whom BHR is already present.

#### Animal studies

Early work *in vivo* in guinea pigs using parainfluenza type 3 (P-3) did not find an alteration by this virus of isolated guinea pig airway contractile responses to carbachol, histamine or specific antigen [99]. However, substance P release following capsaicin inhalation enhanced airway responses in P-3 infected but not uninfected animals [100], and Dusser *et al.* [101] suggested that this effect may be mediated by virus-induced decreases in neutral endopeptidase, the enzyme responsible for degradation of substance P. This tachykinin and others may be involved in the evolution of 'neurogenic inflammation', a process that

may amplify existing allergic inflammation. Recently it has been shown that the severity of the P-3 infection in guinea pigs is an important factor determining the degree of BHR that will develop in this model [102]. Similar studies have not been performed using other cold viruses and additional investigations will indicate the role of tachykinins and their catabolism in the mucosa in virus-associated BHR increases and reversible airway obstruction.

#### Rhinovirus studies in humans

Studies in human subjects have studied the effects of various viruses although most investigators have concentrated on HRVs. Empey *et al.* [15] conducted a prospective study in normal adult subjects and demonstrated increases in BHR after wild-type viral infection presumed to be mostly rhinoviral in origin. Airway reactivity to histamine was increased at the time of clinical viral infection and could be blocked by the use of anticholinergic premedication, leading the authors to postulate that neural mechanisms are implicated in the pathogenesis of BHR increases. A similar study by Jenkins and Breslin [103] in normal and asthmatic subjects failed to confirm the above earlier findings, although individual responses suggested some increases in BHR; however such increases were not significant for the group as a whole.

Other experimental studies *in vivo* have assessed BHR after infection with HRV (Table 2). Halperin *et al.* [104] first studied normal and atopic volunteers infected with HRV HH and followed this up with a study in 23



**Table 3.** Reasons for variable increases in bronchial responsiveness following experimental viral inoculation in human subjects

1	Size of the inoculum
2	Inoculation protocol: nasal drops/nasal drops and aerosol
3	Subjects' pulmonary status: normal/atopic/asthmatic/smoker
4	Variable background bronchial inflammation and effects of steroid medication
5	Presence/absence of neutralizing antibody and the development of mild/moderate/severe cold
6	Different viral strains—? differing pathogenicity/tropism
7	Different bronchial provocation methods: histamine/methacholine/allergen/bradykinin
8	Pulmonary function measurements: proximal vs peripheral airways

asthmatic subjects [90]. They used small doses of inoculum (6–20 tissue culture infective dose—50% [TCID<sub>50</sub>]) and none of the patients in the first study and only nine out of 21 subjects in the later study had severe colds as assessed by their symptom scores. No overall increases in BHR were demonstrated, although four asthmatics (out of 21 infected) exhibited a decrease in FEV<sub>1</sub>, coupled to increases in BHR and a frequency of wheezing similar to that found in previous studies of asthma exacerbations associated with viral infections [105]. Significant increases in BHR did occur and the results were interpreted by the authors as suggesting that not all HRVs induce exacerbations of disease in asthmatics and that the role of viral infections in adult wheezing remains uncertain.

Other workers have suggested that alternative mechanisms may be operative in normal versus atopic subjects. An early study of HRV16 infection in normal volunteers could not demonstrate significant changes in BHR, although again all subjects had only mild colds [106]. Using HRV16, this time in 10 atopic rhinitics, a later study from this group demonstrated increases in histamine and antigen responsiveness which persisted for at least 4 weeks [88]. In addition, late asthmatic reactions (LARs) developed in 8 of 10 subjects during the period of HRV16 infectivity independently of the development of BHR during the cold. The presence of an atopic diathesis thus appeared to render subjects more vulnerable. These findings may be of particular importance for a large susceptible population of atopic asthmatics who could be at particular risk to develop severe exacerbations of asthma after a preceding bout of 'cold and flu'. The relevant pathophysiological mechanisms underlying these results remain to be explored.

Viruses including HRVs may damage the epithelium of the airways and expose sensory nerve-endings [14], cause loss of possible protective mediators such as prosta-

glandins E<sub>2</sub> and I<sub>2</sub> and epithelium derived relaxant factors [16] and loss of neutral endopeptidases with a local increase in tachykinins [19]. Summers *et al.* [107] theorized that this possibility may be reflected by an increase in BHR to a probable sensory nerve stimulant such as bradykinin [108]. Twenty-seven normal and atopic subjects were infected with HRV2 or HRV EL; 20 developed mild colds but no increases in BHR to either histamine or bradykinin. Even in six subjects who responded with a more severe 'clinical' cold there were no increases noted on challenge and the findings of Busse *et al.* [88] could not be corroborated. However, the negative results may be explained by some or all of the following factors: low infecting doses coupled to a different inoculation protocol were applied, different strains of HRVs were used, subjects developed only mild colds and tachyphylaxis may have developed to endogenous bradykinin.

#### *Other viruses and vaccination studies*

Studies using Influenza A virus have demonstrated the development of BHR more predictably in normal subjects [109,110] and asthmatics [110]. Clearly there are not only variations in potency within a virus strain such as HRVs, but also differences between individual viruses of different groups. Influenza A causes marked epithelial damage which may result in a more marked tendency for BHR to develop; a situation that may be more pronounced with wild type strains. What is not clear is whether comparable mechanisms determine pulmonary responses to the various types of viruses or whether alternative mechanisms are activated by certain strains. Comparative studies of BHR induced by individual viruses have not been performed and may give some indication of the distinctive inherent bronchial pathogenicity of some strains.

Early studies of killed Influenza vaccines demonstrated increased methacholine sensitivity in asthmatic but not in normal individuals [111] after vaccination. Live attenuated Influenza A vaccine increased histamine induced changes in specific airway conductance 3 days after inoculation; again this response could be abrogated by pretreatment with beta-agonist and anticholinergic medication [110]. These observations could not be confirmed by a later study [112] using another attenuated strain and a different protocol; inoculation was followed by very mild symptoms in only one subject and no symptoms were present in the other 12 who had serological evidence of infection. In additional studies, use of homologous Influenza A virus (which is more similar to wild type infection) demonstrated increased BHR in response to histamine provocation with maximal responses noted on the second day after infection; this had disappeared by the

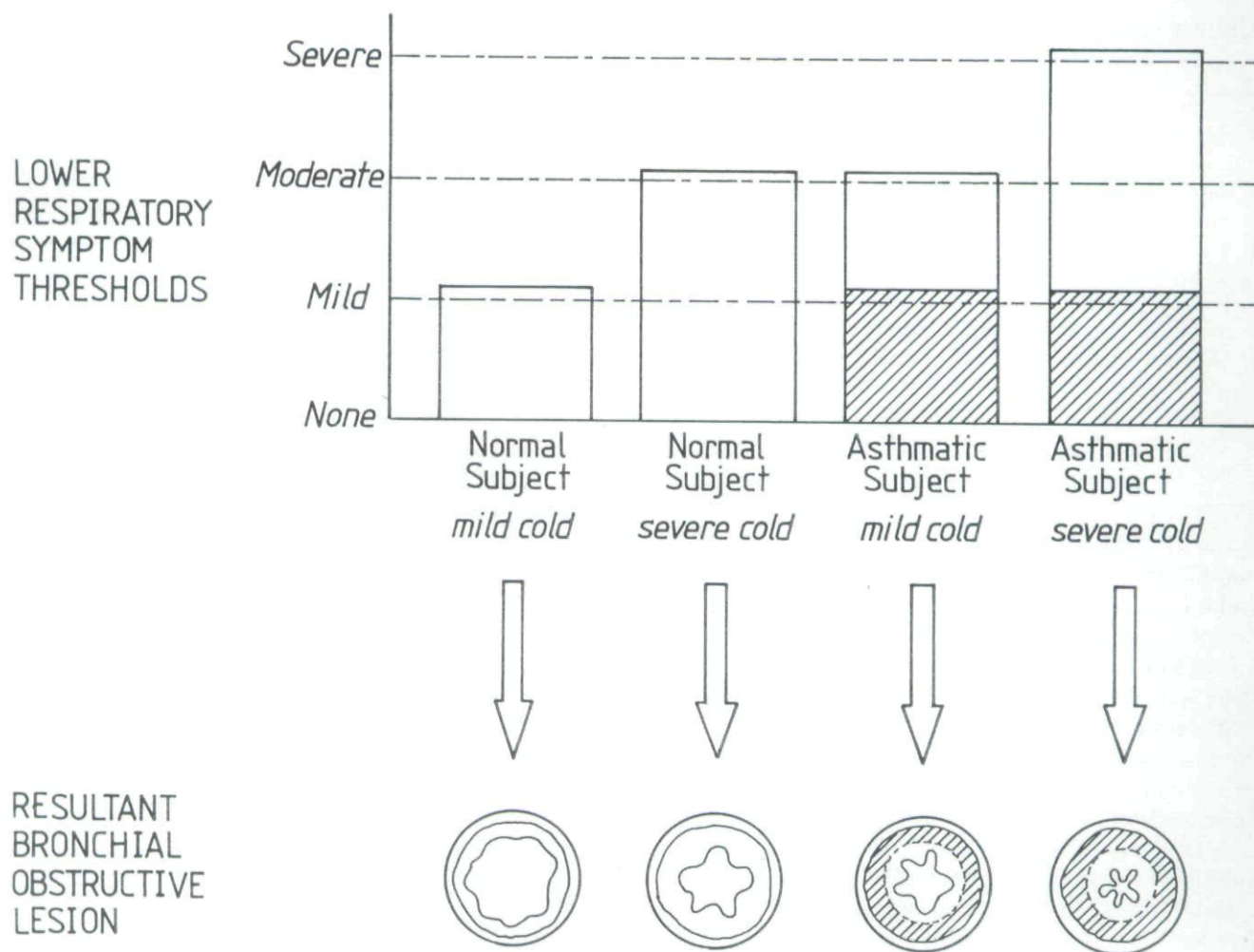


Fig. 1. Diagram of the additive effects of inflammation from a viral infection and inflammation from underlying asthma, in relation to various symptom thresholds, and the resultant obstructive lesion in the lower airway. □, inflammation from cold. ▨, inflammation from asthma.

tenth day [109]. Significantly, inoculation had been done by aerosol as well as with nasal drops making lower respiratory tract deposition of virus more likely. Respiratory signs and symptoms were notably absent in spite of increases in BHR. Such increases in BHR may not be restricted to respiratory viruses as suggested by a study of live measles vaccination in children with asthma which indicated that airway responses to methacholine and allergen were amplified; a finding that had disappeared on rechallenge 9 weeks later [113].

The varying degrees of change in BHR reported from different studies of experimental HRV infection can be explained by large differences in design, differences in instilled doses and inoculation protocols and host factors such as age and the presence or absence of atopy (Table

3). The absence of significant lower airway symptoms in the negative studies may be related to the absence of increased BHR after infection, because there is a direct relationship between such symptoms and the clinical severity of HRV infection [1]. The magnitude of changes in BHR may thus be affected by the severity of viral infection as reflected by the presence of symptoms such as cough, wheeze and chest tightness.

For anatomical reasons epithelial damage to the airways may only cause significant impairment of function in small airways; this may only be reflected by changes in reactivity as assessed by specific measurements of small airway function. A study by Picken *et al.* [114] supported this possibility demonstrating changes in frequency dependent compliance after viral infection.

Measurements of parameters reflecting changes in small airways in subjects with only mild viral infection may therefore demonstrate increases in BHR that would not otherwise be observed.

### Summary

The upper and lower airways have complimentary roles in the ultimate object of supplying the body with oxygen whilst removing waste products of metabolism. Pathology in one area may trigger a response in another, the physiology of which, in the case of virus-induced asthma exacerbations remains poorly characterized. Viral infection of the upper airways by common cold viruses frequently triggers a response in the lower airways leading to prolonged morbidity, especially in subjects with significant pre-existing airway disease. The induction or amplification of BHR may be an important mechanism whereby asthmatic symptoms are produced although the cellular and tissue events or reflex mechanisms activated by viral illnesses and underlying BHR changes are poorly defined and may be dependent on the type and the severity of infection.

Children and asthmatics tend to develop frequent colds setting in motion a sequence of events culminating in airway obstruction and symptoms of wheezing, coughing and chest tightness. This may reflect independent inflammatory changes caused by a simply additive effect of viral damage to the mucosa superimposed upon pre-existing allergic inflammation (Fig. 1). Few if any symptoms will develop in normal subjects with a mild cold whereas significant symptoms may ensue if the cold is severe and induces marked lower airway swelling, secretions and smooth muscle contraction; pathology to which children who have small calibre airways may be particularly susceptible. In asthmatics even a mild cold frequently induces exacerbation of symptoms, while serious life-threatening asthma attacks may occur associated with a severe cold. Some studies have suggested that this effect is not only additive but also synergistic and brought about by release of the mediators already present in increased quantities, the induction of IgE synthesis, or by the potentiation of neural and epithelial damage. The combined effect of both asthma and viruses may thus be amplified and result in a sustained and refractory period of airway obstruction, severe symptoms and unstable asthma.

As most hospital admissions for asthma occur over the winter months and soon after the start of the school terms [115], spread of viruses through the community to susceptible individuals may be the single most important cause of sustained exacerbations of asthma. Definition of the pathological and physiological mechanisms involved

will lead to better understanding and may thus provide a basis for prevention and the development of effective forms of treatment for virus-induced asthma.

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