Infections

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A common feature of asthma and COPD is the important role of infection in triggering exacerbations. Infections have also been implicated in the etiology of the two diseases. This chapter reviews the epidemiological evidence implicating infectious pathogens as triggers and will discuss the mechanisms of interaction between the host–pathogen response and preexisting airway pathology that result in an exacerbation.

ASTHMA

Asthma affects 20–33% of children in the UK.[1] It is a multifaceted syndrome involving atopy, bronchial hyperreactivity and IgE and non-IgE mediated acute and chronic immune responses. The asthmatic airway is characterized by an infiltrate of eosinophils and of T lymphocytes expressing the type 2 cytokines IL-4, IL-5 and IL-13. Trigger factors associated with acute exacerbations of asthma include exposure to environmental allergens, especially animals, molds, pollens and mites, cold, exercise and drugs. The link between respiratory infection and asthma exacerbations is well established, although incompletely understood. In the 1950s this association was attributed to bacterial allergy,[2] but it is now clear that the majority of exacerbations are due to viral rather than bacterial infection.

Epidemiology

Viral respiratory tract infections are a major cause of wheezing in infants and in adult patients with asthma. Their role may have been underestimated in early epidemiological studies because of difficulties in isolation and identification.[3] The introduction of PCR to such studies has implicated viral infection in the majority of asthma exacerbations.

Indirect evidence from population studies has established a significant correlation between the seasonal variation in wheezing episodes in young children and peaks of virus identification.[4] Seasonal patterns of identification of respiratory viruses are associated with peaks in hospital admissions for both children and adults with asthma indicating a role for such infections in severe asthma attacks.[5] Direct evidence implicating viral infection in asthma exacerbations has been provided by studies showing an increased rate of virus detection in individuals suffering asthma attacks. Viruses have been detected in 10–85% of asthma exacerbations in children[4,6–8] and in 10–44% in adults.[9,10] The highest rates of identification are in those studies where subjects were followed prospectively allowing collection of clinical specimens early in the course of the illness, where PCR-based methods of diagnosis were used in addition to serology and culture, and where the methodology used allowed for detection of rhinoviruses. The rate of detection of viruses between exacerbations when individuals are asymptomatic is only of the order of 3–12%. In contrast a study of transtracheal aspirates in adult asthmatics during exacerbations[11] yielded sparse bacterial cultures with no correlation to clinical illness and no difference from those of normal subjects.

In almost all studies of asthmatics the predominant viruses are rhinoviruses (RV), RSV and parainfluenza viruses. RV alone are detected in around 50% of virus-induced asthma attacks. Adenoviruses, enteroviruses and coronaviruses are also detected but less frequently. Influenza is only found during annual epidemics.

Experimental virus infection

The effects of respiratory virus infection in the nasal mucosa and upper respiratory tract have been extensively investigated. More recently the effects of such viruses in the lower respiratory tract have been studied, but detailed knowledge of the pathogenetic mechanisms involved remains limited. Experimental respiratory virus infection in human volunteers is limited by concerns of safety.[12] Most studies have therefore focused on the experimental innoculation of rhinovirus in allergic rhinitic or mild asthmatic individuals or normal control subjects.[13–25] Such studies provide a useful model of natural virus infection in asthma and offer the advantages of patient selection and monitoring, under controlled conditions before, during and after infection, of RV-induced effects including asthma symptomatology, changes in the use of medication, lung function and airway pathology/immunology.

In general, the clinical, physiological and cellular responses to experimental RV infection in asthma are

relatively mild and do not necessarily mimic exactly the events after a natural common cold. It has been suggested that this requires a more complex model including both virus infection and preexisting increase in allergic airway inflammation. Indeed, recent epidemiological evidence confirms a synergistic interaction between virus infection and allergen exposure in precipitating hospital admissions for asthma. Other trigger factors that may interact with infection include air pollution. A study of asthmatic children demonstrated an increased risk of developing an asthmatic episode within 7 days of an upper respiratory tract infection if the nitrogen dioxide level was greater than 28 µg/m³.[26]

Most studies of experimental virus infection in allergic subjects are performed outside the relevant season for allergen exposure. One recent attempt to provide a model combining allergen exposure and virus infection utilized RV infection in subjects with allergic rhinitis. Individuals received three high-dose allergen challenges in the week prior to innoculation to try to mimic combined allergen exposure and virus infection.[27] Interestingly, prior allergen challenge in this model, somewhat unexpectedly, appeared to protect against a RV cold with delayed nasal leukocytosis, delayed rise of the pro-inflammatory cytokines IL-6 and IL-8 and a delayed, less severe clinical course. There was an inverse correlation between nasal lavage eosinophilia and the severity of cold symptoms. The explanation proposed by the authors of this study is that limited high-dose allergen challenge may not reproduce the effects of chronic low-dose allergen exposure and may stimulate the production of anti-inflammatory mediators such as IL-10, or antiviral cytokines such as IFN-γ or TNF-α. Further development of models of combined allergen exposure and virus infection is clearly required.

Mechanisms of virus-induced asthma exacerbations

The interaction of respiratory virus infection and chronic asthmatic airway inflammation results in respiratory symptoms that are more severe than those suffered by nonasthmatic individuals. The detailed immunological mechanisms underlying this interaction are currently unclear. Current hypotheses for the pathogenesis of virus-induced asthma exacerbations are summarized in **Table 39.1.** The disease syndrome following infection by virus is a consequence both of direct harmful effects of the virus itself and of immunopathology resulting from the host–immune response. In an asthmatic individual, exacerbation may occur because of functional interaction between viral pathology and asthmatic pathology, i.e. through different mechanisms with the same end effect on function, or by sharing the same pathogenetic mechanism in an additive or even in a synergistic fashion. Preexisting asthmatic inflammation might interfere with an effective anti-viral response and thus allow the virus itself to cause increased airway damage. Alternatively, virus infection might increase the sensitivity of the asthmatic airway to trigger factors, such as allergen exposure. In fact, it is likely that virus-induced asthma exacerbations occur because of a combination of

these types of interaction. The increased severity of symptoms, including lower respiratory symptoms, seen in subjects with allergic rhinitis, but without asthma, during viral infection suggests that the atopic phenotype itself is important in determining the clinical syndrome following infection by respiratory viruses. Alternatively, it is possible that virus infection in some way amplifies subclinical allergic lower airway inflammation already present prior to infection.

Table 39.1 summarizes some of the current hypotheses proposed to explain the mechanisms of exacerbation of asthma following respiratory virus infection. The evidence supporting these hypotheses is reviewed in detail below.

Rhinovirus infection of the lower airway

Whereas other respiratory viruses such as influenza, parinfluenza, RSV and adenovirus are well recognized causes of lower airway syndromes, such as pneumonia and bronchiolitis and are capable of replication in the lower airway, until recently there was uncertainty as to whether RV infection occurred in the lower airway, as well as in the upper respiratory tract. Although the possibility of nasopharyngeal contamination cannot be ruled out, RV has been detected in lower airway clinical specimens such as sputum,[28] tracheal brushings[22] and BAL[29] by both RT-PCR and culture. RV has

Table 39.1. Current hypotheses for the pathogenesis of virus-induced asthma exacerbations

Epithelial disruption	Reduced ciliary clearance
	Increased permeability
	Loss of protective functions
Mediator production	Kinins
	Complement
	Arachidonic acid metabolites
	Nitric oxide
	Reactive oxygen products
Induction of inflammation	Cytokines
	Chemokines
	Immune cell activation
	Adhesion molecule induction
IgE dysregulation	Increased total IgE
	Antiviral IgE production
Airway remodeling	Airway smooth muscle
	Fibroblasts
	Myofibroblasts
	Growth factors
Alterations of neural responses	Increased cholinergic sensitivity
	Neuropeptide metabolism modulation
	β-Adrenergic receptor dysfunction

been cultured in cell lines of bronchial epithelial cell origin[30] and replication has been demonstrated in primary cultures of bronchial epithelial cells.[31,32] The preference of RV for culture at 33°C rather than 37°C has been used as an argument against lower airway infection, but there is now evidence that replication does occur at lower airway temperatures.[33] Finally the use of *in situ* hybridization has demonstrated RV in bronchial biopsies of subjects following experimental infection.[31] These data confirm that RV infection of the lower airway does occur and directly implicate lower airway infection in the pathogenesis of asthma exacerbations.

Physiological effects of experimental rhinovirus infection

Subjects with asthma and/or allergic rhinitis exhibit increased pathophysiological effects as a result of RV infection as compared with nonatopic nonasthmatic controls. With detailed monitoring, it is possible to detect reductions in both peak flow[34] and home recordings of FEV_1 in atopic asthmatic patients in the acute phase of experimental RV16 infection.[20] There is an enhanced sensitivity to histamine and allergen challenge after RV16 innoculation in nonasthmatic atopic rhinitic subjects.[15,25] RV16 increases asthma symptoms, coinciding with an increase in the maximal bronchoconstrictive response to methacholine up to 15 days after infection.[16] There is a significant increase in sensitivity to histamine in asthmatic subjects after RV16 infection, most pronounced in those with severe cold symptoms.[21]

Effects of viruses on airway epithelial cells

Respiratory viruses enter into and replicate within epithelial cells lining the lower airway. Entry is dependent on interaction with specific receptors, for example the adhesion molecule ICAM-1 in the case of the major group rhinoviruses and the LDL receptor in the case of the minor group rhinoviruses. Influenza viruses bind sialic acid residues via hemaglutinin.

The extent of epithelial cell destruction observed in the airway varies according to virus type. Influenza typically causes extensive necrosis,[35] whereas RV causes little or only patchy damage. Destruction of epithelial cells results in an increase in epithelial permeability, increased penetration of irritants and allergens, and exposure of the extensive network of afferent nerve fibers. These effects may contribute to the increased bronchial hyperresponsiveness induced by virus infection.[16]

There is increasing evidence that the epithelium does not simply act as a physical barrier but has important regulatory roles. Epithelial cells contribute to the immune response following virus infection through the production of cytokines and chemokines (**Fig. 39.1**). They may also act as antigen presenting cells particularly during secondary respiratory viral infections. Epithelial cells express MHC class I and the costimulatory molecules B7-1 and B7-2 and this expression is upregulated *in vitro* by RV16.[36]

Inflammation is a central event both in asthma and in viral infections. The processes involved include interacting cascades from the complement, coagulation, fibrinolytic and kinin systems of the plasma, as well as cell-derived cytokines, chemokines and arachidonic acid metabolites. Our understanding of the interaction of viruses with these cascades in asthma is incomplete and it is likely that different viruses interact with each system to different extents. However, it is reasonable to believe that in all cases the
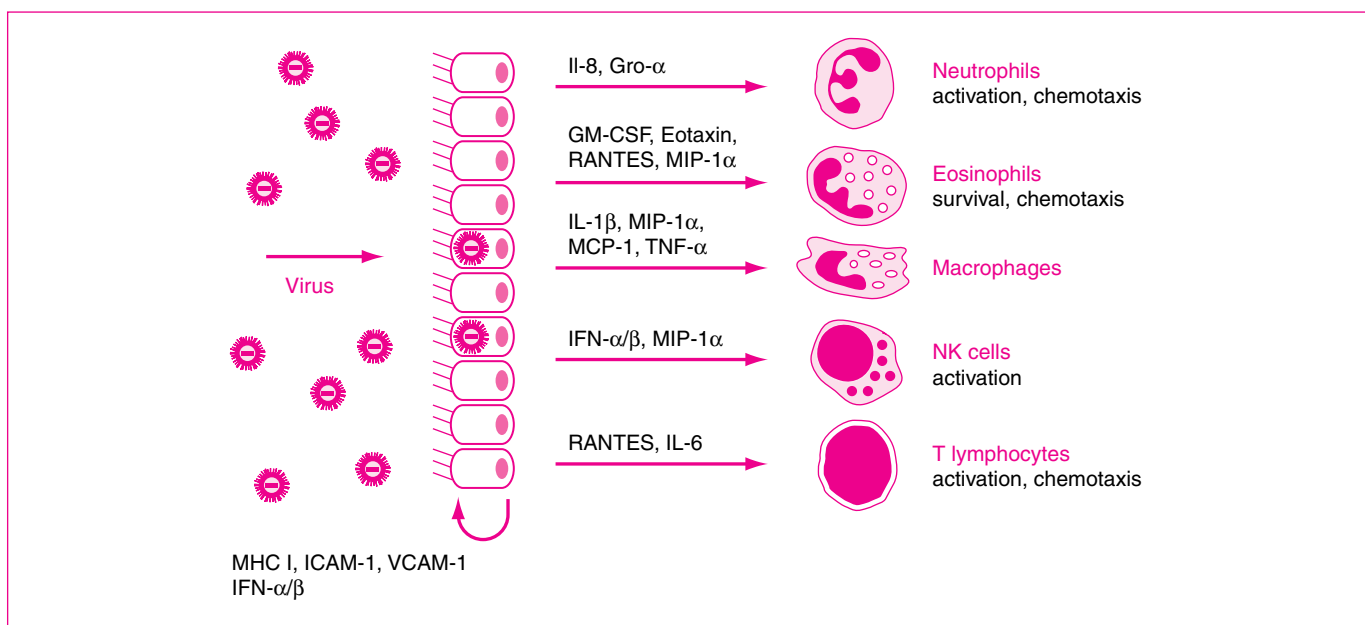
Fig. 39.1. Airway epithelial cells participate in the immune response to respiratory virus, producing a variety of cytokines and chemokines with actions on other cells. In addition, the migration of inflammatory cells is aided by the up-regulation of adhesion molecules and interferons help to establish an antiviral state in neighboring epithelial cells. Up-regulation of MHC class I may facilitate presentation of viral antigens.

initial trigger of the inflammatory reactions is epithelial cell–virus interaction.

A multitude of inflammatory mediators are generated or act on the epithelial surface. Bradykinin, a 9 amino acid peptide generated from plasma precursors as part of the inflammatory process has been shown to be present in nasal secretions of RV-infected individuals.[37] Bradykinin given intranasally is able to reproduce some of the symptoms of the common cold, such as sore throat and rhinitis.[38] Although the presence of kinins in the lungs of virus-infected individuals has not been reported they are present in both the upper and lower airways in allergic reactions.[39–41]

Some viruses may also cause complement-mediated damage. Complement components bind to epithelial cells both *in vitro* and *in vivo* during RSV infections. C3a and C5a are increased in human volunteers infected with influenza A virus.[42]

NO is produced by diverse sources including epithelial, endothelial and smooth muscle cells. In human airways, NO appears to be important in relaxation of the human airway smooth muscle.[43] In experimental animals, parainfluenza virus-induced hyperreactivity correlates with a deficiency in constitutive NO production.[44] On the other hand, in inflamed tissues NO reacts with superoxide anion generating peroxynitrite, a highly toxic compound suggesting a dual role for this mediator. Increased levels of exhaled NO are found in nonasthmatic volunteers following natural colds,[45] as well as in asthmatic patients after experimental RV infection.[46] In the latter study an inverse association between NO increase and worsening of airway hyperresponsiveness was demonstrated arguing in favor of a protective role for this substance. This is further supported by the observation that NO reduces cytokine production and viral replication in an *in vitro* model of RV infection.[47]

The up-regulation of ICAM-1 in the asthmatic airway is one explanation for the increased severity of RV infection. RV has itself been shown to further up-regulate ICAM-1 in bronchial biopsies following experimental RV infection.[48] In nasal epithelial cells obtained by brushings from atopic subjects, basal levels of ICAM-1 were increased relative to nonatopic subjects and were elevated in the relevant season for peak allergen exposure. Nasal epithelial cells from atopic subjects showed further up-regulation after *in vitro* culture with allergen. The highest basal level of expression of ICAM-1 was found on nasal polyp epithelial cells and this was increased further after infection with RV14. Viral titers recovered after RV14 infection were significantly higher for polyp epithelial cells than for nonatopic and atopic non-polyp epithelial cells.[49]

In vitro RV increases expression of both ICAM-1 and VCAM-1 in cultures of primary bronchial epithelial cells and in the A549 respiratory epithelial cell line via a mechanism involving the transcription factor NF-κB.[50,51] Inhibition of the up-regulation of ICAM-1 might be expected to modify favorably the course of RV infection. One effect of corticosteroids is to inhibit NF-κB.[52] In both A549 cells and in primary bronchial epithelial cells pretreatment with three different corticosteroids, hydrocortisone, dexamethasone and mometasone furoate, inhibits RV16-induced increases in ICAM-1 surface expression, mRNA and promoter activation without alteration of virus infectivity or replication.[53] Disappointingly, a study of inhaled corticosteroids in asthmatics prior to experimental RV infection failed to show a reduction of virus-induced ICAM-1 expression in bronchial biopsies,[48] but it is possible that a longer course and/or a higher dose of inhaled steroid or administration of oral steroids might have demonstrated a significant effect.

Viral infection of the respiratory tract results in significant changes in the pattern of cytokine expression by a number of cell types, both by cells of the immune system, which may be increased in number and activation status, and by cells often considered to be structural, but which in fact contribute significantly to the immune response, such as epithelial cells. Efficient orchestration of the immune response by cytokines is essential for eradication of virus. Modification of cytokine expression in the airway may contribute to the increased severity of virus infection in asthma.

In vitro studies of bronchial epithelial cell lines have demonstrated the production of a wide range of proinflammatory cytokines such as IL-1, IL-6, IL-11, IFN-α, IFN-γ, TNF-α and GM-CSF and the chemokines IL-8, RANTES and MIP-1α in response to RV and RSV.[30,54,55] *In vivo* these cytokines can be found in nasal lavage in association with RV infection.[56]

The specific roles of individual cytokines in the human lower airway during viral infection are not well understood, but increasing information is becoming available. IL-1, TNF-α and IL-6 share pro-inflammatory properties, such as the induction of the acute phase response and the activation of both T and B lymphocytes. IL-1 enhances the adhesion of inflammatory cells to endothelium, facilitating chemotaxis.[57] TNF-α is a potent antiviral cytokine, but *in vitro* increases the susceptibility of cultured epithelial cells to infection by the major group rhinovirus RV14 through up-regulation of ICAM-1.[30] IL-6 has been shown to stimulate IgA-mediated immune responses. IL-11 may also be important in virus-induced asthma.[58] It appears to cause bronchoconstriction by a direct effect on bronchial smooth muscle.[55] *In vivo* IL-11 is elevated in nasal aspirates from children with colds, levels correlating with the presence of wheezing. Similarly the chemokine MIP-1α is increased in nasal secretions during natural viral exacerbations of asthma.[59]

Viral up-regulation of cytokines may be mediated through certain key transcription factors. Increases in IL-6 and IL-8 production by cultured epithelial cells due to RV is dependent on NF-κB[60,61] as is the induction of IL-1, -6, -8, -11 and TNF-α by RSV.[62,63]

Effects of viruses on airway smooth muscle cells

Studies utilizing isolated rabbit tissues and human cultured airway smooth muscle cells suggest that, for RV16, exposure to the virus may have a direct effect on smooth muscle cells, resulting in increased contractility to acetylcholine and impaired relaxation to isoproterenol. This effect is dependent

on ICAM-1 and appears to involve an autocrine signaling mechanism, including up-regulation of production of IL-5 and IL-1β by the airway smooth muscle itself.[64] Whether rhinovirus reaches airway smooth muscle cells in sufficient quantity to produce a significant effect by this mechanism *in vivo* is as yet unknown. The effects of other respiratory viruses on smooth muscle require further investigation.

The cellular immune response to virus infection in the lower airway

A variety of leucocytes show changes in number, site of accumulation and activation state in response to virus infection. Since these cells are also implicated in asthmatic inflammation of the lower airway they provide potential sites of interaction between the immunopathologies of virus infection and asthma.

Monocytes/macrophages

Alveolar macrophages are present in large numbers in the lower airway. They make up around 90% of the cells seen in BAL from normal volunteers.[24] They are ideally placed for early phagocytosis of virus particles and are likely to play an important role in the immune response through antigen presentation to T cells and through the production of cytokines and other mediators. RV has been shown to enter human monocytes and macrophages which express high levels of the major RV receptor ICAM-1. It has not been possible to demonstrate RV replication within alveolar macrophages, although low grade productive infection has been shown in the monocyte cell line THP-1,[65] but entry into monocytes does result in activation and the production of both IL-8[65] and TNF-α.[66] Similarly infection of human monocytes *in vitro* with influenza A causes alterations in structure and activation status and the production of IL-1β, IL-6, TNF-α, IFN-α and IFN-β,[67] effects dramatically potentiated by subsequent exposure to bacterial LPS.

Dendritic cells

Dendritic cells are key cells in antigen presentation both of allergens and pathogens with a capacity to induce primary immune responses. They may also play a role in the regulation of the type of T cell-mediated immune response.[68] There is increasing knowledge of the immunobiology of these cells, but they are not well studied in the context of viral exacerbations of asthma.

Lymphocytes

Bronchial biopsies demonstrate increases in cells positive for CD3, CD4 and CD8 within the epithelium and submucosa of both normal and asthmatic subjects following experimental RV infection.[17] Such increases coincide with peripheral lymphopenia suggesting increased recruitment of T cells to the airway, although alternative mechanisms such as reduced apoptosis might also contribute. Since T cells are believed to be key cells in the pathogenesis of asthma the effects of viruses on T cells are of particular importance.

The normal CD4+ T cell response to virus infection is thought in general to be of the T helper 1 (Th1) type. The major type 1 cytokine is IFN-γ which, in addition to IFN-α and IFN-β from monocytes and macrophages, plays a role in establishing an "antiviral state" in neighboring cells. IFN-γ has varied effects on the pathogenesis of asthma. It appears to increase basophil and mast cell histamine release,[69] but on the other hand inhibits the expression of type 2 cytokines. Production of IFN-γ is increased in PBMC[70] and in nasal secretions[56] during RV colds and in human and animal models of influenza, parainfluenza and RSV infection.[71,72]

Asthma is believed to be characterized by Th2-type inflammation. Many studies have demonstrated mutual inhibition of Th1 and Th2 cells.[73,74] It is therefore possible within an airway with a preexisting Th2-type allergic asthmatic microenvironment that there may be inhibition of the normal effective Th1-type antiviral immune responses or that responses may be skewed towards inappropriate and potentially harmful Th2 types (**Fig. 39.2**).

In a recent study of experimental RV16 infection in subjects with allergic rhinitis or asthma, the balance of airway type 1 and type 2 cytokines in sputum induced by viral infection was found to be related to clinical symptoms and viral clearance. Although protein could not be detected in sputum due to the presence of inhibitors of the ELISA assay used, there were increases in mRNA, as determined by semi-quantitative RT-PCR, for both IL-5 and IFN-γ. An inverse correlation was demonstrated between the ratio of IFN-γ mRNA to IL-5 mRNA and peak cold symptoms. In addition subjects with RV16 still detectable 14 days after inoculation had lower IFN-γ/IL-5 ratios during the acute phase of the cold than those subjects who had cleared the virus.[75]

CD8+ T cells are important effector cells in specific cell-mediated antiviral immunity. These cells also demonstrate polarization of cytokine production, the major cytokine produced by Tc1 cells being IFN-γ and they are believed to play a role in the regulation of CD4 Th1/Th2 balance.[76] In a murine model of asthma the induction of bystander CD4+ Th2 immune responses to ovalbumin resulted in a switch of virus-peptide-specific CD8+ T cells in the lung to the production of Tc2 cytokines including IL-5 with, after challenge with virus peptide, the induction of airway eosinophilia.[77] If such a mechanism occurs in man it would suggest a means whereby CD8 antiviral function could be inhibited at the same time as CD8 amplification of allergic inflammation through IL-5 induction of airway eosinophilia.

Recruitment of T cells from the blood into the airway is at least in part under the influence of chemokines, including those whose production by epithelial cells is up-regulated following virus infection. Th1 and Th2 cells appear to show differential expression of chemokine receptors. Studies of T cell clones demonstrate increased expression of CXCR3 (receptor for IP-10, I-TAC and Mig) and CCR5 (MIP-1β) in human Th1 cells and increased expression of CCR4 (TARC and MDC) and to a lesser extent CCR3 (eotaxin
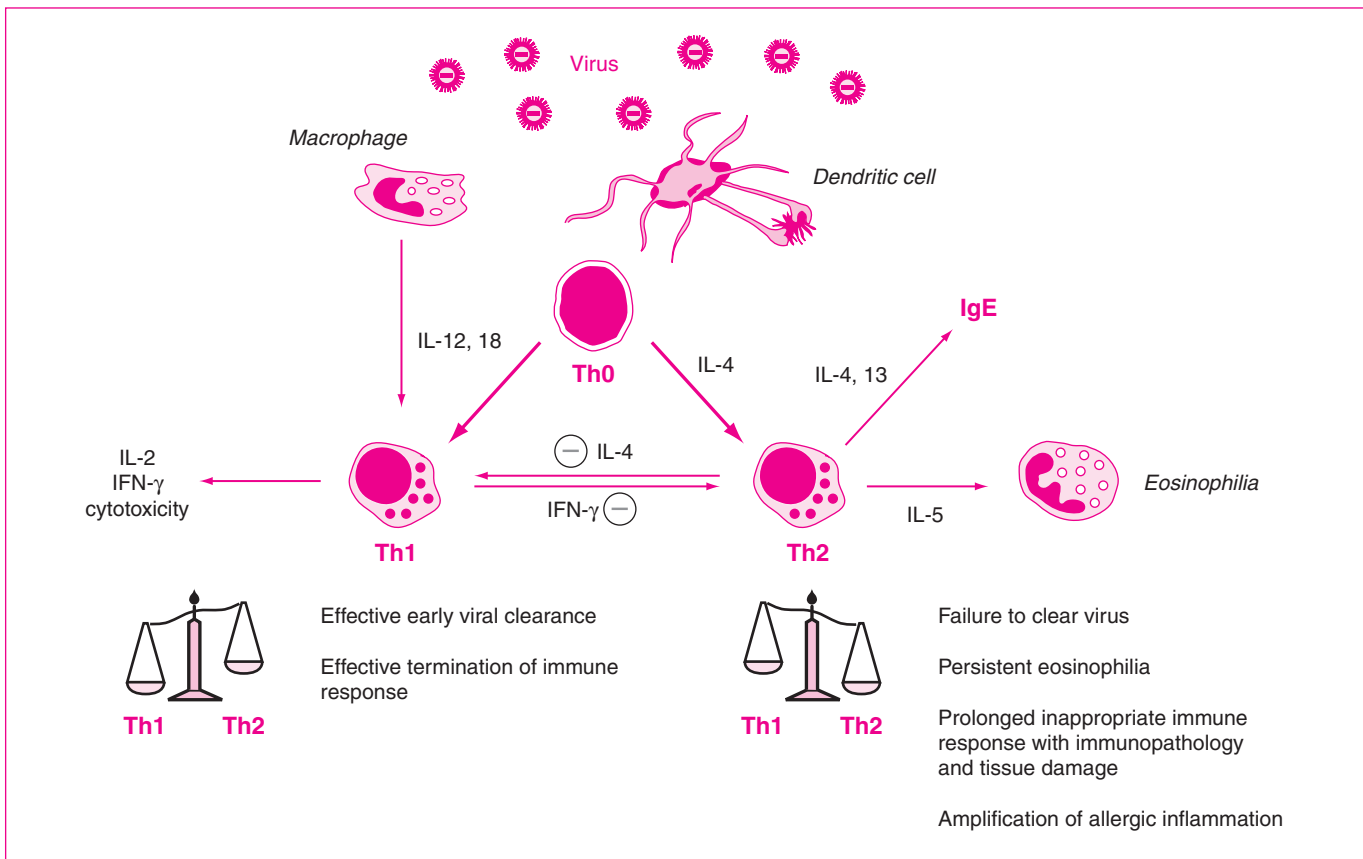
Fig. 39.2. Preexisting asthmatic airway inflammation may modify a predominantly Th1 antiviral immune response, favoring a Th2 or mixed response, which may provide less efficient viral clearance and result in prolonged virus-induced inflammation, increased associated immunopathology and increased tissue damage.

and MCP-3) in Th2 cells, with selective migration of cells in response to the appropriate chemokines. CCR1 (RANTES, MIP-1α, MCP-3) and CCR2 (MCP-1,2,3,4) were found on both Th1 and Th2 cells.[78] Bronchial biopsies from asthmatics show high levels of expression of CCR4 and significant levels of CCR8 by T cells.[79] Increased recruitment of T cells to the airway as a result of virus-induced chemokine production by epithelial cells could amplify preexisting allergic inflammation. If the asthmatic airway microenvironment influences the pattern of chemokine expression following virus infection then this could alter the Th1/Th2 balance of the antiviral immune response.

Mast cells/basophils
These cells are important sources of inflammatory mediators characteristic of allergic inflammation in asthma. Mast cell basal and stimulated histamine release increases after virus infection.[72] Airway mast cell numbers are up-regulated in a rat model of parainfluenza infection. Several viruses can enhance basophil IgE-mediated histamine release, but the role of this cell in human asthma is controversial.

Mast cells are also important sources of inflammatory mediators. Their function and localization suggest an early

interaction with viruses. LTC_4 is among the major mediators responsible for the late phase of bronchospasm in asthma. During RSV infection increased levels of LTC_4 were found in the nasopharyngeal secretions of infants.[80] Levels correlated well with the symptoms of the disease with concentrations in infants presenting with bronchiolitis being 5-fold higher than in those with only upper respiratory tract symptomatology.

Eosinophils
Eosinophils are increased in bronchial epithelium in biopsies taken from both normal and asthmatic human volunteers following experimental RV infection; in a small study, eosinophilic inflammation persisted for up to 6 weeks in asthmatic subjects.[17] In allergic rhinitic subjects, experimental RV infection increases BAL eosinophils following segmental allergen challenge, an effect similarly persisting for 6 weeks,[24] and increased levels of eosinophil cationic protein are found in the sputum of RV-infected subjects.[19]

Eosinophils accumulate in the airway under the influence of IL-5, GM-CSF, IL-8, RANTES and eotaxin.[81] Expression of the potent eosinophil chemoattractant RANTES is increased in nasal secretions of children with natural virus-induced asthma exacerbations.[59] RANTES is up-regulated

in primary nasal epithelial cell cultures by RSV[82] and RV.[32] GM-CSF is believed to be important in eosinophil production in the bone marrow and in prolonging eosinophil survival.[81] However, levels do not appear to be increased during viral upper respiratory tract infections.[59,83] Levels of eotaxin in nasal lavage rise after experimental RV16 infection.[84]

These data suggest a pathogenic role for eosinophils in virus-induced asthma. However, a protective role is also possible. In allergic rhinitic subjects infected with RV after high-dose allergen challenge the severity and duration of cold symptoms were inversely related to the nasal lavage eosinophil count prior to infection.[27] Eosinophils may contribute to antigen presentation during virus infection. Eosinophils purified from PBMC and pretreated with GM-CSF bind RV16 via ICAM-1 and present viral antigen to RV16-specific T cells, inducing proliferation and secretion of IFN-γ. Eosinophils have antiviral actions in parainfluenza-infected guinea pigs.[85] Eosinophil-derived neurotoxin and cationic protein have ribonuclease activity and reduce RSV infectivity.[86] The role of the eosinophil in the antiviral immune response thus requires further evaluation.

Neutrophils
Neutrophils are recruited early in viral infection in response to the production of the chemokine IL-8 by epithelial cells and by activated neutrophils, and are a prominent feature of severe asthma. Induced sputum studies in asthmatic and nonasthmatic volunteers demonstrate a significant increase in sputum neutrophils at day 4 of a natural cold, correlating with sputum IL-8.[87] Similar results were obtained in induced sputum taken 2 and 9 days after experimental RV16 infection in asthmatic subjects. Intracellular staining demonstrated that the increase in cells positive for IL-8 at day 2 could be attributed to the increase in IL-8-positive neutrophils.[19] The chemokine IL-8 is a potent chemoattractant for neutrophils, but also acts on lymphocytes, basophils and primed eosinophils. An increase in IL-8 has been found in nasal lavage from children with natural colds.[56] Experimental infection of atopic asthmatics with RV16 resulted in elevated IL-8 in nasal lavage and this correlated with cold and asthma symptom scores and a fall in histamine PC20.[21] Sputum from asthmatics with asthma exacerbations has both elevated IL-8 and neutrophilia.[88] A study of experimental virus-induced asthma in children also demonstrated elevated IL-8 and neutrophilia in nasal aspirates during the acute phase of infection and levels of neutrophil myeloperoxidase correlated with symptom severity.[89] Such studies suggest a prominent role for the neutrophil in tissues damaged during virus-induced asthma.

Natural killer cells
NK cells are an important part of the innate immune response, their function being the elimination of a variety of target cells including virus-infected cells and the modulation of adaptive immunity towards viruses.[90] Cell killing by NK cells may occur through natural killing, antibody-dependent cellular cytotoxicity (ADCC) or apoptotic killing of Fas-positive target cells via membrane bound FasL. The ability to directly kill virus-infected cells is regulated by a balance between inhibitory and activating receptors.[91] Killer inhibitory receptors (KIRs), Ig-like receptors that recognize HLA-A, B or C molecules, and the lectin-like CD94/NKG2A receptor that interacts with HLA-E allow NK cells to recognize cells expressing normal self MHC class I.[92] Loss of inhibition occurs if potential target cells have lost class I expression following virus infection or if they display abnormal class I/peptide complexes.

NK cells are rapid and efficient producers of cytokines such as IFN-γ, important both in early viral infection in the antigen-independent activation of antigen-presenting cells such as macrophages, dendritic cells and epithelial cells, and for biasing the development of CD4+ Th1 and CD8+ Tc1 cells.

The function of NK cells in the asthmatic airway is as yet unexplored. It may be that in an airway environment rich in type 2 cytokines that NK type 1 function and effective antiviral activity are inhibited. If this is the case, then a key component of the early immune response would be deficient and viral clearance would be impaired.

B lymphocytes and interaction of viruses with IgE dependent mechanisms
An elevated serum total and allergen-specific IgE are features of "extrinsic" or atopic asthma. IgE-mediated mechanisms are certainly important in the pathophysiology of extrinsic asthma. Recent studies suggest a similar airway pathology in both extrinsic and "intrinsic" nonatopic asthma,[93] where there is an absence of specific serum IgE and negative skin prick tests to aeroallergens. It has been suggested that there may be the production of local IgE to as yet unknown environmental allergens in intrinsic asthma.

Up-regulation of total IgE or virus/allergen-specific IgE locally or systemically during respiratory virus infection would be expected to contribute to the duration and severity of symptoms of an asthma exacerbation.

Intranasal challenge with RV39 results in an increase in total serum IgE in allergic rhinitic subjects, but no increase in preexisting allergen-specific IgE.[94] In children with asthma, during infection with influenza A there was no change in total IgE, but increases were observed in specific serum IgE to house dust mite and in ex-vivo proliferative and IL-2 responses of lymphocytes challenged with house dust mite allergen.[95] In a study of RSV infection in infants the development of serum RSV-specific IgE occurred more frequently in atopics and correlated with clinical wheezing, histamine levels in nasal secretions and hypoxia.[96] There is no information as yet on the presence of local virus-specific IgE in the airway during asthma exacerbations.

COPD

Increasing interest in the clinical features and pathogenesis of COPD reflects the worldwide importance of the disease.

More than 14 million patients are affected in the United States alone. It is predicted to become the third leading cause of death worldwide by 2020.[97] National and global initiatives have been launched and management guidelines have been published.[98,99]

The frequency of exacerbations is a major factor in the quality of life of patients with COPD.[100] Problems exist in defining an acute exacerbation. One definition is an acute tracheobronchitis, generally infectious in etiology, that occurs in a patient with established COPD. An important element of this definition is that other causes of respiratory deterioration frequently encountered in this patient group, such as congestive cardiac failure, cardiac arrhythmias, pneumothorax, pneumonia and pulmonary thromboembolism must be excluded.[101] The typical clinical features of an exacerbation include increased dyspnea, wheezing, cough, sputum production and worsened gas exchange. Although noninfectious causes of exacerbations such as allergy, air pollution or inhaled irritants including cigarette smoke may be important, acute airway infections are the major precipitants.[102] The infection and consequent host inflammatory response result in increased airway obstruction.

Epidemiology

At least a third of exacerbations may be caused by viral infections. In a study of 186 patients rhinoviruses, influenza virus, parainfluenza virus and coronavirus were significantly associated with COPD exacerbations. Patients did not seem to have increased susceptibility to these viruses but viral infection had more serious consequences if it occurred.[103]

The role of bacteria in precipitating exacerbations is controversial. Bacteria may have a primary role in the development of an exacerbation or represent a secondary superinfection of an initial viral process. Various bacterial species are present in the airways of 25–50% of patients, even when the COPD is stable but increased frequency of recovery of bacteria during exacerbations suggests that they play a role.[104,105] Significant bacterial infection has been suggested when there is an abundance of neutrophils in the sputum[106] and when the sputum is purulent and green (due to neutrophil myeloperoxidase).[107]

The major bacterial organisms associated with COPD exacerbations are nontypable *Haemophilis influenzae*, *Streptococcus pneumoniae* and *Moraxella (Branhamella) catarrhalis*.[108,109] *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* may play a part.[110] Evidence also suggests that in more severe patients with a baseline FEV_1 of 35% predicted or less, Gram-negative bacteria especially *Enterobacteriaceae* and *Pseudomonas* play an important part in acute exacerbations.[111]

Although the results of placebo-controlled trials show conflicting results, overall the effects of antibiotic treatment also suggest an etiological role for bacteria in exacerbations in some patients. A meta-analysis of nine studies showed a small overall benefit when antibiotics were used for COPD exacerbations.[112] The largest study included 362 exacerbations in 173 outpatients.[106] Compared with placebo, the rate of symptom resolution and improvement of peak expiratory flow during exacerbations was slightly but significantly faster when patients were treated with cotrimoxazole, amoxicillin or doxycycline. More importantly, treatment failures as defined by respiratory deterioration were nearly twice as likely in the placebo group. Benefit from antibiotics was most evident for patients with most symptoms (dyspnea, increased sputum volume and sputum purulence).

Guidelines for the use of antibiotics in acute exacerbations of COPD are unclear because of the difficulties in defining the role of bacterial infection in an individual case. The American Thoracic Society statement on COPD[99] suggests using antibiotics if there is evidence of infection (fever, leukocytosis, CXR changes), but not all patients with bacterial bronchial infection have fever (this is more common in viral infection or pneumonia) and few have CXR changes. The European Respiratory Society recommends antibiotics if the sputum is purulent, using standard antibiotics as first line, and sputum culture if these fail.[113]

The major bacterial pathogens isolated during bronchial infections all form part of the commensal flora in the nasopharynx. Bronchial infections occur in patients with abnormal airways with reduced host defenses. Persistence of bacteria within the bronchial tree may come about through toxins that impair mucociliary clearance, enzymes that break down local immunoglobulin, products that alter immune effector cell function, adherence to mucous and damaged epithelium or other mechanisms of avoiding immune surveillance.[114]

Bronchial infections usually remain confined to the mucosa. Many will resolve spontaneously without antibiotic treatment. Persistent bacterial infection usually reflects the severity of the impairment of the lung defenses, rather than the virulence of the micro-organism. The damage to lung tissue caused by the host inflammatory response to chronic bacterial infection may be more important than the damage caused directly by the bacteria themselves.[102]

Evidence for a role for bacterial infection in pathogenesis/progression of COPD – the vicious cycle hypothesis

Bacterial infection has a definite role in the pathogenesis of other chronic lung diseases, such as cystic fibrosis and bronchiectasis where bacterial infection is chronic, causing not only acute exacerbations but also influencing long-term prognosis.[102]

In these diseases chronic bacterial infection sets up a vicious cycle in which the host inflammatory response is unable to clear the bacteria and instead promotes continued infection and tissue damage.[114] Neutrophils produce proteinases and reactive oxygen species, and lung antiproteinase defenses are overwhelmed. Both proteinase enzymes and reactive oxygen species cause damage to the epithelium, stimulating mucous production and impairing mucociliary clearance. Neutrophil elastase stimulates epithelial cell production of the chemokine IL-8 which

attracts further neutrophils and in addition impairs phago-cytosis by destroying antibody and cleaving complement receptors from neutrophils and complement components from bacteria. Similar cellular mechanisms operate in COPD. Neutrophils are also stimulated by cigarette smoke.

COPD patients may be chronically colonized by bacteria between exacerbations, bacterial numbers then increasing during exacerbations. In a study using bronchoscopic pro-tected brush specimens,[104] ten of 40 COPD patients were colonized with bacteria when stable. During exacerbations 50% had bacteria present and when present, bacterial numbers were greater. When protected brush specimens were taken during severe acute exacerbations of COPD requiring ventilation,[115] bacteria were detected in 50%, but it was not possible to distinguish patients more likely to have bacteria on the basis of clinical features or other investigations.

Bacterial colonization in the stable state represents an equilibrium in which the number of bacteria present in the bronchial tree is contained by the host defenses, but not eliminated. During an exacerbation this equilibrium is upset and bacterial numbers increase, inciting an inflammatory response. Change will usually occur because of a change in the host rather than altered virulence of the bacteria, for example as a result of viral infection.

Evidence for a role for viruses in exacerbations of COPD

Many exacerbations of COPD occur without the hallmarks of bacterial infection – increased volume or purulence of sputum. Between 33 and 70% of exacerbations are associ-ated with symptoms of the common cold. The frequency of exacerbations requiring hospitalization is higher in the win-ter. One explanation for this could be the increased frequency of respiratory viruses at this time of the year. A recent study of 321 exacerbations in 83 patients with moderate to severe COPD using new diagnostic methods including RT-PCR shows a high incidence of viral infection.[116] Viruses were detected in nasal aspirates at exacerbation in almost 40% of cases. Rhinovirus was the most common, occurring in 58% of cases where a virus was present. The presence of virus was associated with increased dyspnea, cold symptoms and sore throat and with prolonged recovery from exacerbation. Earlier studies relying on serology and virus culture quote lower virus detection rates of 15–20%.[103,117–119]

It has been suggested that persistent virus infection con-tributes to the progression of COPD. In particular, aden-ovirus appears to persist in a latent form in which viral proteins are produced without replication of complete virus. Such latent infection may amplify lung inflammation due to cigarette smoke.[120] Adenoviral E1A DNA persists in human lungs from patients with COPD compared with patients of similar age, sex and smoking history who do not have COPD.[121] The E1A protein has been demonstrated in air-way epithelial cells from smokers.[122] It is able to amplify many host genes through attachment to the DNA-binding sites of transcription factors.[123] Airway epithelial cells transfected with E1A produce excess inflammatory cytokines such as IL-8,[124] and surface adhesion molecules such as ICAM-1[125] after in-vitro challenge by an NF-κB-dependent mechanism.[126]

RSV has been identified in induced sputum from patients with stable COPD. These individuals have a higher plasma fibrinogen and serum IL-6, a higher pCO_2 and increased frequency of exacerbations.[116] This suggests either that low grade persistent RSV infection contributes to COPD sever-ity or that patients with more severe COPD are less able to clear RSV from the airway.

The immunology of virus infection in COPD is not well understood. Fewer data are available than for virus infection in asthma, since this has not been the subject of human experimental infection studies. Such studies are clearly needed in view of the increasing evidence for a major role for viruses in causing COPD exacerbations.

THERAPY

Currently much of the treatment of infective exacerbations of both asthma and COPD is symptomatic, consisting of increased bronchodilators, or supportive in the form of oxy-gen and in severe cases noninvasive or invasive ventilatory measures. Corticosteroids are widely used in inhaled or oral form for their anti-inflammatory actions and have a major role in asthma. They are also effective in many infective exacerbations of COPD even if the patient, when stable, shows little evidence of steroid responsiveness. The effects of corticosteroids are the result of actions at many points in various inflammatory cascades. Whilst this undoubtedly contributes to their beneficial effects it also results in signif-icant side-effects, in particular if systemic steroid treatment is prolonged or frequent. In addition systemic steroids may interfere with the antiviral immune response resulting in reduced viral clearance.[127]

Specific antibiotic therapy is available for bacterial infec-tions and is indicated where there is good evidence of such infection or when the exacerbation is severe and bacterial involvement is a possibility.

However, as discussed above, the majority of infective asthma exacerbations are of viral rather than bacterial origin and viruses are also common in exacerbations of COPD.

Vaccination

The success of vaccination to prevent respiratory virus infections has been limited by significant variation within the major virus types causing disease. There are 102 serotypes of rhinovirus and no effective vaccine has been introduced. The influenza viruses display antigenic shift and drift. New vaccines must be developed every 2–3 years to cover the strains prevalent at the time. When a new pan-demic strain arises there is a delay before sufficient quanti-ties of vaccine can be made available. In spite of such problems combined influenza vaccination is of value in indi-viduals with chronic respiratory diseases, especially in the

elderly. Vaccination against RSV experienced a major set-back when the use of formalin-inactivated virus in young babies resulted in increased disease severity following subsequent virus infection.

Antivirus approaches

There are two main approaches to therapy for a viral exacerbation. The first is to use antiviral agents with direct actions against the virus itself. Because of the large number of viruses producing similar clinical syndromes the use of specific antiviral drugs requires rapid accurate diagnostic methods such as PCR.

The second approach is to identify key components of the antiviral immune response and design drugs that will either enhance beneficial antiviral aspects of the immune response or inhibit components that lead to immunopathology. Understanding the complexities of the antiviral immune response, in particular how it may be altered in the context of preexisting chronic airway diseases such as asthma or COPD, is an essential first step.

Simple nonspecific treatments for the common cold do exist although their efficacy is debated. Vitamin C and zinc gluconate[128] both may shorten the duration of a cold by 1–2 days. The inhalation of humidified hot air provides symptomatic relief.[129]

Nasal IFN-α is an effective treatment for the common cold,[130] but must be given either prior to or shortly after exposure to the virus. It is also expensive and is associated with significant local side-effects such as bleeding and discharge. These problems have limited its clinical use.

Amantadine and rimantadine are effective against influenza A. They inhibit the viral M2 ion channel, important for uncoating and release of the virus genome into the host cell. The use of amantadine has been limited by CNS side-effects, such as dizziness and insomnia; fewer such side-effects are seen with rimantadine. Both drugs are indicated for use during epidemics of influenza A both for treatment and for prophylaxis in high-risk groups including patients with asthma or COPD. Neither is active against influenza B. Two new neuraminidase inhibitors, zanamivir and oseltamivir, are active against both influenza A and B.[131–133] These agents are 67–82% effective in preventing infection when used as prophylaxis during the influenza season and, as treatment, they reduce the duration of illness by 1–1.5 days if started within 36–48 hours of the onset of illness. Zanamivir must be given by inhalation, whereas oseltamivir can be given orally.

Ribavarin is a nucleoside analogue, active against RSV *in vivo* and also against influenza *in vitro*. Oral preparations have limited benefit in influenza due to rapid metabolism, but inhaled ribavirin may be effective in reducing symptoms and viral shedding. Because of its toxicity, it is not appropriate for asthma or COPD. Its use is restricted to infants and children in the first 3 days of RSV bronchiolitis.

RSV-enriched immunoglobulin is effective as prophylaxis for infants at high risk of RSV bronchiolitis and trials with RSV neutralizing monoclonal antibodies are in progress, but these therapies are not indicated for patients with asthma or COPD.

Rhinoviruses are a major target for drug treatment. It has been estimated that rhinoviruses result in between six and ten colds per year in young children and between two and five per year in adults. As yet no effective agent is available for clinical use. Capsid binding/canyon inhibitors block the binding of rhinoviruses to their host cell receptor (ICAM-1 in the case of the major group). These drugs can be extremely potent but their clinical usefulness is limited by serotype specificity and the rapid development of resistance. Alternative targets include soluble ICAM-1 which inhibits major rhinovirus infection *in vitro* and conserved viral enzymes such as protein 3D, the RNA-dependent RNA transcriptase, protein 2C, the associated ATP-helicase, and the cysteine protease 3C.

SUMMARY

Infection, in particular by respiratory viruses, is a common trigger of exacerbations of asthma and COPD. Our knowledge of the mechanisms of virus-induced exacerbations remains incomplete. In the case of asthma, investigation of the interactions between preexisting asthmatic airway inflammation and the antiviral immune response, and between virus infection and allergen exposure have been furthered by the use of the model of experimental rhinovirus infection in human volunteers. Similar studies are required in COPD exacerbations for which fewer data are currently available. Current therapy for virus-induced exacerbations of asthma and COPD relies on increased treatment of preexisting disease. Corticosteroids form the major anti-inflammatory component of such therapy, but their use can be associated with significant side-effects, especially if used systemically and in high doses. Antibiotics are indicated for bacterial infection. Antiviral agents do exist, in particular for influenza viruses, but the effective use of such drugs in asthma and COPD requires viral diagnosis and commencement of treatment early in the course of an exacerbation or the targeting of high-risk groups for prophylaxis. Clinically effective broad spectrum agents are not yet available for the rhinoviruses which are the most common cause of exacerbations. Alternative strategies for drug development may involve the identification of key factors common to exacerbations induced by a range of different viruses. Increased knowledge of the host–virus interaction is required to design treatments that will increase virus clearance and minimize immunopathology.

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