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37 CHAPTER

Infections

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INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common diseases and both result in significant morbidity and mortality. Although they share some clinical features and although they may coexist in the same individual, they are distinct disease syndromes with different pathogenetic mechanisms. In each case much of the morbidity and mortality is associated with exacerbations of disease, in response to a variety of trigger factors. 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 will review 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 United Kingdom [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 80–85% of asthma exacerbations in children [4–10] and in 75–80% in adults [11–14]. 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 instead of or 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 [15] 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), influenza, RSV, and parainfluenza viruses. RV alone are detected in around 50% of virus-induced asthma attacks. Adenoviruses, enteroviruses, metapneumoviruses, bocaviruses, 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. The effects of such viruses in the lower respiratory tract have been studied but detailed knowledge of the pathogenetic mechanisms involved in asthma exacerbations remains limited. Experimental respiratory virus infection in human volunteers is limited to mild disease by concerns of safety [16]. Most such studies have therefore focused on the experimental innoculation of rhinovirus in allergic rhinitic or mild asthmatic individuals and normal control subjects [17–28]. 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 administration of active and placebo medication, of ability to sample the lower airway with timing from onset of infection accurately defined and the study of RV-induced effects including asthma symptomatology, lung function, and airway pathology/immunology.

Recent epidemiological evidence confirms a synergistic interaction between virus infection and allergen exposure in precipitating hospital admissions for asthma [29, 30]. 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 $\mu g/m^3$ [9, 31].

Most studies of experimental virus infection in allergic subjects are performed outside the relevant season for allergen exposure. One 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 [32]. Interestingly, prior allergen challenge in this model, somewhat unexpectedly, appeared to protect against an RV cold with delayed nasal leukocytosis, increased generation of the proinflammatory 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. In work by de Kluijver *et al.* the effects of a 10-day period of low dose allergen exposure in house-dust mite sensitive and/or experimental RV16 infection were studied. No synergistic or additive effects were observed as regards lung function parameters [33]. Further development of models of experimental combined allergen exposure and virus infection is clearly required.

We have recently adopted the approach of infecting asthmatic volunteers with RV and then sending them home to continue their normal allergen exposure in the natural environment [34]. We investigated physiologic, virologic, and immunopathologic responses to experimental rhinovirus infection in blood, induced sputum, and bronchial lavage in 10 atopic mild asthmatic and 15 nonatopic normal volunteers. Rhinovirus infection induced significantly greater lower respiratory symptoms, lung function impairment, increases in bronchial hyperreactivity, and eosinophilic lower airway inflammation in asthmatic compared to normal subjects. We also saw trends to increased neutrophils and lymphocytes in the lower airway in asthmatics, and coincident reductions in blood lymphocytes, suggesting trafficking to the airway. In asthmatic, but not normal subjects, virus load was significantly related to lower respiratory symptoms, bronchial hyperreactivity, and reductions in blood total and $CD8^+$ lymphocytes and lung function impairment was significantly related to neutrophilic and eosinophilic lower airway inflammation. This study demonstrated increased rhinovirus-induced clinical illness severity in asthmatic compared to normal subjects, provided evidence of strong relationships between virus load, lower airway virus-induced inflammation, and asthma exacerbation severity and suggests that this approach could provide a very good model in which to examine asthma exacerbation pathogenesis as well as treatment interventions.

Rhinovirus infection of the lower airway

Whereas other respiratory viruses such as influenza, parainfluenza, 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 or solely 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 [35], tracheal brushings [26], and BAL [36] by both RT-PCR and culture. RV has been cultured in cell lines of bronchial epithelial cell origin [37] and replication has been demonstrated in primary cultures of bronchial epithelial cells [38–40]. 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 [41]. Finally the use of *in situ* hybridization has conclusively demonstrated RV replication in bronchial biopsies of subjects following experimental infection [38] and recent immunochemistry data suggests a preference for basal cells [42]. These data

confirm that RV infection of the lower airway does occur and directly implicate lower airway infection in the pathogenesis of asthma exacerbations.

A mouse model of rhinovirus-induced asthma exacerbation

Investigation into the pathogenesis of rhinovirus infections and rhinovirus-induced asthma exacerbations has been severely hampered for the ~50 years since their discovery, as it has been believed that rhinoviruses only infect humans and chimpanzees. However, a mouse model of rhinovirus infection has recently been successfully developed for the first time. New methods of purification and concentration of rhinoviruses, were used to show that for the minor group of rhinoviruses (the ~10% that use the LDL receptor as their mode of entry into cells), wild-type BALB/c mice can be successfully infected and that most of the disease-related outcomes observed in humans were reproduced in this unique new model. These outcomes include induction of both innate and acquired immune responses, induction of mucin synthesis and secretion, induction of both acute neutrophilic and prolonged lymphocytic airway inflammation, and induction of chemokines responsible for chemoattraction of neutrophils, lymphocytes and dendritic cells as well as a range of proinflammatory cytokines. Mice transgenic for a chimera of ICAM-1, the receptor for the major group (~90%) of rhinoviruses, in which the rhinovirus-binding domains were human, but the remainder of the molecule mouse were then developed. This transgenic mouse was then able to be infected by major group strains, thus generating mouse models capable of being infected by all rhinovirus serotypes. Finally an established mouse model of allergic airway inflammation was used to demonstrate that rhinovirus infection of this model resulted in rhinovirus-induced exacerbation of allergic airway inflammation. The asthma-related outcomes exacerbated by infection in this model include exacerbation of airway hyperresponsiveness, exacerbation of mucin synthesis and secretion (MUC5AC and MUC5B), exacerbation of neutrophilic, eosinophilic, and lymphocytic airway inflammation, and augmented induction of both Th1 (IFN-γ) and Th2 (IL-4 and -13) cytokines. The development of this novel mouse model of rhinovirus-induced asthma exacerbations, should allow mechanisms of disease to be investigated *in vivo* and true causation be established *in vivo*. [43].

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 to nonatopic, nonasthmatic controls. With detailed monitoring, it is possible to detect reductions in both peak flow [44] and home recordings of FEV_1 [24] in atopic asthmatic patients in the acute phase of experimental RV16 infection. There is an enhanced sensitivity to histamine and allergen challenge after RV16 innoculation in nonasthmatic atopic rhinitic subjects [19, 45]. RV16 increases asthma symptoms, coinciding with an increase in the maximal bronchoconstrictive response to methacholine up to 15 days after infection [20]. There is also a significant increase in sensitivity to histamine in asthmatic subjects after RV16 infection, most pronounced in those with severe cold symptoms [25] and our recent study confirmed that these reductions in lung function and increases in symptoms and airway hyperresponsiveness were observed only in asthmatic, but not in normal subjects [34].

Components of the antiviral immune response

Current concepts of a typical antiviral immune response, as reviewed in detail elsewhere [46, 47], result from research in human volunteers and patients but also in experimental animals, especially inbred mice. Results of animal studies may not be directly applicable to the outbreed human population but ethical considerations often limit direct investigation of the human immune system. All immune responses are a combination of nonspecific (innate) and specific (adaptive) immunity.

Nonspecific or innate [48] elements include: phagocytes such as neutrophils and macrophages that engulf and destroy viruses; natural killer (NK) cells that recognize and destroy virus-infected cells on the basis of reduced HLA class I expression; cells including NK cells, neutrophils, macrophages, mast cells, basophils, epithelial cells that release cytokines, such as interferons, with immunoregulatory or antiviral actions; components of body fluids such as complement, defensins, and surfactant proteins that are capable of neutralizing viruses independently of, or in combination with, antibodies.

Complement

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 [49]. There is little information on the role of complement in immunity to RV. Recent data suggests that the RV 3C protease cleaves the complement factors C3 and C5 which may interfere with the destruction of virus-infected cells [50]. For other viruses, for example influenza, the complement system forms an important link between the innate and specific immune systems. Mice deficient for the third component of complement are highly susceptible to primary influenza, showing reduced priming of T-helper cells and cytotoxic T-cells in lung draining lymph nodes and severely impaired recruitment into the lung of virus-specific $CD4^+$ and $CD8^+$ effector T-cells producing IFN-γ [51]. Activation of the complement cascade may be necessary for the function of other innate antiviral proteins such as serum mannose-binding protein [52]

Defensins

The α and β defensins are small cationic antimicrobial peptides which have the capacity to kill bacteria, fungi, and enveloped viruses by disruption of the microbial membrane.

In vivo they are probably most important in phagocytic vacuoles and on the surface of skin and mucosal epithelia. In addition to their direct antibiotic role, defensins are increasingly being found to have immunomodulatory actions [53] and to play a role in cell recruitment through activation of certain chemokine receptors, for example hBD3 and CCR6 on dendritic cell (DC).

Specific immunity involves production of antibody by B-lymphocytes and the activities of cytotoxic T-cells following processing and presentation of viral antigens by additional cells of the immune system, the most important of which are probably dendritic cells. Immunological memory modifies the overall response to reinfection by previously encountered virus and alters the timing and magnitude of contributions due to different components.

Time course of innate and adaptive immunity in primary and secondary infections

In primary infection, viruses replicate in the respiratory tract reaching peak levels at around days 2–4. At this time type I interferons are first detected, peaking around days 2–3 and falling to become undetectable once active replication has ceased. Interferons activate NK cells, first detectable around day 3 and peaking around day 4. In addition to destruction of virally infected cells NK cells release cytokines including IFN-γ that activate additional inflammatory cells in the airway including macrophages. Such nonspecific immune mechanisms are essential in early defense against virus in the first few days. In addition, the innate immune system plays a role in stimulating specific immunity and may influence the nature of the specific response, for example whether this is characterized by type 1 or type 2 cytokines.

Meanwhile, viral antigens are processed locally and in regional lymph nodes by dendritic cells and presented to T-cells. $CD4^+$ and $CD8^+$ T-cells are detectable from around day 4 then generally decline as infection resolves to become undetectable by day 14. However, memory $CD4^+$ and $CD8^+$ responses may persist for life. T-cell recruitment is dependent on the production of chemokines and on alterations in the expression of adhesion molecules on the endothelium of inflamed tissues. Time is also required to generate B-cell responses. Mucosal IgA may be detected around day 3, serum IgM from days 5–6 and IgG days 7–8, increasing in amount and avidity over the next 2–3 weeks. IgA falls normally to low or undetectable levels over 3–6 months. Serum IgG may remain detectable for life. Specific immune mechanisms such as $CD8^+$ T-cells and immunoglobulin are responsible for the eradication of infectious virus usually by 7–10 days after infection.

Secondary infection with the same virus results in rapid mobilization of B- and T-cell specific immunity with an earlier T-cell peak coinciding with the NK cell peak around days 3–4. If reinfection is with the same serotype a rapid increase in levels of preexisting neutralizing antibodies may limit viral replication to such an extent that infection is clinically silent. Because this results in fewer infected cells there is relatively less activation of nonspecific immunity and it may be difficult to detect a $CD8^+$ T-cell response.

Following experimental infection of seronegative subjects with RV2 [54] serum-specific antibodies are detectable at 1–2 weeks, reach a maximum at 5 weeks, persist for at least a year and may remain elevated many years after infection. Local specific antibody levels may be lost more rapidly. High levels of serum neutralizing antibody or specific IgA protect against reinfection with the same rhinovirus serotype. However, since it appears relatively late, recovery from illness for seronegative hosts which usually occurs at 7–10 days must be due to other components of the immune response. In seropositive subjects preexisting serum neutralizing antibodies to RV39 and to RV-Hanks modify experimental infections in human subjects [55, 56]. Local IgA and IgG passing from the vasculature into the pulmonary interstitium contribute to viral clearance. However, the 100+ RV serotypes mean that repeated infection with RV to which an individual lacks appropriate antibodies is common.

T-cell responses to RV demonstrate MHC class I restricted cross-reactivity between serotypes due to specificity for conserved epitopes within the capsid proteins VP 1–3 [57]. RV16- and RV49-specific T-cell clones from human peripheral blood demonstrate recognition of both serotype specific and shared viral epitopes [58]. Vigorous proliferation of and IFN-γ production by PBMC in response to RV16 in seronegative subjects is associated with reduced viral shedding after inoculation [59], thus T-cells responses also appear protective.

Interactions between virus infection and asthmatic airway inflammation

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 [34, 60] and case-control studies have demonstrated clear synergistic interactions between virus infection and allergen exposure in increasing risk of exacerbation [29, 30]. The detailed immunological mechanisms underlying this interaction are currently being investigated, but recent data suggest deficient production of type I (β), type II (γ), and type III (λ) IFNs, as well as Th1 cytokines (IL-12) and anti-inflammatory cytokines (IL-10), are likely to increase virus-induced lower airway inflammation [34, 61, 62]. These deficiencies are also accompanied by augmented production of Th2 cytokines suggesting that perhaps allergen-induced inflammation is also increased in the pathogenesis of virus-induced asthma exacerbations.

Bronchial inflammation is likely therefore a central event for virus-induced asthma exacerbations. 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 initial trigger of the inflammatory reactions is epithelial cell–virus interaction.

TABLE 37.1 Current hypotheses for the pathogenesis of virus-induced asthma exacerbations.

Epithelial disruption	Reduced ciliary clearance Increased permeability Loss of protective functions Kinins
Mediator production	Complement Arachidonic acid metabolites Nitric oxide Reactive oxygen products Cytokines
Induction of inflammation	Chemokines Immune cell activation Adhesion molecule induction Impaired innate IFN production
Immune dysregulation	Impaired apoptosis Impaired Th1 immunity Impaired IL-10 production Augmented Th2 immunity Increased total IgE
IgE dysregulation	Antiviral IgE production Airway smooth muscle
Airway remodeling	Fibroblasts Myofibroblasts Growth factors Increased cholinergic sensitivity
Alterations of neural responses	Neuropeptide metabolism modulation β-adrenergic receptor dysfunction

Table 37.1 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.

The role of the airway epithelial cell

The airway epithelium is an important component of antiviral defense. In addition to its function as a physical barrier to the entry of viruses, the responses of epithelial cells (EC) following viral infection, whether or not this results in destruction of the cell, contribute to both innate and adaptive antiviral immune responses. Information regarding the effects of RV on EC comes from *in vivo* studies and from *in vitro* models using either cultured primary airway EC or cell lines of epithelial cell origin such as A549, BEAS-2B, and H292.

EC contribute to the immune response following virus infection through the production of cytokines and chemokines (Fig. 37.1). 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 [63] (Fig. 37.2).

The extent of epithelial cell destruction observed in the airway varies according to virus type. Influenza typically causes extensive necrosis [64], whereas RV causes little or only patchy damage. Destruction of epithelial cells results in both an increase in epithelial permeability, and increased penetration of irritants and allergens, and exposure of the extensive network of afferent nerve fibers. Both effects may contribute to increased bronchial hyperresponsiveness.

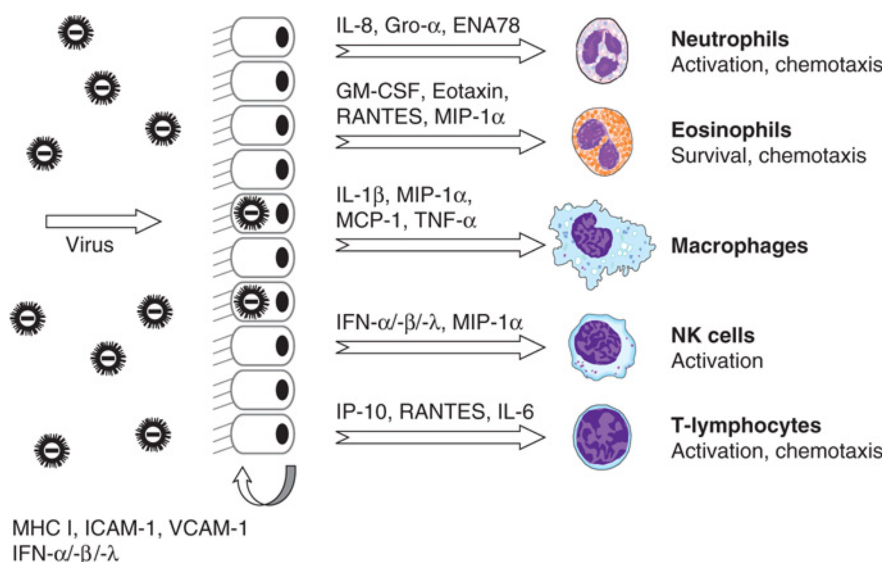

FIG. 37.1 Airway epithelial cells participate in the immune response to respiratory virus, producing a variety of cytokines and chemokines with actions on other cells. In addition the migration of inflammatory cells is aided by the upregulation of adhesion molecules and interferons help to establish an antiviral state in neighboring epithelial cells. Upregulation of MHC class I may facilitate presentation of viral antigens.

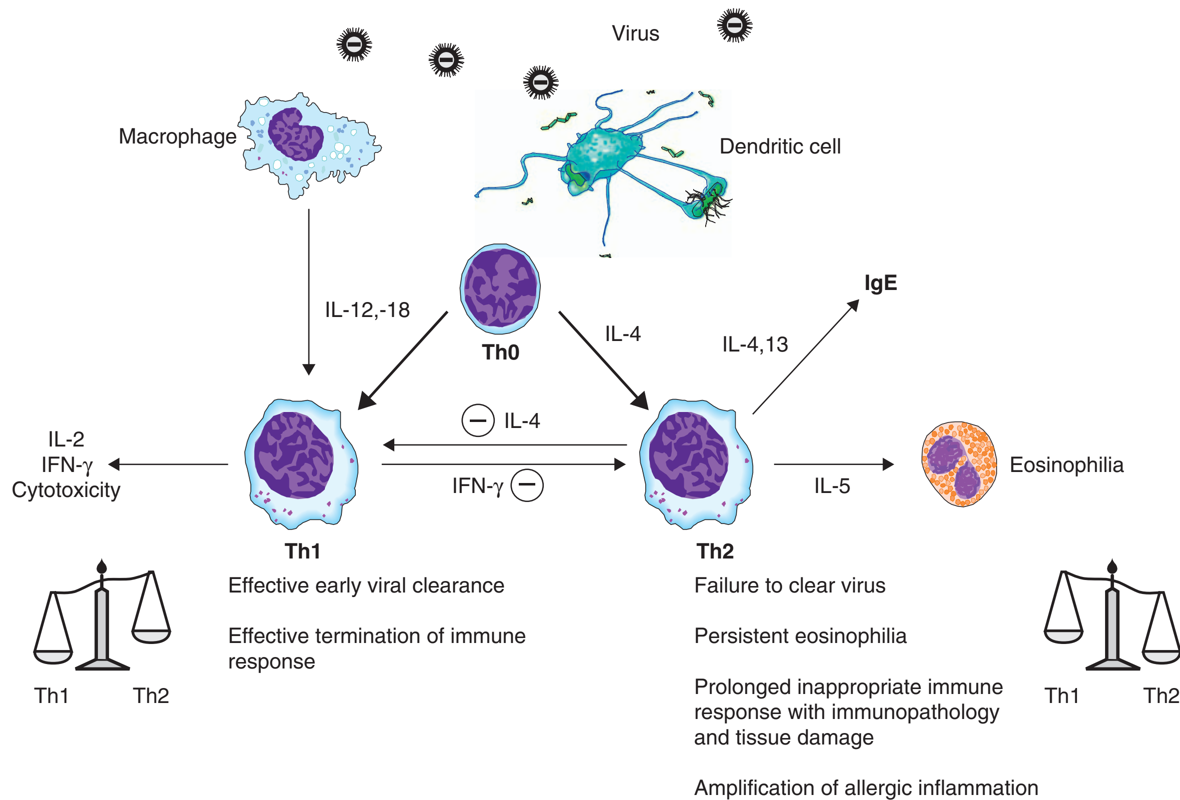

FIG. 37.2 Preexisting asthmatic airway inflammation may modify a predominantly Th1 antiviral immune response, favoring a Th2 or mixed response which may provide less efficient viral clearance and result in prolonged virus-induced inflammation, increased associated immunopathology and increased tissue damage.

In vivo RV causes some shedding of infected ciliated EC [65], but the extent of viral infection of the epithelium may be incomplete even in the nose [66]. *In vitro* studies exposing monolayer cultures of nasal epithelial cells to respiratory viruses at 10^3–10^4 $TCID_{50}$/ml demonstrate no detectable CPE (carboxypeptidase E) with RV or coronavirus in contrast to the extensive destruction with influenza and adenovirus [67]. *Ex vivo* infection of cells from both the upper and the lower respiratory tract suggest that less than 10% of cells in the epithelium are infected by RV [68], however the extent of virus-induced epithelial damage may be considerably greater in asthmatic than in normal subjects, as asthmatic epithelium has been shown to be much more susceptible *in vitro* [61]. *In vivo* studies of degrees of epithelial damage would be technically challenging, but could generate interesting findings.

Receptors for entry of RV into host cells

Viruses enter into and replicate within airway EC. Entry is dependent on the interaction with host cell surface proteins which function as receptors. In the case of the major group RV this is ICAM-1 [69] and infection can be blocked by antibodies to ICAM-1 or with soluble ICAM-1 [37]. There is relatively limited expression of ICAM-1 in airway epithelium prior to RV infection [70] and this may explain the patchy nature of infection. The upregulation of ICAM-1 in the asthmatic airway is one possible explanation for the increased severity of RV infection in asthma. RV upregulates expression of its own receptor ICAM-1 both *in vitro* and *in vivo*. Following experimental infection with RV, ICAM-1 expression is upregulated in nasal epithelium within 24 h, declining by day 5 [71]. RV has similar effects on EC from the lower airway. RV has been shown to upregulate ICAM-1 in primary bronchial EC *in vitro* [72] and ICAM-1 is upregulated in bronchial biopsies following experimental infection of asthmatic subjects with RV16 [73].

There are two forms of ICAM-1: membrane bound (mICAM-1) which favors viral infection by acting as a virus receptor and soluble (sICAM-1) which binds virus outside the cell and can thereby inhibit virus infection. RV infection of EC is reported to alter the balance in favor of further infection by inducing mRNA for mICAM-1 whilst suppressing that of sICAM-1 [74]. The LDL receptor is the receptor for the minor group RV. RV2 infection of primary human tracheal EC (PHTEC) is blocked by an antibody to the LDL receptor and is also reported to upregulate LDL-R expression [48].

ICAM-1 expression by human nasal EC is upregulated *in vitro* by exposure to a number of inflammatory cytokines and mediators including IL-1β, IL-8, IFN-γ, TNF-λ, and the eosinophil-derived proteins MBP and ECP [75]. IL-1β in particular may be important in RV-induced induction of

ICAM-1. Antibodies to IL-1β but not TNF-α decreased viral replication and ICAM-1 expression by PHTEC [76]. Not all respiratory epithelial cell lines behave in the same way as primary EC – for example A549 cells express ICAM-1 at lower levels constitutively and show upregulation by IFN-γ and TNF-α but not by ECP or MBP. The effect of IFN-γ is complex. Whilst IFN-γ upregulates ICAM-1 in uninfected cells this cytokine inhibits ICAM-1 upregulation by RV14 in H292 cells and its presence results in reduced viral titers [72, 77].

A preexisting elevation of ICAM-1 expression in the asthmatic airway may contribute to increased symptom severity of RV infection. Type 2 cytokines (IL-4, IL-5, IL-13) upregulate ICAM-1 in H-292 cells [78]. Allergen challenge results in upregulation of ICAM-1 on conjunctival and nasal EC in atopics [79]. In nasal brushing EC from atopics, basal ICAM-1 levels were increased relative to nonatopics and elevated in the relevant allergen season. Nasal EC from atopics showed further upregulation after *in vitro* culture with allergen. The highest basal ICAM-1 was found on nasal polyp EC and this was increased further after RV14 infection. Viral titers after RV14 infection were significantly higher for polyp EC than for nonatopic and atopic nonpolyp EC [80].

Modification of EC ICAM-1 expression is therefore of possible therapeutic benefit. *In vitro* RV increases expression of ICAM-1 and VCAM-1 in primary bronchial EC (PBEC) cultures and in A549 cells via a mechanism involving NF-κB [5, 81, 82]. One of the actions of corticosteroids is inhibition of NF-κB [83]. In both A549 cells and in PBEC pretreatment with three 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. Dexamethasone suppresses ICAM-1 in PHTEC and inhibits RV infections [84]. Dexamethasone does not inhibit infection of PHTEC by minor group RV2 [85]. Disappointingly, a study of inhaled corticosteroids in asthmatics prior to experimental RV infection failed to show reduced virus-induced ICAM-1 expression in bronchial biopsies [73] 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.

Other drugs which affect EC ICAM-1 include reducing agents [86], the H_1 receptor antagonists desloratidine/loratidine which inhibit RV-induced ICAM-1 upregulation in HPBEC and in A549 cells [87] and erythromycin which inhibits infection of PHTEC by both major group RV14 and minor group RV2 through effects including ICAM-1 reduction, blockage of RV RNA entry into endosomes and small reductions in LDL receptor expression [88].

RV induction of EC production of cytokines and chemokines

EC can activate and recruit a variety of other cell types such as lymphocytes, eosinophils, and neutrophils through the production of chemokines and cytokines (Fig. 37.1). Such cells are important components of the antiviral response but may also contribute to airway inflammation and dysfunction in asthma.

Type 1 interferons

Interferons (IFN) play an important role in innate resistance to viruses [89], acting on virus-infected cells and surrounding cells to produce an antiviral state characterized by the expression and antiviral activity of IFN-stimulated genes (ISGs). There are three main types of IFN, type 1 (IFN-α, IFN-β, IFN-ω, IFN-τ), type 2 (IFN-γ) and the recently discovered type 3 (IFN-λs [90, 91]). EC can produce both type 1 and type 3 IFNs. There are 14 IFN-α genes but only 1 IFN-β gene. IFN-β synthesis involves NF-κB, ATF/JUN and the interferon regulatory factors (IRFs) (up to 10 of which are currently identified), activation of which occurs in response to virus-specific signals including dsRNA, a product of the replication of ssRNA viruses such as RV, RSV, and influenza.

IFN-β and IFN-α4 are expressed early through the action of IRF3. Activation of the IFN intracellular signaling pathway is required for induction of IRF7 which is required for transcription of the full range of IFNs. DNA microarray analysis has shown that following binding to their receptors on target cells IFNs trigger a complex signaling pathway (mainly JAK-STAT) resulting in the transcription of hundreds of ISGs [92].

Several ISGs have been well studied. These include the dsRNA-activated serine/threonine protein kinase (PKR) which reduces cellular mRNA translation and transcriptional events, two enzymes involved in mRNA degradation, 2′5′oligoadenylate synthetase (OAS) and RNase L, the myxovirus resistance (Mx) proteins and RNA-specific adenosine deaminase (ADAR) which is involved in RNA editing. These ISGs inhibit virus replication at a number of levels and not surprisingly, viruses have evolved mechanisms to resist the actions of IFNs, for example blocking of PKR by the influenza NS1 protein [93]. IFNs also upregulate cellular expression of MHC class I and II molecules therefore increasing antigen presentation to $CD8^+$ and $CD4^+$ T-cells and enhancing cellular immune responses.

One recent study has examined type 1 interferon production by primary bronchial epithelial cells from normal and asthmatic subjects infected *ex vivo* with RV [40]. Asthmatic EC following infection released a higher titer of virus into culture supernatant and exhibited impaired apoptosis and a greater degree of necrotic cell death, favoring release of virus from dying cells. This was accompanied by lower concentrations of IFN-β after infection. Addition of IFN-β to asthmatic ECs inhibited virus replication to levels observed in ECs from normal subjects. This study suggests that the production of IFN-β is deficient in asthmatic ECs and that replacement/augmentation of IFN-β to boost the innate immune response could be a novel approach to treatment of virus-induced asthma exacerbations [94].

Type III interferons

A new family of interferons, called type III IFN-λs, and characterized by three elements: λ1, λ2, and λ3, also termed IL-29, IL-28A, and IL-28B, has recently been described

[90, 91]. The three highly homologous IFN-λ proteins demonstrate limited (about 20%) homology to type I IFNs [95]. Human IFN-λs bind to a unique heterodimeric receptor (IFN-λR), composed of CRF2-12 (also designated IFN-λR1), and CRF2-4 (also designated IL-10R2) shared with other class II cytokine-receptor ligands including IL-10, IL-22, and IL-26 [90].

Viral infection induces upregulation of IFN-λ mRNA in epithelial cells, peripheral blood mononuclear cells (PBMCs), and dendritic cells [90, 91, 96–99]. IFN-λs exhibit some similar biological properties to type I IFNs: they induce Jak/STAT pathways that lead to the upregulation of several antiviral proteins and enzymes including 2′,5′-OAS and MxA, have antiviral activity *in vitro* [90, 91, 97] and have also exhibited antviral activity in an *in vivo* model of vaccinia virus-infected mice [100]. Based on current knowledge, it thus appears that both IFN-α/-β and IFN-λ ligand-receptor systems can independently induce an antiviral state by engaging similar participants of the antiviral response, though the signaling pathways involved in IFN-λ production are currently largely unknown. Recent data suggest that IFN-λ may be involved in antiviral responses against RV. *In vitro* RV infection of a bronchial epithelial cell line (BEAS-2B) led to IFN-λ production and this cytokine demonstrated a dose-dependent antiviral effect against RV [62]. Moreover, IFN-λ production occurred after *in vitro* infection of primary bronchial epithelial cells, macrophages, and BAL cells from healthy volunteers.

We have recently investigated the production of IFN-λs in response to RV in primary bronchial ECs and in BAL cells (90% macrophages) from normal and asthmatic subjects [62]. Production of IFN-λ1 and IFN-λ2–3 was deficient in ECs and BAL cells from asthmatic subjects after *in vitro* RV infection and induction of IFN-λs by RV infection of ECs was strongly inversely related to RV replication. To determine whether IFN-λ production was important in determining responses to RV infection *in vivo*, the same volunteers were then experimentally infected with RV16, the severity of symptoms and reductions in lung function were monitored and the virus load was determined in BAL. *In vitro* production of IFN-λs by RV infection of BAL cells was strongly inversely correlated with both common cold symptoms and *in vivo* virus load and strongly positively correlated with severity of falls in lung function, in asthmatic and normal volunteers experimentally infected with RV16. Asthmatic patients, in whom *in vitro* IFN-λ production in BAL cells was significantly lower than in normal subjects, exhibited increased common cold symptoms and reductions in lung function and virus load after *in vivo* RV16 infection. In marked contrast normal subjects had robust IFN-λ responses, less severe cold symptoms, lower virus load, and no significant changes in lung function. These results document the importance of IFN-λ in the host defense against RV infection *in vitro* and *in vivo* and indicate that deficient IFN-λ production is likely to be important in the pathogenesis of virus-induced asthma exacerbations.

Proinflammatory cytokines and chemokines

Viral infection of the respiratory tract results in significant changes in the pattern of cytokine expression by a number of cell types, by both 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 EC. 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 EC lines or macrophages 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 granulocyte-macrophage colony stimulating factor (GM-CSF) and the chemokines IL-8, ENA-78, RANTES, and IP-10 and macrophage inflammatory protein (MIP)-1α in response to RV and RSV [37, 101–103]. *In vivo* these cytokines can be found in nasal lavage in association with RV infection [104].

The specific roles of individual cytokines in the human lower airway during viral infection are not well understood, but increasing information is becoming available. Such cytokines and chemokines activate and recruit a variety of other cells including lymphocytes, eosinophils, and neutrophils. IL-1, TNF-α, and IL-6 share proinflammatory 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 [105]. TNF-α is a potent antiviral cytokine but *in vitro* increases the susceptibility of cultured epithelial cells to infection by RV14 through upregulation of ICAM-1 [37]. IL-6 has been shown to stimulate IgA-mediated immune responses. IL-11 may also be important in virus-induced asthma [106]. It appears to cause bronchoconstriction by a direct effect on bronchial smooth muscle [102]. Production of this cytokine by human stromal cells *in vitro* is increased by RV14, RSV, and parainfluenza type 3 but not by cytomegalovirus (CMV), herpes simplex virus (HSV)-2 or adenovirus. *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 [107]. Studies in MIP-1α knock-out mice suggest that it mediates pneumonitis due to influenza [108]. The other chemokines IL-8 and ENA-78, will recruit and activate neutrophils, while RANTES and IP-10 will do the same for lymphocytes [103].

Viral upregulation of cytokines and chemokines may be mediated through certain key transcription factors. Increases in IL-6 and IL-8 production by cultured epithelial cells due to RV was dependent on NF-κB [82, 107, 109] and further upstream, protein kinase R (PKR)-mediated RV-induced RANTES, IL-8, and IL-6 [110]. Rhinovirus induction of IL-8 was shown to require IkappaB kinase-beta (IKKβ) and the transcription factor NF-IL-6 as well as NF-κB. Similar observations have been made with regard to the induction of IL-1, -6, -8, -11, and TNF-α by RSV [111, 112], thus the potential role of inhibition of NF-κB in this context has generated considerable interest.

In addition to the induction of IL-1α and IL-1β RV infection results in substantial increases in IL-1ra both *in vivo* and *in vitro*. This is a relatively late effect, occurring

48–72 h after infection and may contribute to symptom resolution [113].

Kinins and nitric oxide

A multitude of inflammatory mediators are generated or act on the epithelial surface. Bradykinin, a-nine-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 [114]. Bradykinin given intranasally is able to reproduce some of the symptoms of the common cold such as sore throat and rhinitis [115]. 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 [114–118].

Nitric oxide (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 [119]. Nitric oxide (NO) may be important in a range of respiratory diseases [120] including asthma [121] and in virus infection [122]. NO is produced both by the constitutive enzymes, nitric oxide synthase (NOS)1, and NOS3 and by the inducible, calcium-independent NOS2 expressed by airway EC [123] and macrophages.

In asthma there is increased NOS2 expression and an elevated level of exhaled NO [124] that falls with corticosteroid therapy; the level of exhaled NO correlates with sputum eosinophilia and methacholine responsiveness [125]. In contrast in stable COPD, exhaled NO levels are not different from normal subjects [126] although there may be an increase during exacerbations [127]. In fact *in vitro* cigarette smoke reduces cytokine-induced NOS2 mRNA expression in the LA-4 murine cell line, in A549 cells and in PHBEC [128].

The relative importance of the beneficial antimicrobial activity of NO versus the potentially disadvantageous suppression of IFN-γ may be dependent on the specific pathogen. NOS2 knock-out mice show an increased susceptibility to infections [129], perhaps because release of NO may be important for NK cell-mediated target cell killing [130]. However NO may also possess antiviral activity. *In vitro* RV induces NOS expression in HPBEC [131]. There is increased expression of NOS2 mRNA in cultured HPBEC after RV16 infection [132]. NO inhibits RV-induced production of IL-6, IL-8, and GM-CSF and viral replication in a human respiratory epithelial cell line [132, 133]. RSV also induces NOS2 and increases nitrite levels in supernatant from A549 cells, from HPBEC culture and in BAL fluid from RSV-infected BALB/c mice, effects opposed by IL-4 and dexamethasone but unaffected by IL-13 or IFN-γ [134]. Replication of RSV in Hep2 cells is inhibited following transfection with a retroviral construct containing NOS and this inhibition is abolished by the NOS inhibitor, NG-methyl-l-arginine [135]. Replication of influenza A and B in Mabin Darby kidney cells is severely impaired by the NO donor, *S*-nitroso-*N*-acetylpenicillamine [136].

Overall there is evidence that *in vivo* increased lower airway NO production may be of benefit in virus-induced asthma exacerbations. Work in a guinea pig model suggests that one mechanism for increased airway hyperresponsiveness during respiratory virus infection is through inhibition of NOS enzymes and a loss of NO-related relaxation of airway smooth muscle [137]. Studies of human asthmatics would also suggest that NO has a protective role in virus-induced exacerbations. Following experimental RV16 infection patients with the greatest increase in exhaled NO had smaller increases in histamine airway responsiveness [131].

In experimental animals parainfluenza virus-induced hyperreactivity correlates with a deficiency in constitutive NO production [137]. Increased levels of exhaled NO are found in nonasthmatic volunteers following natural colds [138] as well as in asthmatic patients after experimental RV infection [131]. 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 [133]. Interestingly, studies of viral upper respiratory tract infections have failed to demonstrate an increase in nasal NO after experimental RSV, RV, and influenza infections [139]. In normal subjects experimental influenza infection increased oral NO 8 days postinfection but had no effect on nasal NO [140]. This raises the possibility that during respiratory virus infection induction of NO is selective for the lower respiratory tract.

Signaling pathways

The responses of airway EC to virus infection are consequences of the interactions between virus and the intracellular signaling pathways of the host cell [141]. Knowledge of the mechanisms involved for rhinoviruses is currently very limited. Activation of signaling pathways may be dependent on cell surface receptor (ICAM-1, LDL-R) binding or may occur during viral replication within the cell. The need for replicative virus is demonstrated by the inhibition of RV induction of EC cytokines after UV inactivation. One product of replication, common to ssRNA viruses such as RV and also RSV and influenza, is dsRNA, which has been shown to activate components of signaling pathways including dsRNA-dependent protein kinase PKR, IKKβ, NF-κB, and p38 mitogen-activated protein kinase with resultant induction of IL-6, IL-8, and RANTES [110, 142]. Activation of EC by dsRNA may be direct or indirect through the interferon system as discussed above. It has also been reported that dsRNA and virus infections activate EC through binding to TLR3 [143, 144].

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 upregulation of production of

IL-5 and IL-1β by the airway smooth muscle itself [145]. A more recent study demonstrated that RV induction of IL-6 and IL-8 was increased in smooth muscle cells from asthmatic compared to normal subjects [146]. 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 leukocytes 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 [28]. 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 [147]. Replication also occurred in THP-1-derived macrophages but was limited in monocyte-derived macrophages which are relatively resistant to viral replication at least partly because of higher levels of type I interferon production [148]. RV entry into monocytes results in activation and the production of both IL-8 [147] and TNF-α [149]. In monocyte and THP-1-derived macrophages RV induction of TNF-α is NF-κB dependent [148].

A recent study reported that infectious but not UV-inactivated RV-increased TNF-α and IL-8 release by macrophages derived from resected lung tissue. Interestingly, infectious rhinovirus-impaired LPS and lipoteichoic acid-induced TNF-α and IL-8 secretion by macrophages as well as the macrophage phagocytic response to labeled bacterial particles [150]. This RV-induced impairment of cytokine responses to bacterial LPS and lipoteichoic acid and of phagocytosis in alveolar macrophages could lead to impairment of antibacterial host defense may have important implications in the pathogenesis of exacerbations of respiratory diseases including both asthma [151] and COPD [152]. In contrast, 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 [153], effects dramatically potentiated by subsequent exposure to bacterial LPS.

Dendritic cells

Dendritic cells are key cells in IFN production, as well as in antigen presentation both of allergens and pathogens with a capacity to induce both primary and secondary immune responses. They may also play a role in the regulation of the type of T-cell-mediated immune response [154]. RV infection has been shown to induce production of the dendritic cell attracting chemokine MIP-3α [43] and to increase in number in the lung during RSV infection [155], suggesting they are recruited to the lung during respiratory virus infections. However, they have also been shown to be produced from local precursors during RSV infection [156]. Plasmacytoid dendritic cells are likely protective against infection as they have been shown to limit virus replication in RSV infections, as well as reducing airway inflammation and airway hyperresponsiveness [157]. In contrast, others have reported induction of the high affinity IgE receptor on dendritic cells during Sendai virus infection, and linked this with induction of mucus cell metaplasia and airway hyperreactivity [158]. There is thus increasing knowledge of the immunobiology of these cells during respiratory infections but their role in the context of viral exacerbations of asthma remains unclear and further studies are needed.

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 [21] and we have recently demonstrated a trend toward increased numbers of lymphocytes in BAL from asthmatic compared to normal subjects ($p = 0.06$)[34]. Such increases coincided with peripheral blood lymphopenia, and reductions in blood total lymphocytes and $CD8^+$ T-cells correlated strongly with virus load only in asthmatic subjects[34] suggesting increased recruitment of T-cells to the asthmatic airway may be important in the context of asthma exacerbations. 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.

T-cell recruitment into the airway is at least partly under the influence of chemokines, including those whose production by EC is upregulated by viruses. The nature and the effectiveness of the specific immune response may be influenced by the balance of chemokine production by airway EC. This balance may in turn be influenced by preexisting chronic inflammation as found in asthma.

Studies of cloned T-cells suggest that Th1 and Th2 cells show differential expression of chemokine receptors. There is 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 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 [159]. Bronchial biopsies from asthmatics show high levels of expression of CCR4 and significant levels of CCR8 by T-cells [160].

Increased recruitment of T-cells to the airway as a result of virus-induced chemokine production by EC 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.

CD4$^+$ T-cells

The CD4$^+$ T-cell response to virus infection is thought to be of the T-helper 1 (Th1) type. It is thought that an effective antiviral immune response is characterized by the production of type 1 cytokines such as IFN-γ. IFN-γ, in addition to IFN-α, IFN-β, and IFN-λ from monocytes and macrophages, plays a role in establishing an "antiviral state" in neighboring cells. IFN-γ has a complex role in the pathogenesis of asthma. It appears to increase basophil and mast cell histamine release [161] but on the other hand inhibits the expression of type 2 cytokines. Production of IFN-γ is increased in PBMC [162] and in nasal secretions [104] during RV colds and in human and animal models of influenza, parainfluenza, and RSV infection [119, 163, 164]. There are exceptions where the antiviral response exhibits a Th2 character or a mixture of Th1/Th2. In animal models of RSV, different proteins of the virus may induce either Th1 or Th2 type responses and priming with such proteins prior to infection with whole virus can influence the character, effectiveness, and associated immunopathology of the immune response [165].

Asthma is believed to be characterized by type 2 inflammation. Many studies have demonstrated mutual inhibition of Th1 and Th2 cells [166, 167]. It is therefore possible within an airway with a preexisting type 2 allergic asthmatic microenvironment that there may be inhibition of the normal effective type 1 antiviral immune response or that the system may be skewed toward type 2 responses.

Papadopoulos *et al.* have shown that type 1 responses to RV are deficient in individuals with asthma [168]. PBMC taken from asthmatics and exposed *in vitro* to RV show lower levels of IFN-γ and IL-12 and higher levels of IL-4 and IL-10 in culture supernatants than cells from normal subjects. The IFN-γ/IL-4 ratio was three times lower in the asthmatic group [168].

In a study by Gern *et al.* of experimental RV16 infection in subjects with allergic rhinitis or asthma, the balance of airway Th1 and Th2 cytokines in induced 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 [169].

We have recently investigated the production of type 1 and type 2 cytokines from BAL cells in asthmatic and normal subjects. We found that production of the type 1 cytokines IL-12 and IFN-γ were suppressed in the asthmatics, while production of the type 2 cytokines IL-4, -5 and, -13 were all increased [34]. Importantly CD4$^+$ T-cell production of IFN-γ was strongly inversely correlated with virus load and reductions in lung function in the asthmatic subjects when they then underwent RV experimental infection, suggesting that CD4$^+$ T-cell production of IFN-γ is protective in the context of RV-induced asthma [34]. Conversely, CD4$^+$ T-cell production of each of IL-4, -5, and -13 was positively correlated with lower respiratory symptom severity, suggesting CD4$^+$ T-cell production of each of IL-4, -5, and -13 are associated with more severe exacerbations. These data are novel and important, but causal roles cannot be established in such human challenge studies. Investigation of the possible causal role of each these cytokines *in vivo* is now required using the newly developed mouse model [43].

CD8$^+$ T-cells

CD8$^+$ T-cells are important effector cells in specific cell-mediated antiviral immunity. They also demonstrate polarization of cytokine production, the major Tc1 cytokine again being IFN-γ and are believed to regulate CD4 Th1/Th2 balance [170]. In a murine asthma model induction of bystander CD4$^+$ Th2 responses to ovalbumin resulted in a switch of virus-peptide specific lung CD8$^+$ T-cells to production of Tc2 cytokines including IL-5 with, after virus peptide challenge, induction of airway eosinophilia [171]. If this occurs in man it suggests 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. The role of CD8$^+$ T-cell production of type 1 and type 2 cytokines in virus-induced asthma exacerbations requires investigation.

$\gamma\delta$-TCR$^+$ T-cells

$\gamma\delta$-TCR$^+$ T-cells are a minor subset of T-cells expressing receptors distinct from the $\alpha\beta$ receptors found on the majority of T-cells involved in adaptive immunity. There appear to be at least two types of $\gamma\delta$-TCR$^+$ T-cells. The first type is found in the lymphoid tissue of all vertebrates and displays highly diversified receptors. The second type, intraepithelial $\gamma\delta$-TCR$^+$ T-cells, display receptors of limited diversity. It has been suggested that this second subset recognize molecules expressed only by nearby infected cells. Candidate ligands are heat-shock proteins, MHC class IB molecules and unorthodox nucleotides and phospholipids. Antigen is recognized directly rather than as processed peptide presented by MHC. Recognition of molecules expressed as a consequence of infection rather than pathogen-specific molecules themselves would place $\gamma\delta$-TCR$^+$ T-cells at the intersection of innate and adaptive immunity [172].

However, exaggerated responses to various pathogens and self tissues have been found in studies of mice deficient in $\gamma\delta$-TCR$^+$+ T-cells rather than deficiencies in control of pathogens. Such work has suggested that at least some $\gamma\delta$-TCR$^+$+ T-cells have a regulatory role in modulating immune responses [173], a function consistent with their demonstrated ability to secrete regulatory cytokines when activated.

It has been reported that γδ-TCR$^+$ T-cells are more numerous in the asthmatic airway [174]. A recent study found a greater capacity for production of IL-5 and IL-13 in bronchoalveolar lavage γδ-TCR$^+$ T-cells from asthmatic subjects [175]. If virus infection results in the release of molecules from epithelial cells that activate γδ-TCR$^+$ T-cells in the respiratory mucosa, such cells could provide a source of type 2 cytokines that influence the nature of the subsequent immune response. The role of γδ-TCR$^+$ T-cells in virus-induced asthma exacerbations requires investigation.

Eosinophils

Eosinophils are increased in bronchial epithelium in biopsies taken from normal and asthmatic volunteers following experimental RV infection; in a small study eosinophilic inflammation persisted for up to 6 weeks in asthmatic subjects [21] and in our recent study eosinophil numbers in the BAL were significantly (threefold) increased in asthmatic compared to normal subjects during the acute RV infection and correlated significantly with reductions in lung function only in the asthmatic subjects [34]. In allergic rhinitis experimental RV infection increases BAL eosinophils following segmental allergen challenge, again persisting for 6 weeks [28], and increased levels of ECP are found in the sputum of RV-infected subjects [23] and during naturally occurring acute exacerbations of asthma [13]. Eosinophils accumulate in the airway under the influence of IL-5, GM-CSF, IL-8, RANTES, and eotaxin [176]. Of these only IL-5 has not been shown to be produced by airway EC *in vitro* after infection by RV. Expression of RANTES is increased in nasal secretions of children with natural virus-induced asthma [107]. RANTES is upregulated in primary nasal EC cultures by RSV [177] and RV [178]. GM-CSF is important in bone marrow eosinophil production and in eosinophil survival [176] but levels are not increased during viral upper respiratory tract infections [107, 179, 180]. Levels of eotaxin in nasal lavage rise after experimental RV16 infection [181]. 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 NL eosinophil count prior to infection [32]. Eosinophils may contribute to viral antigen presentation. Eosinophils 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 [182]. Eosinophils have antiviral actions in parainfluenza-infected guinea pigs [183]. EDN and ECP have ribonuclease activity and reduce RSV infectivity [184]. The role of the eosinophil in the antiviral immune response thus requires further evaluation.

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 [185]. Airway mast cell numbers are upregulated 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. Leukotriene (LT) C_4 is among the 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 [186]. Levels correlated well with the symptoms of the disease with concentrations in infants presenting with bronchiolitis being fivefold higher than in those with only upper respiratory tract symptomatology. Cultured alveolar macrophages can be infected with parainfluenza virus and respond with an increase in arachidonic acid metabolism. Several of the products of this pathway are known inducers of airway constriction, including LTC_4, LTD_4, $PGF_{2\alpha}$, and thromboxanes and/or stimulants of mucous secretion such as $PGF_{2\alpha}$, LTB_4, and 5-hydroxyeicosatetraenoic acid [187]. RV infection has been shown to induce prostaglandin and LT synthetic enzymes in bronchial biopsies in normal subjects, as well as trends for increased numbers of mast cells ($p = 0.07$) bronchoalveolar lavage fluid cysteinyl-leukotriene levels ($p = 0.13$), but these outcomes have not been studied in asthmatic subjects[188].

Neutrophils

Neutrophils are recruited early during respiratory viral infection in response to the production of IL-8, Gro-α, and ENA-78 by EC and activated neutrophils are a prominent feature of severe asthma. Induced sputum IS in asthmatics and nonasthmatics demonstrates a significant increase in neutrophils at day 4 of a natural cold, correlating with sputum IL-8 [189]. Similar results were obtained in IS taken 2 and 9 days after experimental RV16 infection in asthmatics. Intracellular staining demonstrated an increase in cells positive for IL-8 at day 2 attributable to increased IL-8 positive neutrophils [25]. The chemokine IL-8 is a potent chemoattractant for neutrophils but also acts on lymphocytes, basophils, and primed eosinophils. Increased IL-8 has been found in NL from children with natural colds [104]. Experimental RV16 infection of asthmatics resulted in elevated NL IL-8, correlating with cold/asthma symptom scores and histamine PC20 [25]. IS from asthmatics with exacerbations has both elevated IL-8 and neutrophilia [23, 190]. A study of experimental infection in asthmatic children also demonstrated elevated IL-8 and neutrophilia in NL during the acute infection and levels of neutrophil myeloperoxidase correlated with symptom severity [191]. In asthma, exacerbations in asthmatic adults [13] those with virus infection had increased sputum neutrophils and increased neutrophil elastase and more severe clinical disease. Such studies suggest a prominent role for the neutrophil in tissue damage during virus-induced asthma.

Natural killer cells

Natural killer (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 toward viruses [192]. 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 [193]. 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 [194]. 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. Cytokines and chemokines shown to enhance the activities of NK cells *in vitro* and *in vivo* include IFN-α/-β, IFN-γ, TNF-α, IL-2, IL-12, IL-15, IL-18, MIP-1α MIP-1β, MCP-1, 2, 3, and RANTES. Transforming growth factor (TGF)-β and IL-10 inhibit NK cell activity [195]. Type 2 cytokines may also modulate NK function, increasing NK type 2 activities and decreasing NK type 1 activities. Human NK cells cultured in medium supplemented with IL-4 differentiated into NK type 2 cells, secreting IL-5 and IL-13 and when cultured in the presence of IL-12, differentiated into NK type 1 cells secreting IL-10 and IFN-γ. IL-4 and IL-13 have also been shown to suppress IL-2-induced cytolytic and proliferative activities and IFN-γ production of human NK cells [196]. NK cell production of IL-5 is enhanced by IL-4 and reduced by IL-10 and IL-12 [197]. In a mouse model of asthma, intracellular staining of NK cells has demonstrated IL-5 production and depletion of NK cells resulted in reduced airway eosinophilia [198].

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. In addition, if NK type 2 function is favored by the asthmatic microenvironment, production of type 2 cytokines by NK cells in response to virus infection might be one mechanism for amplification of allergic inflammation. These hypotheses are as yet untested in human studies of experimental virus infection.

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 [199] 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.

Upregulation 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 [200]. 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 [201]. 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 [202]. 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 [203]. National and global initiatives have been launched and management guidelines have been published [204, 205].

The frequency of exacerbations is a major factor in the quality of life of patients with COPD [206]. 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 [207]. The infection and consequent host inflammatory response result in increased airway obstruction.

Epidemiology

It is likely that two-thirds to three-quarters of COPD 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 [208]. Between 60% and 70% of exacerbations are associated with preceding symptoms of a common cold. The frequency of exacerbations requiring hospitalization is higher in the winter. 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 [209]. 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% [208, 210–212]. Other studies of more severe exacerbations using more comprehensive PCR methods confirmed the importance of virus infection, with viruses being detected in around 50% of exacerbations [152, 213]. Because these studies all involve sampling relatively late in the course of illness, it is likely that these detection rates underestimate the true importance of virus infections.

The role of bacteria in precipitating exacerbations is also somewhat controversial. Bacteria may have a primary role in the development of an exacerbation and/or represent a secondary superinfection of an initial viral process. Various bacterial species are present in the airways of 25–40% of patients, even when the COPD is stable but increased frequency of recovery of bacteria during exacerbations (~55%) as well as higher bacterial loads during exacerbations both suggest that they play an important role in a significant number [152, 214, 215]. Significant bacterial infection has been suggested when there is an abundance of neutrophils in the sputum [216] and when the sputum is purulent and green (due to neutrophil myeloperoxidase) [217]. Bacteria may contribute to the pathogenesis of an exacerbation due to increased bacterial loads of bacteria already colonizing diseased airways, however in addition to this, acquisition of new bacterial strains has also been shown to be important, increasing the risk of exacerbation over twofold [218].

The major bacterial organisms associated with COPD exacerbations are nontypable *Haemophilis influenzae*, *Streptococcus pneumoniae*, and *Moraxella (Branhamella) catarrhalis* [219, 220]. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* may play a part [221, 222]. 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 [223].

Recent studies have addressed the role of coinfection with both bacteria and viruses – one study showed this to occur in 25% of exacerbations, and that patients with dual infection had more marked lung function impairment and longer hospitalizations [152]. Another reported that exacerbations with both cold symptoms (a marker of putative viral infection) and a bacterial pathogen, the FEV_1 fall was greater and symptom count was higher than those with a bacterial pathogen alone [224]. Thus even in exacerbations in which viruses are detected, bacteria can also contribute to exacerbation severity.

Although the results of placebo-controlled trials show conflicting results, overall the effects of antibiotic treatment also support 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 [225]. The largest study included 362 exacerbations in 173 outpatients [216]. 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 co-trimoxazole, 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 [205] 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 [226].

Evidence for a role for bacterial infection in pathogenesis/progression of COPD

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 [207].

In these diseases chronic bacterial infection occurs as the host immune response is unable to clear the bacteria, the continuous infection leads to continuous inflammatory responses and continuous tissue damage [207]. Host and bacterial factors attract and activate neutrophils, which produce proteinases and reactive oxygen species. Lung antiproteinase defenses are overwhelmed. Both proteinase enzymes and reactive oxygen species cause damage to the epithelium, stimulating mucus production and impairing mucociliary clearance. Neutrophil elastase stimulates epithelial cell production of the chemokine IL-8 which attracts further neutrophils and in addition impairs phagocytosis by destroying antibody and cleaving complement receptors from neutrophils and complement components from bacteria. Neutrophils are also stimulated by cigarette smoke.

Identification of bacteria during exacerbation is also associated with increased levels of inflammatory mediators in BAL and/or sputum. These include reactive oxidant species, IL-8, TNF-α, neutrophil elastase, LTB_4, and myeloperoxidase and many others. These clearly have potential to cause considerable tissue damage, as well as further recruitment and activation of inflammatory cells. COPD patients, particularly those at the more severe end of the disease spectrum, may also be chronically colonized by bacteria between exacerbations, bacterial numbers then increasing during exacerbations. In a study using bronchoscopic protected brush specimens [214] 10 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 [227] 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.

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 breakdown local immunoglobulin, products that alter immune effector cell function, adherence to mucus and damaged epithelium, or other mechanisms of avoiding immune surveillance [207, 228].

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 pathogenesis/progression of COPD

Exacerbations associated with viral infections also have increased levels of many inflammatory mediators also found in bacterial exacerbations including TNF-α, IL-8, neutrophil elastase, myeloperoxidase, and LTB_4. In contrast to bacterial exacerbations where neutrophils and neutrophil products predominate, during a viral exacerbation both neutrophils and eosinophils are present and eosinophil products such as ECP are also increased [152]. Other mediators implicated include ENA-78, RANTES, and endothelin–1 [229].

It has also been suggested that persistent virus infection contributes to the progression of COPD. In particular, adenovirus 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 [230]. 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 [231]. The E1A protein has been demonstrated in airway epithelial cells from smokers [232]. It is able to amplify many host genes through attachment to the DNA-binding sites of transcription factors [233]. Airway epithelial cells transfected with E1A produce excess inflammatory cytokines such as IL-8 [234] and surface adhesion molecules such as ICAM-1 [235] after *in vitro* challenge by an NF-κB-dependent mechanism [236].

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 [209]. This suggests either that low grade persistent RSV infection contributes to COPD severity 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. Less data is available than for virus infection in asthma since this has not been a major subject of human experimental infection studies. In a small safety study of four patients, inoculation with low dose RV16 resulted in symptomatic colds, viral replication, significant increases in lower respiratory tract symptoms, and reductions in PEF and FEV_1 typical of an acute exacerbation of COPD [237]. Further studies are clearly needed in view of the increasing evidence for a major role for viruses in causing COPD exacerbations.

Therapy for infective exacerbations of asthma and COPD

Currently much of the treatment of infective exacerbations of asthma and COPD is symptomatic, consisting of increased bronchodilators, either short-acting β_2-agonists in inhaled or intravenous form or anticholinergics or theophyllines, or supportive in the form of oxygen and in severe cases noninvasive or invasive ventilatory measures. Corticosteroids are widely used in inhaled or oral form for their anti-inflammatory actions. 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 significant local and systemic side effects, in particular if oral steroid treatment is prolonged or frequent. In addition systemic steroids may interfere with the antiviral immune response resulting in reduced viral clearance [238].

In persistent asthma, control of disease is achieved predominantly with inhaled corticosteroids. There is a role for additional drugs such as long-acting β_2-agonists and leukotriene antagonists. The long-acting β_2-agonists in particular appear to increase the effectiveness of inhaled corticosteroids allowing the dose needed to achieve control to be reduced [239]. There is also evidence that these drugs in combination with inhaled corticosteroids may further reduce exacerbation frequency [240]. The leukotriene antagonists appear to be most effective in treating or preventing exacerbations in children [241, 242].

Regular corticosteroid treatment is however only partially effective at preventing exacerbations. In adult asthma inhaled steroids reduce exacerbation frequency by only 40% [243]. In school age children inhaled steroids are ineffective at reducing exacerbation frequency, duration, or severity [244]. In preschool age children with virus-induced wheeze oral steroids are ineffective even in those with primed eosinophils [245].

Specific antibiotic therapy is available for bacterial infections 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 serotyped strains of rhinovirus and several more that have not been serotyped and no effective vaccine has been introduced. A decavalent vaccine [246] developed in the 1970s was

ultimately of limited efficacy. The influenza viruses display antigenic shift and drift. Vaccines must be modified every 2–3 years to cover the strains prevalent at the time. Vaccination against RSV experienced a major setback in the 1960s when the use of formalin-inactivated virus in young babies resulted in increased disease severity following subsequent virus infection [247]. Eighty percent of vaccinated children required hospitalization when subsequently infected with RSV, as compared to 5% of controls. The lungs of two vaccinated children who died contained eosinophilic infiltrates. It has been suggested that formalin inactivation may have modified epitopes within the RSV G and F surface glycoproteins, resulting in a modified immune response to subsequent infection with enhanced immunopathology [248]. Vaccinated individuals demonstrate a number of differences from individuals who have suffered natural RSV infection including a lack of specific mucosal antibodies and deficient neutralizing and fusion-inhibiting serum antibodies [249]. There are also differences in the cell-mediated immune response with some vaccinated individuals demonstrating peripheral eosinophilia and exaggerated lymphocytic proliferative responses to RSV [250]. To protect against RSV infection a successful vaccine would need to provide more effective protection than natural infection, which is itself frequently followed by reinfection [251], and would have to be administered early in infancy to have an effect on infant bronchiolitis.

Treatment for virus-induced asthma exacerbations

Simple nonspecific treatments for the common cold do exist although their efficacy is debated. Vitamin C and zinc gluconate [252] both may shorten the duration of a cold by 1–2 days. The inhalation of humidified hot air provides symptomatic relief [253]. Nasal IFN-α is an effective treatment for the common cold [254] 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. However IFN therapy for virus-induced asthma exacerbations may be more useful in view of the deficiencies identified by recent studies. Further because of the large number of viruses producing similar clinical syndromes, the general antiviral properties of IFNs would provide significant advantage over the use of specific antiviral drugs.

Antivirals

Specific antiviral agents exist for influenza. Amantidine and rimantidine are effective against influenza A. The use of amantidine has been limited by CNS side effects such as dizziness and insomnia; fewer such side effects are seen with rimantidine. Both drugs are indicated during epidemics for treatment and prophylaxis in high risk groups including asthmatics. Neither is active against influenza B. Two neuraminidase inhibitors, zanamivir and oseltamivir, are active against both influenza A and B [255–257]. These agents are effective in preventing infection when used as prophylaxis during the influenza season and, as treatment, they reduce the duration of illness if started within 36–48 h of the onset of illness. Zanamivir must be given by inhalation whereas oseltamivir can be given orally. Ribavirin is a nucleoside analog active against RSV *in vivo* and also against influenza *in vitro*. Nebulized ribavirin therapy is licensed for use in hospitalized infants and children in the first 3 days of RSV bronchiolitis. It is however expensive and of unproven benefit on clinical outcome. Because of its toxicity it is not appropriate for asthma. RSV enriched immunoglobulin was effective as prophylaxis for infants at high risk of RSV bronchiolitis [258] but has been superseded by RSV neutralizing monoclonal antibodies [259].

Antirhinoviral agents

RV are a major target for drug treatment. It has been estimated that rhinoviruses result in 6–10 colds per year in young children [260]. As yet no effective agent is available for clinical use. Capsid-binding/canyon inhibitors block RV binding to host cell receptor (ICAM-1 in the case of the major group). One example in phase 3 clinical trials is pleconaril (Picovir). These drugs can be extremely potent but their clinical usefulness is often limited by toxicity, the need for rapid initiation of therapy and the possible development of resistance. Alternative targets include soluble ICAM-1 which inhibits major rhinovirus infection and conserved viral enzymes such as protein 3D, the RNA-dependent RNA transcriptase, protein 2C, the associated ATP-helicase, and the cysteine protease 3C.

New approaches

Alternative approaches to direct antiviral therapy are suppression of virus-induced inflammation, or strategies that promote innate or type 1 immune responses in individuals with excessive type 2 responses. 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 is an essential first step. Further work is needed to elucidate the important sites of interaction between the immunological networks of asthma and of virus infection. Greater knowledge is required if we are to identify key targets for therapeutic intervention, the aim of which will be to minimize immunopathology whilst maintaining or enhancing the host anti-viral immune response.

References

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H, and ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 368: 733–43, 2006.
2. Stevens FA. Acute asthmatic episodes in children caused by upper respiratory bacteria during colds, with and without bacterial sensitization. J Allergy 24: 221–26, 1953.

3. Bardin PG, Johnston SL, Pattemore PK. Viruses as precipitants of asthma symptoms. II. Physiology and mechanisms. *Clin Exp Allergy* 22: 809–22, 1992.
4. McIntosh K, Ellis EF, Hoffman LS, Lybass TG, Eller JJ, Fulginiti VA. The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *J Pediatr* 82: 578–90, 1973.
5. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, Cunningham A, Robinson BS, Myint SH, Ward ME et al. The relationship between upper respiratory infections and hospital admissions for asthma: A time-trend analysis. *Am J Respir Crit Care Med* 154: 654–60, 1996.
6. Minor TE, Dick EC, DeMeo AN, Ouellette JJ, Cohen M, Reed CE. Viruses as precipitants of asthmatic attacks in children. *JAMA* 227: 292–98, 1974.
7. Horn ME, Brain EA, Gregg I, Inglis JM, Yealland SJ, Taylor P. Respiratory viral infection and wheezy bronchitis in childhood. *Thorax* 34: 23–28, 1979.
8. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, Symington P, O'Toole S, Myint SH, Tyrrell DA. Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 310: 1225–29, 1995.
9. Chauhan AJ, Inskip HM, Linaker CH, Smith S, Schreiber J, Johnston SL, Holgate ST. Personal exposure to nitrogen dioxide (NO_2) and the severity of virus-induced asthma in children. *Lancet* 361: 1939–44, 2003.
10. Freymuth F, Vabret A, Brouard J, Toutain F, Verdon R, Petitjean J, Gouarin S, Duhamel JF, Guillois B. Detection of viral, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infections in exacerbations of asthma in children. *J Clin Virol* 13: 131–39, 1999.
11. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 307: 982–86, 1993.
12. Beasley R, Coleman ED, Hermon Y, Holst PE, O'Donnell TV, Tobias M. Viral respiratory tract infection and exacerbations of asthma in adult patients. *Thorax* 43: 679–83, 1988.
13. Wark PA, Johnston SL, Moric I, Simpson JL, Hensley MJ, Gibson PG. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J* 19: 68–75, 2002.
14. Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, Whitehead BF, Gibson PG. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med* 172: 433–39, 2005.
15. Berman SZ, Mathison DA, Stevenson DD, Tan EM, Vaughan JH. Transtracheal aspiration studies in asthmatic patients in relapse with "infective" asthma and in subjects without respiratory disease. *J Allergy Clin Immunol* 56: 206–14, 1975.
16. Gwaltney JMJ, Hendley O, Hayden FG, McIntosh K, Hollinger FB, Melnick JL, Turner RB. Updated recommendations for safety-testing of viral inocula used in volunteer experiments on rhinovirus colds. *Prog Med Virol* 39: 256–63, 1992.
17. Halperin SA, Eggleston PA, Beasley P, Suratt P, Hendley JO, Groschel DH, Gwaltney JM. Exacerbations of asthma in adults during experimental rhinovirus infection. *Am Rev Respir Dis* 132: 976–80, 1985.
18. Bardin PG, Fraenkel DJ, Sanderson G, Dorward M, Lau LC, Johnston SL, Holgate ST. Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy* 24: 457–64, 1994.
19. Lemanske RFJ, Dick EC, Swenson CA, Vrtis RF, Busse WW. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *J Clin Invest* 83: 1–10, 1989.
20. Cheung D, Dick EC, Timmers MC, de Klerk EP, Spaan WJ, Sterk PJ. Rhinovirus inhalation causes long-lasting excessive airway narrowing in response to methacholine in asthmatic subjects *in vivo*. *Am J Respir Crit Care Med* 152: 1490–96, 1995.
21. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 151: 879–86, 1995.
22. Grunberg K, Kuijpers EA, de Klerk EP, de Gouw HW, Kroes AC, Dick EC, Sterk PJ. Effects of experimental rhinovirus 16 infection on airway hyperresponsiveness to bradykinin in asthmatic subjects *in vivo*. *Am J Respir Crit Care Med* 155: 833–38, 1997.
23. Grunberg K, Smits HH, Timmers MC, de Klerk EP, Dolhain RJ, Dick EC, Hiemstra PS, Sterk PJ. Experimental rhinovirus 16 infection. Effects on cell differentials and soluble markers in sputum in asthmatic subjects. *Am J Respir Crit Care Med* 156: 609–16, 1997.
24. Grunberg K, Timmers MC, de Klerk EP, Dick EC, Sterk PJ. Experimental rhinovirus 16 infection causes variable airway obstruction in subjects with atopic asthma. *Am J Respir Crit Care Med* 160: 1375–80, 1999.
25. Grunberg K, Timmers MC, Smits HH, de Klerk EP, Dick EC, Spaan WJ, Hiemstra PS, Sterk PJ. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects *in vivo* [see comments]. *Clin Exp Allergy* 27: 36–45, 1997.
26. Halperin SA, Eggleston PA, Hendley JO, Suratt PM, Groschel DH, Gwaltney JM. Pathogenesis of lower respiratory tract symptoms in experimental rhinovirus infection. *Am Rev Respir Dis* 128: 806–10, 1983.
27. Calhoun WJ, Swenson CA, Dick EC, Schwartz LB, Lemanske RFJ, Busse WW. Experimental rhinovirus 16 infection potentiates histamine release after antigen bronchoprovocation in allergic subjects. *Am Rev Respir Dis* 144: 1267–73, 1991.
28. Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. *J Clin Invest* 94: 2200–8, 1994.
29. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: Case-control study. *BMJ* 324: 763, 2002.
30. Murray CS, Poletti G, Kebadze T, Morris J, Woodcock A, Johnston SL, Custovic A. Study of modifiable risk factors for asthma exacerbations: Virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 61: 376–82, 2006.
31. Linaker CH, Coggon D, Holgate ST, Clough J, Josephs L, Chauhan AJ, Inskip HM. Personal exposure to nitrogen dioxide and risk of airflow obstruction in asthmatic children with upper respiratory infection. *Thorax* 55: 930–33, 2000.
32. Avila PC, Abisheganaden JA, Wong H, Liu J, Yagi S, Schnurr D, Kishiyama JL, Boushey HA. Effects of allergic inflammation of the nasal mucosa on the severity of rhinovirus 16 cold. *J Allergy Clin Immunol* 105: 923–32, 2000.
33. de Kluijver J, Evertse CE, Sont JK, Schrumpf JA, van Zeijl-van der Ham CJ, Dick CR, Rabe KF, Hiemstra PS, Sterk PJ. Are rhinovirus-induced airway responses in asthma aggravated by chronic allergen exposure? [see comment]. *Am J Respir Crit Care Med* 168: 1174–80, 2003.
34. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, Contoli M, Sanderson G, Kon OM, Papi A, Jeffery PK, Stanciu L, Johnston SL. Rhinovirus induced lower respiratory illness is increased in asthma and related to viral load and Th1/2 cytokine and IL-10 production. *ProcNatl AcadSci USA* 24, 2008, (in press).
35. Horn ME, Reed SE, Taylor P. Role of viruses and bacteria in acute wheezy bronchitis in childhood: A study of sputum. *Arch Dis Childhood* 54: 587–92, 1979.
36. Gern JE, Galagan DM, Jarjour NN, Dick EC, Busse WW. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med* 155: 1159–61, 1997.
37. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. *J Clin Invest* 96: 549–57, 1995.
38. Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST et al. Rhinoviruses infect the lower airways. *J Infect Dis* 181: 1875–84, 2000.

39. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, Love R, Gern JE. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am J Respir Cell Mol Biol* 20: 1220–28, 1999.
40. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 201: 937–47, 2005.
41. Papadopoulos NG, Sanderson G, Hunter J, Johnston SL. Rhinoviruses replicate effectively at lower airway temperatures. *J Med Virol* 58: 100–4, 1999.
42. Jakiela B, Brockman-Schneider R, Amineva S, Lee WM, Gern JE. Basal cells of differentiated bronchial epithelium are more susceptible to rhinovirus infection. *Am J Respir Cell Mol Biol* 38(5): 517–23, 2008.
43. Bartlett NW, Walton RP, Edwards MR, Aniscenko J, Caramori G, Zhu J, Glanville N, Choy KJ, Jourdan P, Burnet J et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med* 14: 199–204, 2008.
44. Bardin PG, Fraenkel DJ, Sanderson G, van Schalkwyk EM, Holgate ST, Johnston SL. Peak expiratory flow changes during experimental rhinovirus infection. *Eur Respir J* 16: 980–85, 2000.
45. Gern JE, Calhoun W, Swenson C, Shen G, Busse WW. Rhinovirus infection preferentially increases lower airway responsiveness in allergic subjects. *Am J Respir Crit Care Med* 155: 1872–76, 1997.
46. Whitton JL, Oldstone MBA. Immune response to viruses. In: Fields BN, Knipe DN, Howley PM. *Fields Virology*, 345–74. Philadelphia, PA: Lippincott-Raven, 1996.
47. Yewdell JW, Bennink JR. Immune responses to viruses. In: Richman DR, Whiteley RJ, Hayden FG. *Clinical Virology*, 271–306. New York: Churchill Livingstone, 1997.
48. Suzuki T, Yamaya M, Kamanaka M, Jia YX, Nakayama K, Hosoda M, Yamada N, Nishimura H, Sekizawa K, Sasaki H. Type 2 rhinovirus infection of cultured human tracheal epithelial cells: Role of LDL receptor. *Am J Physiol Lung Cell Mol Physiol* 280: L409–20, 2001.
49. Bjornson AB, Mellencamp MA, Schiff GM. Complement is activated in the upper respiratory tract during influenza virus infection. *Am Rev Respir Dis* 143: 1062–66, 1991.
50. Amineva SP, Gern JE. Rhinovirus 3C protease cleaves the C3 and C5 complement factors. *Am J Respir Crit Care Med* 167: A212, 2003.
51. Kopf M, Abel B, Gallimore A, Carroll M, Bachmann MF. Complement component C3 promotes T-cell priming and lung migration to control acute influenza virus infection. *Nat Med* 8: 373–78, 2002.
52. Anders EM, Hartley CA, Reading PC, Ezekowitz RA. Complement-dependent neutralization of influenza virus by a serum mannose-binding lectin. *J Gen Virol* 75: 615–22, 1994.
53. Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just microbicidal. *Trends Immunol* 23: 291–96, 2002.
54. Barclay WS, al-Nakib W, Higgins PG, Tyrrell DA. The time course of the humoral immune response to rhinovirus infection. *Epidemiol Infect* 103: 659–69, 1989.
55. Alper CM, Doyle WJ, Skoner DP, Buchman CA, Seroky JT, Gwaltney JM, Cohen SA. Prechallenge antibodies: Moderators of infection rate, signs, and symptoms in adults experimentally challenged with rhinovirus type 39. *Laryngoscope* 106: 1298–305, 1996.
56. Alper CM, Doyle WJ, Skoner DP, Buchman CA, Cohen S, Gwaltney JM. Prechallenge antibodies moderate disease expression in adults experimentally exposed to rhinovirus strain hanks. *Clin Infect Dis* 27: 119–28, 1998.
57. Hastings GZ, Francis MJ, Rowlands DJ, Chain BM. Epitope analysis of the T cell response to a complex antigen: Proliferative responses to human rhinovirus capsids. *Eur J Immunol* 23: 2300–5, 1993.
58. Gern JE, Dick EC, Kelly EA, Vrtis R, Klein B. Rhinovirus-specific T cells recognize both shared and serotype-restricted viral epitopes. *J Infect Dis* 175: 1108–14, 1997.
59. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol* 105: 692–98, 2000.
60. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: A longitudinal cohort study. *Lancet* 359: 831–34, 2002.
61. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 201: 937–47, 2005.
62. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, Kebadze T, Mallia P, Stanciu LA, Parker HL et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med* 12: 1023–26, 2006.
63. Papi A, Stanciu LA, Papadopoulos NG, Teran LM, Holgate ST, Johnston SL. Rhinovirus infection induces major histocompatibility complex class I and costimulatory molecule upregulation on respiratory epithelial cells. *J Infect Dis* 181: 1780–84, 2000.
64. Hers JF. Disturbances of the ciliated epithelium due to influenza virus. *Am Rev Respir Dis* 93 (Suppl 77), 1966.
65. Turner RB, Hendley JO, Gwaltney JM. Shedding of infected ciliated epithelial cells in rhinovirus colds. *J Infect Dis* 145: 849–53, 1982.
66. Turner RB, Winther B, Hendley JO, Mygind N, Gwaltney JM. Sites of virus recovery and antigen detection in epithelial cells during experimental rhinovirus infection. *Acta Otolaryngol Suppl* 413: 9–14, 1984.
67. Winther B, Gwaltney JM, Hendley JO. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. *Am Rev Respir Dis* 141: 839–45, 1990.
68. Mosser AG, Brockman-Schneider R, Amineva S, Burchell L, Sedgwick JB, Busse WW, Gern JE. Similar frequency of rhinovirus-infectible cells in upper and lower airway epithelium. *J Infect Dis* 185: 734–43, 2002.
69. Greve JM, Davis G, Meyer AM, Forte CP, Yost SC, Marlor CW, Kamarck ME, McClelland A. The major human rhinovirus receptor is ICAM-1. *Cell* 56: 839–47, 1989.
70. Winther B, Greve JM, Gwaltney JMJ, Innes DJ, Eastham JR, McClelland A, Hendley JO. Surface expression of intercellular adhesion molecule-1 on epithelial cells in the human adenoid. *J Infect Dis* 176: 523–25, 1997.
71. Winther B, Arruda E, Witek TJ, Marlin SD, Tsianco MM, Innes DJ, Hayden FG. Expression of ICAM-1 in nasal epithelium and levels of soluble ICAM-1 in nasal lavage fluid during human experimental rhinovirus infection. *Arch OtolaryngolHead Neck Surg* 128: 131–36, 2002.
72. Bianco A, Spiteri MA. A biological model to explain the association between human rhinovirus respiratory infections and bronchial asthma. *Monaldi Arch Chest Dis* 53: 83–87, 1998.
73. Grunberg K, Sharon RF, Hiltermann TJ, Brahim JJ, Dick EC, Sterk PJ, van Krieken JH. Experimental rhinovirus 16 infection increases intercellular adhesion molecule-1 expression in bronchial epithelium of asthmatics regardless of inhaled steroid treatment. *Clin Exp Allergy* 30: 1015–23, 2000.
74. Whiteman SC, Bianco A, Knight RA, Spiteri MA. Human rhinovirus selectively modulates membranous and soluble forms of its intercellular adhesion molecule-1 (ICAM-1) receptor to promote epithelial cell infectivity. *J Biol Chem* 278: 11954–61, 2003.
75. Altman LC, Ayars GH, Baker C, Luchtel DL. Cytokines and eosinophil-derived cationic proteins upregulate intercellular adhesion molecule-1 on human nasal epithelial cells. *J Allergy Clin Immunol* 92: 527–36, 1993.
76. Terajima M, Yamaya M, Sekizawa K, Okinaga S, Suzuki T, Yamada N, Nakayama K, Ohrui T, Oshima T, Numazaki Y et al. Rhinovirus infection of primary cultures of human tracheal epithelium: Role of ICAM-1 and IL-1beta. *Am J Physiol* 273: L749–59, 1997.
77. Sethi SK, Bianco A, Allen JT, Knight RA, Spiteri MA. Interferon-gamma (IFN-gamma) down-regulates the rhinovirus-induced expression of intercellular adhesion molecule-1 (ICAM-1) on human airway epithelial cells. *Clin Exp Immunol* 110: 362–69, 1997.

78. Bianco A, Sethi SK, Allen JT, Knight RA, Spiteri MA. Th2 cytokines exert a dominant influence on epithelial cell expression of the major group human rhinovirus receptor, ICAM-1. *Eur Respir J* 12: 619–26, 1998.
79. Canonica GW, Ciprandi G, Pesce GP, Buscaglia S, Paolieri F, Bagnasco M. ICAM-1 on epithelial cells in allergic subjects: A hallmark of allergic inflammation. *Int Arch Allergy Immunol* 107: 99–102, 1995.
80. Bianco A, Whiteman SC, Sethi SK, Allen JT, Knight RA, Spiteri MA. Expression of intercellular adhesion molecule-1 (ICAM-1) in nasal epithelial cells of atopic subjects: A mechanism for increased rhinovirus infection?. *Clin Exp Immunol* 121: 339–45, 2000.
81. Papi A, Johnston SL. Respiratory epithelial cell expression of vascular cell adhesion molecule-1 and its up-regulation by rhinovirus infection via NF-kappaB and GATA transcription factors. *J Biol Chem* 274: 30041–51, 1999a.
82. Papi A, Johnston SL. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kappaB-mediated transcription. *J Biol Chem* 274: 9707–20, 1999b.
83. Barnes PJ, Adcock IM. Transcription factors and asthma. *Eur Respir J* 12: 221–34, 1998.
84. Papi A, Papadopoulos NG, Degitz K, Holgate ST, Johnston SL. Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells. *J Allergy Clin Immunol* 105: 318–26, 2000.
85. Suzuki T, Yamaya M, Sekizawa K, Yamada N, Nakayama K, Ishizuka S, Kamanaka M, Morimoto T, Numazaki Y, Sasaki H. Effects of dexamethasone on rhinovirus infection in cultured human tracheal epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 278: L560–71, 2000.
86. Papi A, Papadopoulos NG, Stanciu LA, Bellettato CM, Pinamonti S, Degitz K, Holgate ST, Johnston SL. Reducing agents inhibit rhinovirus-induced up-regulation of the rhinovirus receptor intercellular adhesion molecule-1 (ICAM-1) in respiratory epithelial cells. *FASEB J* 16: 1934–36, 2002.
87. Papi A, Papadopoulos NG, Stanciu LA, Degitz K, Holgate ST, Johnston SL. Effect of desloratadine and loratadine on rhinovirus-induced intercellular adhesion molecule 1 upregulation and promoter activation in respiratory epithelial cells. *J Allergy Clin Immunol* 108: 221–28, 2001.
88. Suzuki T, Yamaya M, Sekizawa K, Hosoda M, Yamada N, Ishizuka S, Yoshino A, Yasuda H, Takahashi H, Nishimura H et al. Erythromycin inhibits rhinovirus infection in cultured human tracheal epithelial cells. *Am J Respir Crit Care Med* 165: 1113–18, 2002.
89. Samuel CE. Antiviral actions of interferons. *Clinical Microbiology Reviews* 14:778–809, 2001.
90. Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, Langer JA, Sheikh F, Dickensheets H, Donnelly RP. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 4: 69–77, 2003.
91. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol* 4: 63–68, 2003.
92. Katze MG, He Y, Gale M. Viruses and interferon: A fight for supremacy. *Nat Rev Immunol* 2: 675–87, 2002.
93. Bergmann M, Garcia-Sastre A, Carnero E, Pehamberger H, Wolff K, Palese P, Muster T. Influenza virus NS1 protein counteracts PKR-mediated inhibition of replication. *J Virol* 74: 6203–6, 2000.
94. Contoli M, Stanciu L, Message SD, Papi A, Johnston SL. Susceptibility to asthma exacerbations: Antiviral immunity and protection against asthma exacerbations. In: Johnston SL, O'Byrne PM. *Exacerbations of asthma*, 167–85. UK: Informa, 2007.
95. Kotenko SV. The family of IL-10-related cytokines and their receptors: Related but to what extent?. *Cytokine Growth Factor Rev* 13: 223–40, 2002.
96. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. *J Virol* 79: 3851–54, 2005.
97. Osterlund P, Veckman V, Siren J, Klucher KM, Hiscott J, Matikainen S, Julkunen I. Gene expression and antiviral activity of alpha/beta interferons and interleukin-29 in virus-infected human myeloid dendritic cells. *J Virol* 79: 9608–17, 2005.
98. Coccia EM, Severa M, Giacomini E, Monneron D, Remoli ME, Julkunen I, Cella M, Lande R, Uze G. Viral infection and Toll-like receptor agonists induce a differential expression of type I and lambda interferons in human plasmacytoid and monocyte-derived dendritic cells. *Eur J Immunol* 34: 796–805, 2004.
99. Spann KM, Tran KC, Chi B, Rabin RL, Collins PL. Suppression of the induction of alpha, beta, and lambda interferons by the NS1 and NS2 proteins of human respiratory syncytial virus in human epithelial cells and macrophages. *J Virol* 78: 4363–69, 2004.
100. Bartlett NW, Buttigieg K, Kotenko SV, Smith GL. Murine interferon lambdas (type III interferons) exhibit potent antiviral activity *in vivo* in a poxvirus infection model. *J GenVirol* 86: 1589–96, 2005.
101. Becker S, Quay J, Soukup J. Cytokine (tumor necrosis factor, IL-6, and IL-8) production by respiratory syncytial virus-infected human alveolar macrophages. *J Immunol* 147: 4307–12, 1991.
102. Einarsson O, Geba GP, Zhu Z, Landry M, Elias JA. Interleukin-11: Stimulation *in vivo* and *in vitro* by respiratory viruses and induction of airways hyperresponsiveness. *J Clin Invest* 97: 915–24, 1996.
103. Edwards MR, Johnson MW, Johnston SL. Combination therapy: Synergistic suppression of virus-induced chemokines in airway epithelial cells. *Am J Respir Cell Mol Biol* 34: 616–24, 2006.
104. Corne JM, Lau L, Scott SJ, Davies R, Johnston SL, Howarth PH. The relationship between atopic status and IL-10 nasal lavage levels in the acute and persistent inflammatory response to upper respiratory tract infection. *Am J Respir Crit Care Med* 163: 1101–7, 2001.
105. Proud D, Gwaltney JMJ, Hendley JO, Dinarello CA, Gillis S, Schleimer RP. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. *J Infect Dis* 169: 1007–13, 1994.
106. Einarsson O, Geba GP, Zhou Z, Landry ML, Panettieri RAJ, Tristram D, Welliver R, Metinko A, Elias JA. Interleukin-11 in respiratory inflammation. *AnnNY Acad Sci* 762: 89–100, 1995.
107. Teran LM, Seminario MC, Shute JK, Papi A, Compton SJ, Low JL, Gleich GJ, Johnston SL. RANTES, macrophage-inhibitory protein 1alpha, and the eosinophil product major basic protein are released into upper respiratory secretions during virus-induced asthma exacerbations in children. *J Infect Dis* 179: 677–81, 1999.
108. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O. Requirement of MIP-1 alpha for an inflammatory response to viral infection. *Science* 269: 1583–85, 1995.
109. Johnston SL, Papi A, Bates PJ, Mastronarde JG, Monick MM, Hunninghake GW. Low grade rhinovirus infection induces a prolonged release of IL-8 in pulmonary epithelium. *J Immunol* 160: 6172–81, 1998.
110. Edwards MR, Hewson CA, Laza-Stanca V, Lau HT, Mukaida N, Hershenson MB, Johnston SL. Protein kinase R, IkappaB kinase-beta and NF-kappaB are required for human rhinovirus induced pro-inflammatory cytokine production in bronchial epithelial cells. *Mol Immunol* 44: 1587–97, 2007.
111. Mastronarde JG, He B, Monick MM, Mukaida N, Matsushima K, Hunninghake GW. Induction of interleukin (IL)-8 gene expression by respiratory syncytial virus involves activation of nuclear factor (NF)-kappa B and NF-IL-6. *J Infect Dis* 174: 262–67, 1996.
112. Bitko V, Velazquez A, Yang L, Yang YC, Barik S. Transcriptional induction of multiple cytokines by human respiratory syncytial virus requires activation of NF-kappa B and is inhibited by sodium salicylate and aspirin. *Virology* 232: 369–78, 1997.
113. Yoon HJ, Zhu Z, Gwaltney JM, Elias JA. Rhinovirus regulation of IL-1 receptor antagonist *in vivo* and *in vitro*: A potential mechanism of symptom resolution. *J Immunol* 162: 7461–69, 1999.
114. Proud D, Naclerio RM, Gwaltney JM, Hendley JO. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 161: 120–23, 1990.

115. Proud D, Reynolds CJ, Lacapra S, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* 137: 613–16, 1988.
116. Christiansen SC, Proud D, Cochrane CG. Detection of tissue kallikrein in the bronchoalveolar lavage fluid of asthmatic subjects. *J Clin Invest* 79: 188–97, 1987.
117. Christiansen SC, Zuraw BL, Proud D, Cochrane CG. Inhibition of human bronchial kallikrein in asthma. *Am Rev Respir Dis* 13: 1125–31, 1989.
118. Christiansen SC, Proud D, Sarnoff RB, Juergens U, Cochrane CG, Zuraw BL. Elevation of tissue kallikrein and kinin in the airways of asthmatic subjects after endobronchial allergen challenge. *Am Rev Respir Dis* 145: 900–5, 1992.
119. Nijkamp FP, Folkerts G. Nitric oxide and bronchial reactivity. *Clin Exp Allergy* 24: 905–14, 1994.
120. Nevin BJ, Broadley KJ. Nitric oxide in respiratory diseases. *Pharmacol Ther* 95: 259–93, 2002.
121. Fischer A, Folkerts G, Geppetti P, Groneberg DA. Mediators of asthma: Nitric oxide. *Pulm Pharmacol Ther* 15: 73–81, 2002.
122. Akaike T, Maeda H. Nitric oxide and virus infection. *Immunology* 101: 300–8, 2000.
123. Donnelly LE, Barnes PJ. Expression and regulation of inducible nitric oxide synthase from human primary airway epithelial cells. *Am J Respir Cell Mol Biol* 26: 144–51, 2002.
124. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 343: 133–35, 1994.
125. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 53: 91–95, 1998.
126. Rutgers SR, van der Mark TW, Coers W, Moshage H, Timens W, Kauffman HF, Koeter GH, Postma DS. Markers of nitric oxide metabolism in sputum and exhaled air are not increased in chronic obstructive pulmonary disease. *Thorax* 54: 576–80, 1999.
127. Maziak W, Loukides S, Culpitt S, Sullivan P, Kharitonov SA, Barnes PJ. Exhaled nitric oxide in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 157: 998–1002, 1998.
128. Hoyt JC, Robbins RA, Habib M, Springall DR, Buttery LD, Polak JM, Barnes PJ. Cigarette smoke decreases inducible nitric oxide synthase in lung epithelial cells. *Exp Lung Res* 29: 17–28, 2003.
129. Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, Huang FP, Xu D, Muller W, Moncada S, Liew FY. Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* 375: 408–11, 1995.
130. Cifone MG, Ulisse S, Santoni A. Natural killer cells and nitric oxide. *Int Immunopharmacol* 1: 1513–24, 2001, [Review] [103 refs].
131. de Gouw HW, Grunberg K, Schot R, Kroes AC, Dick EC, Sterk PJ. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur Respir J* 11: 126–32, 1998.
132. Sanders SP, Kim J, Connolly KR, Porter JD, Siekierski ES, Proud D. Nitric oxide inhibits rhinovirus-induced granulocyte macrophage colony-stimulating factor production in bronchial epithelial cells. *Am J Respir Cell Mol Biol* 24: 317–25, 2001.
133. Sanders SP, Siekierski ES, Porter JD, Richards SM, Proud D. Nitric oxide inhibits rhinovirus-induced cytokine production and viral replication in a human respiratory epithelial cell line. *J Virol* 72: 934–42, 1998.
134. Kao YJ, Piedra PA, Larsen GL, Colasurdo GN. Induction and regulation of nitric oxide synthase in airway epithelial cells by respiratory syncytial virus. *Am J Respir Crit Care Med* 163: 532–39, 2001.
135. Ali-Ahmad D, Bonville CA, Rosenberg HF, Domachowske JB. Replication of respiratory syncytial virus is inhibited in target cells generating nitric oxide *in situ*. *Front Biosci* 8: A48–53, 2003.
136. Rimmelzwaan GF, Baars MM, de Lijster P, Fouchier RA, Osterhaus AD. Inhibition of influenza virus replication by nitric oxide. *J Virol* 73: 8880–83, 1999.
137. Folkerts G, van der Linde HJ, Nijkamp FP. Virus-induced airway hyperresponsiveness in guinea pigs is related to a deficiency in nitric oxide. *J Clin Invest* 95: 26–30, 1995.
138. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur Respir J* 8: 295–97, 1995.
139. Gentile DA, Doyle WJ, Belenky S, Ranck H, Angelini B, Skoner DP. Nasal and oral nitric oxide levels during experimental respiratory syncytial virus infection of adults. *Acta Otolaryngol* 122: 61–66, 2002.
140. Murphy AW, Platts-Mills TA, Lobo M, Hayden F. Respiratory nitric oxide levels in experimental human influenza. *Chest* 114: 452–56, 1998.
141. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. *Microbiol Mol Biol Rev* 65: 131–50, 2001.
142. Gern JE, French DA, Grindle KA, Brockman-Schneider RA, Konno S, Busse WW. Double-stranded RNA induces the synthesis of specific chemokines by bronchial epithelial cells. *Am J Respir Cell Mol Biol* 28: 731–37, 2003.
143. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413: 732–38, 2001.
144. Hewson CA, Jardine A, Edwards MR, Laza-Stanca V, Johnston SL. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J Virol* 79: 12273–79, 2005.
145. Grunstein MM, Hakonarson H, Maskeri N, Chuang S. Autocrine cytokine signaling mediates effects of rhinovirus on airway responsiveness. *Am J Physiol Lung Cell Mol Physiol* 278: L1146–53, 2000.
146. Oliver BG, Johnston SL, Baraket M, Burgess JK, King NJ, Roth M, Lim S, Black JL. Increased proinflammatory responses from asthmatic human airway smooth muscle cells in response to rhinovirus infection. *Respir Res* 7: 71, 2006.
147. Johnston SL, Papi A, Monick MM, Hunninghake GW. Rhinoviruses induce interleukin-8 mRNA and protein production in human monocytes. *J Infect Dis* 175: 323–29, 1997.
148. Laza-Stanca V, Stanciu L, Message SD, Edwards MR, Gern JE, Johnston SL. Rhinovirus replication in human macrophages induces NFkB dependent tumour necrosis factor alpha production. *J Virol* 80(16): 8248–58, 2006.
149. Gern JE, Dick EC, Lee WM, Murray S, Meyer K, Handzel ZT, Busse WW. Rhinovirus enters but does not replicate inside monocytes and airway macrophages. *J Immunol* 156: 621–27, 1996.
150. Oliver BG, Lim S, Wark P, Laza-Stanca V, King NJ, Black JL, Burgess JK, Roth M, Johnston SL. Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. *Thorax* 43, 2008.
151. Johnston SL. Macrolide antibiotics and asthma treatment. *J Allergy Clin Immunol* 117: 1233–36, 2006.
152. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, Fabbri LM, Johnston SL. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med* 173: 1114–21, 2006.
153. Peschke T, Bender A, Nain M, Gemsa D. Role of macrophage cytokines in influenza A virus infections.. *Immunobiology* 189: 340–55, 1993, [Review] [33 refs].
154. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. [Review] [289 refs]. *Ann Rev Immunol* 18: 767–811, 2000.
155. Beyer M, Bartz H, Horner K, Doths S, Koerner-Rettberg C, Schwarze J. Sustained increases in numbers of pulmonary dendritic cells after respiratory syncytial virus infection. *J Allergy Clin Immunol* 113(1): 127–33, 2004.
156. Wang H, Peters N, Laza-Stanca V, Nawroly N, Johnston SL, Schwarze J. Local CD11c+ MHC class II- precursors generate lung dendritic cells during respiratory viral infection, but are depleted in the process. *J Immunol* 177: 2536–42, 2006.

157. Wang H, Peters N, Schwarze J. Plasmacytoid dendritic cells limit viral replication, pulmonary inflammation, and airway hyperresponsiveness in respiratory syncytial virus infection. *J Immunol* 177(9): 6263–70, 2006.
158. Grayson MH, Cheung D, Rohlfing MM, Kitchens R, Spiegel DE, Tucker J, Battaile JT, Alevy Y, Yan L, Agapov E, Kim EY, Holtzman MJ. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 204(11): 2759–69, 2007.
159. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 187: 129–34, 1998.
160. Panina-Bordignon P, Papi A, Mariani A, Di Lucia P, Casoni G, Bellettato C, Buonsanti C, Miotto D, Mapp C, Villa A, Arrigoni G, Fabbri L, Sinigaglia F. The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 107(11): 1357–64, 2001.
161. Huftel MA, Swensen CA, Borcherding WR, Dick EC, Hong R, Kita H, Gleich GJ, Busse WW. The effect of T-cell depletion on enhanced basophil histamine release after *in vitro* incubation with live influenza A virus. *Am J Respir Cell Mol Biol* 7: 434–40, 1992.
162. Hsia J, Goldstein AL, Simon GL, Sztein M, Hayden FG. Peripheral blood mononuclear cell interleukin-2 and interferon-gamma production, cytotoxicity, and antigen-stimulated blastogenesis during experimental rhinovirus infection. *J Infect Dis* 162: 591–97, 1990.
163. Corne JM, Holgate ST. Mechanisms of virus induced exacerbations of asthma. *Thorax* 52: 380–89, 1997.
164. Folkerts G, Nijkamp FP. Virus-induced airway hyperresponsiveness. Role of inflammatory cells and mediators. *Am J Respir Crit Care Med* 151: 1666–73, 1995.
165. Alwan WH, Kozlowska WJ, Openshaw PJ. Distinct types of lung disease caused by functional subsets of antiviral T cells. *J Exp Med* 179: 81–89, 1994.
166. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17: 138–46, 1996.
167. Romagnani S. The Th1/Th2 paradigm. *Immunol Today* 18: 263–66, 1997.
168. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. *Thorax* 57: 328–32, 2002.
169. Gern JE, Vrtis R, Grindle KA, Swenson C, Busse WW. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *Am J Respir Crit Care Med* 162: 2226–31, 2000.
170. Hussell T, Spender LC, Georgiou A, O'Garra A, Openshaw PJ. Th1 and Th2 cytokine induction in pulmonary T cells during infection with respiratory syncytial virus. *J Gen Virol* 77: 2447–55, 1996.
171. Coyle AJ, Erard F, Bertrand C, Walti S, Pircher H, Le Gros G. Virus-specific CD8+ cells can switch to interleukin 5 production and induce airway eosinophilia. *J Exp Med* 181: 1229–33, 1995.
172. Holtmeier W, Kabelitz D. gammadelta T cells link innate and adaptive immune responses. *Chem Immunol Allergy* 86: 151–83, 2005.
173. Holtmeier W. Compartmentalization gamma/delta T cells and their putative role in mucosal immunity. *Crit Rev Immunol* 23: 473–88, 2003.
174. Spinozzi F, Agea E, Bistoni O, Forenza N, Bertotto A. gamma delta T cells, allergen recognition and airway inflammation. *Immunol Today* 19: 22–26, 1998.
175. Krug N, Erpenbeck VJ, Balke K, Petschallies J, Tschernig T, Hohlfeld JM, Fabel H. Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+), and gammadelta T cells in people with asthma after segmental allergen challenge. *Am J Respir Cell Mol Biol* 25: 125–31, 2001.
176. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 105: 651–63, 2000.
177. Saito T, Deskin RW, Casola A, Haeberle H, Olszewska B, Ernst PB, Alam R, Ogra PL, Garofalo R. Respiratory syncytial virus induces selective production of the chemokine RANTES by upper airway epithelial cells. *J Infect Dis* 175: 497–504, 1997.
178. Schroth MK, Grimm E, Frindt P, Galagan DM, Konno SI, Love R, Gern JE. Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am J Respir Cell Mol Biol* 20: 1220–28, 1999.
179. Noah TL, Henderson FW, Henry MM, Peden DB, Devlin RB. Nasal lavage cytokines in normal, allergic, and asthmatic school-age children. *Am J Respir Crit Care Med* 152: 1290–96, 1995a.
180. Noah TL, Henderson FW, Wortman IA, Devlin RB, Handy J, Koren HS, Becker S. Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 171: 584–92, 1995b.
181. Greiff L, Andersson M, Andersson E, Linden M, Myint S, Svensson C, Persson CG. Experimental common cold increases mucosal output of eotaxin in atopic individuals. *Allergy* 54: 1204–8, 1999.
182. Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, Kelly EA, Gern JE. Eosinophils bind rhinovirus and activate virus-specific T cells. *J Immunol* 160: 1279–84, 1998.
183. Adamko DJ, Yost BL, Gleich GJ, Fryer AD, Jacoby DB. Ovalbumin sensitization changes the inflammatory response to subsequent parainfluenza infection. Eosinophils mediate airway hyperresponsiveness, m(2) muscarinic receptor dysfunction, and antiviral effects. *J Exp Med* 190: 1465–78, 1999.
184. Domachowske JB, Dyer KD, Adams AG, Leto TL, Rosenberg HF. Eosinophil cationic protein/RNase 3 is another RNase A-family ribonuclease with direct antiviral activity. *Nucleic Acids Res* 26: 3358–63, 1998.
185. Folkerts G, Busse WW, Nijkamp FP, Sorkness R, Gern JE. Virus-induced airway hyperresponsiveness and asthma. *Am J Respir Crit Care Med* 157: 1708–20, 1998.
186. Volovitz B, Faden H, Ogra PL. Release of leukotriene C4 in respiratory tract during acute viral infection. *J Pediatr* 112: 218–22, 1988.
187. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: An update. *Pharmacol Rev* 50: 515–96, 1998.
188. Seymour ML, Gilby N, Bardin PG, Fraenkel DJ, Sanderson G, Penrose JF, Holgate ST, Johnston SL, Sampson AP. Rhinovirus infection increases 5-lipoxygenase and cyclooxygenase-2 in bronchial biopsy specimens from nonatopic subjects. *J Infect Dis* 185: 540–44, 2002.
189. Pizzichini MM, Pizzichini E, Efthimiadis A, Chauhan AJ, Johnston SL, Hussack P, Mahony J, Dolovich J, Hargreave FE. Asthma and natural colds. Inflammatory indices in induced sputum: A feasibility study. *Am J Respir Crit Care Med* 158: 1178–84, 1998.
190. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 95: 843–52, 1995.
191. Teran LM, Johnston SL, Schroder JM, Church MK, Holgate ST. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. *Am J Respir Crit Care Med* 155: 1362–66, 1997.
192. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: Function and regulation by innate cytokines. *Ann Rev Immunol* 17: 189–220, 1999, [Review] [253 refs].
193. Moretta L, Biassoni R, Bottino C, Mingari MC, Moretta A. Human NK-cell receptors. *Immunol Today* 21: 420–22, 2000.
194. Mingari MC, Ponte M, Bertone S, Schiavetti F, Vitale C, Bellomo R, Moretta A, Moretta L. HLA class I-specific inhibitory receptors in human T lymphocytes: Interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8+ T cells. *Proc Natl Acad Sci USA* 95: 1172–77, 1998.
195. Biron CA. Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. *Semin Immunol* 10: 383–90, 1998, [Review] [76 refs].
196. Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol* 161: 5821–24, 1998.

197. Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J Immunol* 154: 5144–52, 1995.
198. Walker C, Checkel J, Cammisuli S, Leibson PJ, Gleich GJ. IL-5 production by NK cells contributes to eosinophil infiltration in a mouse model of allergic inflammation. *J Immunol* 161: 1962–69, 1998.
199. Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, Kay AB. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: More similarities than differences. *Immunol Today* 20: 528–33, 1999.
200. Skoner DP, Doyle WJ, Tanner EP, Kiss J, Fireman P. Effect of rhinovirus 39 (RV-39) infection on immune and inflammatory parameters in allergic and non-allergic subjects. *Clin Exp Allergy* 25: 561–67, 1995.
201. Lin CY, Kuo YC, Liu WT, Lin CC. Immunomodulation of influenza virus infection in the precipitating asthma attack. *Chest* 93: 1234–38, 1988.
202. Welliver RC, Wong DT, Sun M, Middleton EJ, Vaughan RS, Ogra PL. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *New Engl J Med* 305: 841–46, 1981.
203. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global burden of disease study. *Lancet* 349: 1498–504, 1997.
204. Anonymous. BTS guidelines for the management of chronic obstructive pulmonary disease. The COPD Guidelines Group of the Standards of Care Committee of the BTS. *Thorax* 52(Suppl 5): S1–28, 1997.
205. Anonymous. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. *Am J Respir Crit Care Med* 152: S77–121, 1995.
206. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 157: 1418–22, 1998.
207. Wilson R. The role of infection in COPD. *Chest* 113: 242S–48S, 1998.
208. Smith CB, Golden CA, Kanner RE, Renzetti AD. Association of viral and *Mycoplasma pneumoniae* infections with acute respiratory illness in patients with chronic obstructive pulmonary diseases. *Am Rev Respir Dis* 121: 225–32, 1980.
209. Seemungal TA, Harper-Owen R, Bhowmik A, Moric I, Sanderson G, Message SD, MacCallum P, Meade TW, Jeffries DJ, Johnston SL, Wedzicha JA. The role of respiratory viral infections in COPD. *Am J Respir Crit Care Med*, 164(9): 1618–23, 2001.
210. Gump DW, Phillips CA, Forsyth BR, McIntosh K, Lamborn KR, Stouch WH. Role of infection in chronic bronchitis. *Am Rev Respir Dis* 113: 465–74, 1976.
211. Tager I, Speizer FE. Role of infection in chronic bronchitis. *New Engl J Med* 292: 563–71, 1975.
212. Greenberg SB, Allen M, Wilson J, Atmar RL. Respiratory viral infections in adults with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 162: 167–73, 2000.
213. Rohde G, Wiethege A, Borg I, Kauth M, Bauer TT, Gillissen A, Bufe A, Schultze-Werninghaus G. Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: A case-control study. *Thorax* 58: 37–42, 2003.
214. Monso E, Ruiz J, Rosell A, Manterola J, Fiz J, Morera J, Ausina V. Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med* 152: 1316–20, 1995.
215. Irwin RS, Erickson AD, Pratter MR, Corrao WM, Garrity FL, Myers JR, Kaemmerlen JT. Prediction of tracheobronchial colonization in current cigarette smokers with chronic obstructive bronchitis. *J Infect Dis* 145: 234–41, 1982.
216. Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Int Med* 106: 196–204, 1987.
217. Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD. *Chest* 117: 1638–45, 2000.
218. Sethi S, Evans N, Grant BJ, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. *N Engl J Med* 347(7): 465–71, 2002.
219. Murphy TF, Sethi S. Bacterial infection in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 146: 1067–83, 1992.
220. Ball P, Tillotson G, Wilson R. Chemotherapy for chronic bronchitis. *Controversies Presse Med* 24: 189–94, 1995.
221. Blasi F, Cosentini R, Schoeller MC, Lupo A, Allegra L. *Chlamydia pneumoniae* seroprevalence in immunocompetent and immunocompromised populations in Milan. *Thorax* 48: 1261–63, 1993.
222. Blasi F, Legnani D, Lombardo VM, Negretto GG, Magliano E, Pozzoli R, Chiodo F, Fasoli A, Allegra L. *Chlamydia pneumoniae* infection in acute exacerbations of COPD. *Eur Res J* 6: 19–22, 1993.
223. Eller J, Ede A, Schaberg T, Niederman MS, Mauch H, Lode H. Infective exacerbations of chronic bronchitis: Relation between bacteriologic etiology and lung function. *Chest* 113: 1542–48, 1998.
224. Wilkinson TM, Hurst JR, Perera WR, Wilks M, Donaldson GC, Wedzicha JA. Effect of interactions between lower airway bacterial and rhinoviral infection in exacerbations of COPD. *Chest* 129(2): 317–24, 2006.
225. Saint S, Bent S, Vittinghoff E, Grady D. Antibiotics in chronic obstructive pulmonary disease exacerbations. A meta-analysis. *JAMA* 273: 957–60, 1995.
226. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M, Higenbottam T, Postma DS. Optimal assessment and management of chronic obstructive pulmonary disease (COPD). The European Respiratory Society Task Force. *Eur Respir J* 8: 1398–420, 1995.
227. Fagon JY, Chastre J, Trouillet JL, Domart Y, Dombret MC, Bornet M, Gibert C. Characterization of distal bronchial microflora during acute exacerbation of chronic bronchitis. Use of the protected specimen brush technique in 54 mechanically ventilated patients. *Am Rev Respir Dis* 142: 1004–8, 1990.
228. Wilson R, Dowling RB, Jackson AD. The biology of bacterial colonization and invasion of the respiratory mucosa. *Eur Respir J* 9: 1523–30, 1996.
229. Sykes A, Mallia P, Johnston SL. Diagnosis of pathogens in exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 4: 642–46, 2007.
230. Hogg JC. Childhood viral infection and the pathogenesis of asthma and chronic obstructive lung disease. *Am J Respir Critl Care Med* 160: S26–28, 1999.
231. Matsuse T, Hayashi S, Kuwano K, Keunecke H, Jefferies WA, Hogg JC. Latent adenoviral infection in the pathogenesis of chronic airways obstruction. *Am Rev Respir Dis* 146: 177–84, 1992.
232. Elliott WM, Hayashi S, Hogg JC. Immunodetection of adenoviral E1A proteins in human lung tissue. *Am J Respir Cell Mol Biol* 12: 642–48, 1995.
233. Liu F, Green MR. Promoter targeting by adenovirus E1a through interaction with different cellular DNA-binding domains. *Nature* 368: 520–25, 1994.
234. Keicho N, Elliott WM, Hogg JC, Hayashi S. Adenovirus E1A upregulates interleukin-8 expression induced by endotoxin in pulmonary epithelial cells. *Am J Physiol* 272: L1046–52, 1997.
235. Keicho N, Elliott WM, Hogg JC, Hayashi S. Adenovirus E1A gene dysregulates ICAM-1 expression in transformed pulmonary epithelial cells. *Am J Respir Cell Mol Biol* 16: 23–30, 1997.
236. Keicho N, Higashimoto Y, Bondy GP, Elliott WM, Hogg JC, Hayashi S. Endotoxin-specific NF-kappaB activation in pulmonary epithelial cells harboring adenovirus E1A. *Am J Physiol* 277: L523–32, 1999.
237. Mallia P, Message SD, Kebadze T, Parker HL, Kon OM, Johnston SL. An experimental model of rhinovirus induced chronic obstructive pulmonary disease exacerbations: A pilot study. *Respir Res* 7(116), 2006.

238. Gustafson LM, Proud D, Hendley JO, Hayden FG, Gwaltney JM. Oral prednisone therapy in experimental rhinovirus infections. *J Allergy Clin Immunol* 97: 1009–14, 1996.
239. Eickelberg O, Roth M, Lorx R. Ligand-independent activation of the glucocorticoid receptor by β2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 274: 1005–10, 1999.
240. Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *New Engl J Med* 337: 1405–11, 1997.
241. Robertson CF, Price D, Henry R, Mellis C, Glasgow N, Fitzgerald D, Lee AJ, Turner J, Sant M. Short-course montelukast for intermittent asthma in children: A randomised controlled trial. *Am J Respir Crit Care Med* 175(4): 323–29, 2007.
242. Bisgaard H, Zielen S, Garcia-Garcia ML, Johnston SL, Gilles L, Menten J, Tozzi CA, Polos P. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. *Am J Respir Crit Care Med* 171: 315–22, 2005.
243. Pauwels RA, Pedersen S, Busse WW, Tan WC, Chen YZ, Ohlsson SV, Ullman A, Lamm CJ, O'Byrne PM START Investigators Group. Early intervention with budesonide in mild persistent asthma: A randomised, double-blind trial. *Lancet* 361: 1071–76, 2003.
244. Doull IJ, Lampe FC, Smith S, Schreiber J, Freezer NJ, Holgate ST. Effect of inhaled corticosteroids on episodes of wheezing associated with viral infection in school age children: Randomised double blind placebo controlled trial. *BMJ* 315: 858–62, 1997.
245. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1–5 years: Randomised controlled trial. *Lancet* 362: 1433–38, 2003.
246. Hamory BH, Hamparian VV, Conant RM, Gwaltney JM. Human responses to two decavalent rhinovirus vaccines. *J Infect Dis* 132: 623–29, 1975.
247. Kim HW, Canchola JG, Brandt CD, Pyles G, Chanock RM, Jensen K, Parrott RH. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 89: 422–34, 1969.
248. Hall CB. Respiratory syncytial virus and parainfluenza virus. *New Engl J Med* 344: 1917–28, 2001.
249. Murphy BR, Prince GA, Walsh EE, Kim HW, Parrott RH, Hemming VG, Rodriguez WJ, Chanock RM. Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. *J Clin Microbiol* 24: 197–202, 1986.
250. Kim HW, Leikin SL, Arrobio J, Brandt CD, Chanock RM, Parrott RH. Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. *Pediatr Res* 10: 75–78, 1976.
251. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 140: 543–46, 1986.
252. Marshall S. Zinc gluconate and the common cold. Review of randomized controlled trials. *Can Fam Physician* 44: 1037–42, 1998.
253. Singh M. Heated, humidified air for the common cold. *Cochrane Database of Syst Rev*4: CD001728, 2004.
254. Hayden FG, Gwaltney JM. Intranasal interferon-alpha 2 treatment of experimental rhinoviral colds. *J Infect Dis* 150: 174–80, 1984.
255. Khare MD, Sharland M. Influenza. *Expert Opinion on Pharmacotherapy* 1: 367–75, 2000.
256. Lalezari J, Campion K, Keene O, Silagy C. Zanamivir for the treatment of influenza A and B infection in high-risk patients: a pooled analysis of randomized controlled trials. *Archives of Internal Medicine* 161: 212–17, 2001.
257. Monto AS, Robinson DP, Herlocher ML, Hinson JMJ, Elliott MJ, Crisp A. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. *JAMA* 282: 31–35, 1999.
258. Rodriguez WJ, Gruber WC, Welliver RC, Groothuis JR, Simoes EA, Meissner HC, Hemming VG, Hall CB, Lepow ML, Rosas AJ et al. Respiratory syncytial virus (RSV) immune globulin intravenous therapy for RSV lower respiratory tract infection in infants and young children at high risk for severe RSV infections: Respiratory Syncytial Virus Immune Globulin Study Group. *Pediatrics* 99: 454–61, 1997, [see comments].
259. Wu H, Pfarr DS, Johnson S, Brewah YA, Woods RM, Patel NK, White WI, Young JF, Kiener PA. Development of motavizumab, an ultra-potent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. *J Mol.Biol* 368(3): 652–65, 2008.
260. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clinical & Experimental Allergy* 22: 325–36, 1992.