

## Review article

Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA<sup>2</sup>LEN and InterAirways document

Viral infections of the respiratory tract are the most common precipitants of acute asthma exacerbations. Exacerbations are only poorly responsive to current asthma therapies and new approaches to therapy are needed. Viruses, most frequently human rhinoviruses (RV), infect the airway epithelium, generate local and systemic immune responses, as well as neural responses, inducing inflammation and airway hyperresponsiveness. Using *in vitro* and *in vivo* experimental models the role of various proinflammatory or anti-inflammatory mediators, antiviral responses and molecular pathways that lead from infection to symptoms has been partly unravelled. In particular, mechanisms of susceptibility to viral infection have been identified and the bronchial epithelium appeared to be a key player. Nevertheless, additional understanding of the integration between the diverse elements of the antiviral response, especially in the context of allergic airway inflammation, as well as the interactions between viral infections and other stimuli that affect airway inflammation and responsiveness may lead to novel strategies in treating and/or preventing asthma exacerbations. This review presents the current knowledge and highlights areas in need of further research.

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Epidemiological studies, with virological confirmation, have convincingly demonstrated that the majority of acute asthma exacerbations, in both children and adults, follow upper respiratory infections (1, 2). On some occasions, particularly young children, common colds are unique precipitants of wheezing and associated symptoms (3). However, much less is known about the

mechanisms of virus-induced asthma exacerbations, than the relatively less frequent allergen-induced events.

An integrated response, aimed at the fast and efficient clearance of the invading pathogen, occurs during a respiratory viral infection. Elements of this response include the respiratory, the immune, as well as the nervous systems (4). The airway epithelium, site of viral

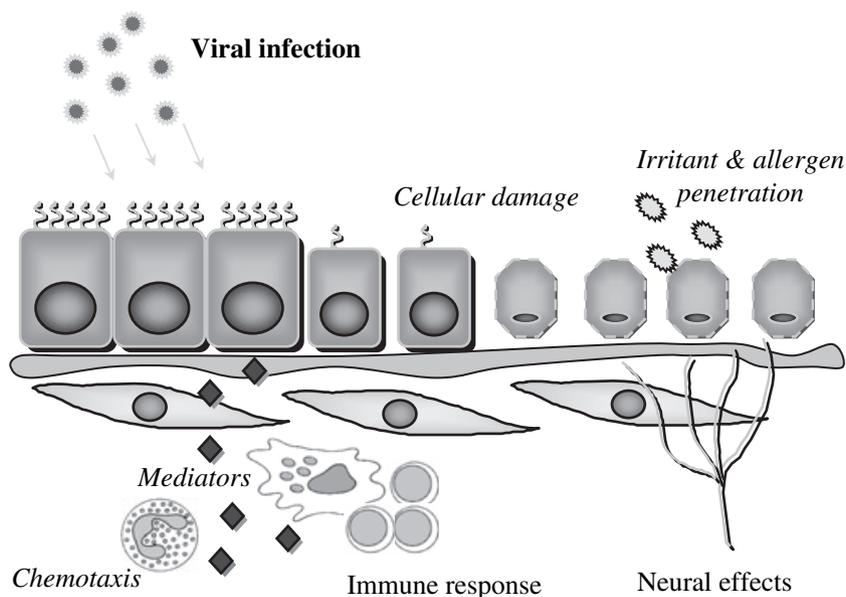


Figure 1. Overview of virus-induced asthma exacerbations: respiratory viruses infect the airway epithelium inducing cellular damage, proinflammatory mediator production, a local and a systemic immune response and may affect neural homeostasis.

replication, generates an innate antiviral response, orchestrating downstream responses involving subepithelial and immune factors. An immune response is generated both locally and systemically, in order to preclude further viral invasion. Neural signals are generated in response to, or in an attempt to control or coordinate the inflammation. Several of these elements are altered in asthmatic individuals, resulting in induction of airway hyperresponsiveness (AHR) and contributing to the development of exacerbations consequent upon the infection (Fig. 1). Although not proven, mouth breathing or postnasal drip may also contribute to the clinical presentation of a respiratory viral infection (5). All respiratory viruses have been associated with asthma exacerbations; there is very little evidence on differential effects between these agents in their capacity to trigger asthma, nevertheless, the majority of relevant evidence comes from human rhinovirus (RV) studies.

### Experimental models of virus-induced asthma

A variety of *in vitro* and *in vivo* models has been used for the study of virus-induced asthma. *In vitro* systems have most frequently been based upon continuous cell lines of bronchial epithelial origin, such as the BEAS-2B, A549, 16HBE and others (6). Cultures of human primary bronchial epithelial cells, obtained by bronchoscopy either by brushing or from biopsies, are preferable to continuous cell lines as they more closely represent *in vivo* conditions (7); more recently, differentiated primary epithelial cells, grown at an air-liquid interface, have been proposed to represent an optimal model for RV infection (8). Lung fibroblasts, either primary or continuous cell lines of embryonic origin (e.g. WI38) have

also been popular, as fibroblasts support viral replication. Occasionally, smooth muscle cells, peripheral blood mononuclear cells (PBMC), monocytes, macrophages and eosinophils have been used either to assess viral replication or to model the antiviral immune response.

Small animal models of virus infections have been in use for a long time, with respect to influenza virus, respiratory syncytial virus, parainfluenza viruses (mostly Sendai virus in the mouse) as well as coronaviruses. Unfortunately, no practical small animal model of RV infection exists; thus for many years experimental RV infection in humans has been the model used to study RV infections. Over the past two decades several experimental studies have been carried out in both asthmatic and normal volunteers, with a good safety record. This approach offers a number of important advantages over studies of natural virus-induced asthma exacerbations, such as the ability to study subjects at baseline before infection; the selection of subjects according to specific criteria such as atopy, asthma severity and medication use; knowledge of the virus type and dose; accurate timing of investigations relative to the timing of infection; and the ability to carry out invasive investigations before, during and after infection, including bronchoscopy for sampling of the lower airway.

Experimental RV infection of asthmatics leads to an increase in lower respiratory tract symptoms typical of a mild asthma exacerbation (9, 10). These are accompanied by objective measures of increased airflow obstruction such as reduced peak expiratory flow (PEF) and forced expiratory volume (FEV)<sub>1</sub> and enhanced sensitivity to histamine (9, 11, 12). Few experimental infection studies have compared RV infection in asthmatic and normal subjects; however, to date it has not been demonstrated

that experimental RV infection in asthmatics is associated with greater changes in lung function than in normals (9, 10), as is the case in naturally acquired infections in adults (13) and infants (14). The lack of a clear difference may be because of the inclusion of subjects with mild asthma only and the small number of subjects studied. Furthermore, the clinical, physiological and inflammatory responses to experimental RV infection in asthmatics are relatively mild compared with exacerbations induced by naturally acquired colds. This may be because of more complex interactions such as concomitant airway inflammation occurring in naturally acquired infections and leading to exacerbations.

### Virus-induced airway hyperresponsiveness

Airway wall oedema, goblet cell metaplasia, altered surfactant function and mucus composition, due to cellular debris and plasma protein excess, modified patency of the small airways and increased epithelial and endothelial permeability, occur during viral infections and may lead to AHR (15, 16). Virus-induced epithelial damage causes narrowing through dead epithelial cells dropping into the lumen, decreased mucociliary clearance, increased exposure of sensory nerves to irritants, and decreased production of bronchodilating (PGE<sub>2</sub>, NO, endopeptidase) and/or decreased metabolism of bronchoconstricting (substance P, neurokinin A) substances (17). Proinflammatory mediators, described below, may also contribute to AHR (18–21).

Viral infection induces greater nonspecific AHR in patients with respiratory allergy than healthy controls

(22, 23). In addition, following a viral infection the response to allergen exposure may be exaggerated, as shown using bronchial provocations with allergen (24, 25). It has been suggested that the defective epithelial repair cycle, characteristic of asthma and strongly correlating with AHR, is amplified by exposure to Th2 cytokines (26). The duration of postviral AHR is around 7 weeks in children with intermittent asthma, comparable with studies prospectively evaluating postviral AHR in experimental animals (27, 28). Although the duration of AHR after a single cold is not affected by the atopic status of the patient, an increased number of symptomatic colds may cumulatively lead to prolonged AHR in atopic children (Fig. 2; 28). Taking into account that the degree of airway responsiveness is indicative of asthma severity and an indirect marker of airway inflammation (29), prolongation of virus-induced AHR may reflect persistent airway inflammation after multiple viral insults (30).

### Structural cells and extracellular matrix

Respiratory viruses enter and replicate in epithelial cells lining the upper as well as the lower airways. Although RV in particular was considered in the past as an upper respiratory pathogen only, studies using polymerase chain reaction and *in situ* hybridization have conclusively shown that RV can also replicate in the lower airways (7, 14). The extent of epithelial cell destruction varies according to the type of virus. Influenza virus may cause extensive epithelial necrosis, whereas RV usually causes little or only patchy epithelial damage; *in vitro*, RV becomes cytotoxic under specific conditions (31). Death/damage of epithelial cells is likely to result in both an increase in epithelial permeability and an exposure of sensory nerve fibres to irritants and allergens. These effects may contribute to the increased AHR induced by respiratory viral infection. Dead epithelial cells, in addition to other inflammatory cells, dropping into the lumen may also contribute to airway obstruction (32).

Nevertheless, the lower airway epithelium does not simply act as a physical barrier but has an essential role in immune/inflammatory responses. Bronchial epithelial cells are major determinants of the inflammatory response through the production of a wide array of cytokines and chemokines, detailed below.

Adhesion molecules expressed on the surface of epithelial and endothelial cells are also involved in local inflammatory cell recruitment. One such molecule is ICAM-1 (CD54), the receptor for the majority of RVs and the natural ligand of the  $\beta$ 2-integrin CD11a. Viral infections upregulate the epithelial expression of ICAM-1 both *in vivo* and *in vitro*, supporting leucocyte infiltration, while potentially facilitating RV attachment and entry in host cells (33). A similar role could be attributed to vascular adhesion molecule-1 (VCAM-1; CD106), which

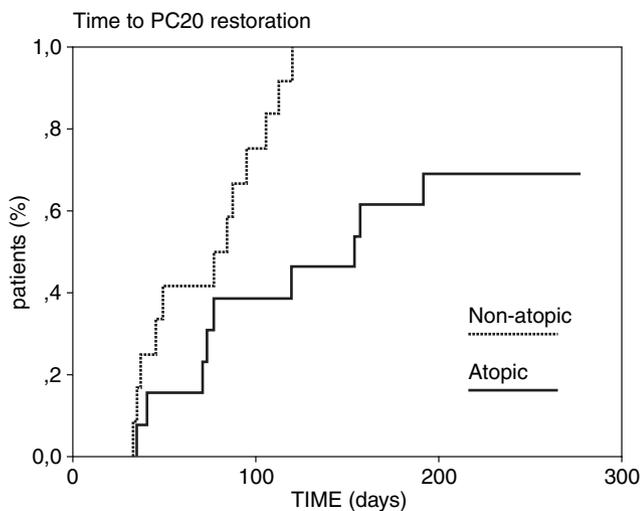


Figure 2. Duration of airway hyperresponsiveness (AHR) in atopic and nonatopic children with asthma, after an initial virus-induced exacerbation. An increased number of symptomatic infections leads to considerably prolonged AHR in the atopic group [adapted from Ref. (28)].

is also upregulated by viral infection and selectively recruits eosinophils (34).

Epithelial cells may also act as antigen-presenting cells as they express major histocompatibility complex I and the costimulatory molecules CD80 (B7.1) and CD86 (B7.2), whose expression are upregulated *in vitro* by RV infection (35).

An important characteristic of the epithelium in asthma is the fact that it is particularly susceptible to injury (36). The imbalance between cell death and proliferation is considered crucial in the pathophysiology and pathology of the disease (37). Viruses may cause or interfere with such imbalance. For example, RV infection is able to delay epithelial repair (Fig. 3; 31). The degree of viral cytotoxicity not only depends upon the type of viral pathogen, but is also affected by the host epithelial cells. RV becomes highly cytotoxic only in sparsely cultured cells, a condition that may resemble the already disrupted epithelium occurring in asthma (31). When epithelial cells from patients with asthma were infected with RV *in vitro*, they were found to be resistant to early apoptosis, an effect caused by lack of the pro-apoptotic interferon (IFN)- $\beta$  (38). Reduced apoptosis was associated with increased viral proliferation within the epithelial cells, resulting into increased cytotoxicity (38). Similar effects were observed when an epithelial cell line was conditioned by exposure to PBMC supernatants: immune cells deriving from atopic asthmatic individuals were unable to protect epithelial cells from RV-induced cytotoxicity (M. Xatzipsalti, unpublished data). Type I IFNs limit virus spread and prime neighbouring cells to an antiviral state. Recently, similar and even stronger effects were shown to occur through the newly described type III, or  $\lambda$ -interferons (39).

In addition to epithelial cells, RVs are capable of infecting airway smooth muscle (ASM) cells *in vitro*, inducing constrictor responsiveness and reducing  $\beta$ -adrenoceptor-mediated relaxation, although the clinical significance of this finding is uncertain (40). Submucosal gland (SMG) hypertrophy and mucus metaplasia with increased expression of the MUC5AC gene are seen in the asthmatic airways. Human SMG cells infected with RV produce IL-8, contributing to neutrophil inflammation and also augment eosinophil transmigration across the airway epithelium through the secretion of eosinophil chemotactic factors (RANTES, GM-CSF; 41). Increased

numbers of mucus-producing goblet cells and increased staining for MUC5AC have also been observed in mice infected with Sendai virus (42) and RSV (43).

The major structural components of the airways, epithelium, underlying fibroblasts but also the matrix, interact dynamically. These structures (constituting the epithelial mesenchymal trophic unit) are physiologically active during lung morphogenesis in the fetus; it has been suggested that they are reactivated in asthma to drive pathological remodelling, in which the epithelium enters a chronic wound response, accompanied by production of profibrogenic growth factors (36). Virus-infected cells secrete increased amounts of fibroblast growth factor (FGF)-2 (44, 45) and vascular endothelial growth factor (VEGF; 46, 47). RV-induced VEGF was able to augment angiogenesis in an *in vitro* model (47). Furthermore, imbalance of matrix metalloproteinases regulating the deposition of collagen may also affect remodelling. Virus-induced proinflammatory cytokines stimulate the production and release of matrix metalloproteinase (MMP)-9 and MMP-2 by epithelial cells (48). This increase may allow extracellular matrix damage, possibly followed by abnormal mucosal repair in asthma.

## Immune cells and function

### Neutrophils

Neutrophils are among the predominant cell types participating in virus-induced asthma through generation of superoxide and/or release of cytokines. In nasopharyngeal aspirates and bronchial lavages, neutrophils are the main cells recruited during the acute stage of natural colds (49), probably via IL-8 or leukotriene B4 (50).

Adult patients, presenting to the emergency department with acute asthma and concomitant virus infection, had increased neutrophils and neutrophil elastase in sputum (51). Neutrophil proteases are potent secretagogues for airway SMGs (52) and can increase mucus production contributing to airway obstruction.

Neutrophils comprise the majority of nonepithelial cells in sputum during acute exacerbations of asthma (53). Experimental RV infection results in an increase in neutrophils in BAL (54) and sputum (55). In this model, peripheral blood neutrophil counts correlate with cold

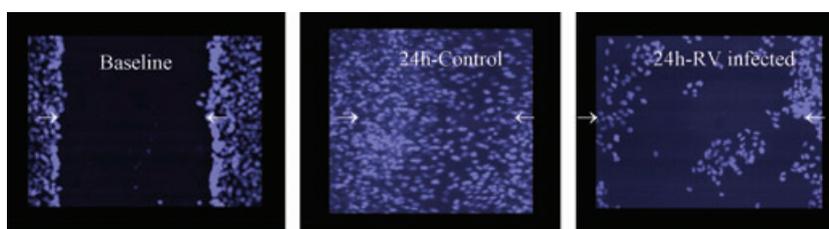


Figure 3. Rhinovirus (RV) infection delays epithelial wound healing. BEAS-2B cells were mechanically damaged and then infected or not with RV. Cells were stained with DAPI 24 h later, revealing a defect in epithelial repair in RV-infected cells (31).

and asthma symptom scores (11), or signs in animals (56). Viruses can also activate neutrophil functions upregulating chemotaxis, adhesion and superoxide production (57).

#### Eosinophils

Viral infections can also trigger increased recruitment and activation of eosinophils, contributing to AHR (58–60).

Rhinovirus experimental infections increase allergen-induced eosinophil numbers in bronchial lavage fluid in rhinitic adults (24) and eosinophil products in sputum supernatants in asthmatic adults (18). *In vitro* experiments indicate that RVs do not activate eosinophils directly (61), but probably through the activity of virus-induced mediators, adhesion molecules (33, 34) or cytokines secreted by T cells, epithelial cells or other airway cells (62).

A marked bronchial eosinophil infiltration has been observed during colds in both normal and asthmatic adults. However, the eosinophil infiltrate was more prolonged in asthmatic patients and still present 6–8 weeks after infection (59). Such differences have not, however, been confirmed in induced sputum (10).

Eosinophil granular proteins (e.g. major basic protein) have been detected in nasal secretions of asthmatic children with wheezing illness caused by RV or RSV (63, 64). In these children, RANTES and GM-CSF, factors affecting eosinophil recruitment, survival, degranulation and superoxide production, were also significantly increased (65).

#### Basophils and mast cells

*In vitro* incubation of basophils with viruses does not in itself cause release of mediators; however, some viruses (e.g. RSV, adenovirus and influenza) have been shown to enhance anti-immunoglobulin (Ig)E-mediated histamine release (66). These effects were shown to occur *ex vivo* with basophils from asthmatic volunteers during the acute phase compared with the convalescent phase of symptomatic colds, the increase in histamine and leukotriene release occurring in response to cross-linking of VLA 4 (67). Basophil activation could be mediated by interferons resulting from upper respiratory tract infection and enhancing histamine release (68). Nevertheless, systemic activation has not been confirmed *in vivo* during common colds (69).

The effect of viruses on mast cells have not been subject of much study, possibly because of the difficulty of obtaining adequate number of cells. Some animal studies on rat and calf are available (70, 71).

#### Dendritic cells

Pulmonary dendritic cells (DCs) express several 'pathogen recognition receptors', such as C-type lectins, mannose receptors and Toll-like receptors (TLRs), for

efficient sensing and sampling of a wide variety of microbial organisms, including viruses. Whereas human plasmacytoid DCs express TLR7, which binds to single-stranded RNA (72) and TLR9, binding to CpG-rich DNA (73), CD11c<sup>+</sup> human myeloid DCs express TLR3, a ligand for double-stranded (ds) RNA and TLR7 (74, 75). TLR 3, 7 and 9 specialize in viral detection and recognize nucleic acids in late endosomes-lysosomes, as these TLRs are localized to intracellular compartments (76).

Stimulation of human myeloid DCs and plasmacytoid DCs with synthetic TLR7 or TLR9 agonists induces the secretion of IL-12 and large amounts of IFN- $\alpha$ , respectively (75, 77). Toll-like receptor-mediated induction or modulation of type-1 interferons occurs *in vitro* after exposure of plasmacytoid DCs to RSV and influenza viruses, or epithelial cells to RV (78–80).

#### T and B lymphocytes

In the absence of infection, DCs isolated from the lungs express inducible costimulatory ligand (ICOSL) and secrete IL-10, selectively inducing regulatory T cells (81). After viral infections such as influenza, an optimal T-helper 1 (Th1) response is induced by lung DCs, leading to antigen-specific effector T cells producing IFN- $\gamma$  and tumour necrosis factor (TNF). Virus-infected cells in the airway are thus recognized by Th1 CD4<sup>+</sup> T cells and cytotoxic Tc1 CD8<sup>+</sup> T cells. This recognition leads to killing of the infected cell and release of effector cell cytokines (such as IFN- $\gamma$ ), which further enhance the antiviral activities of the innate immune defence.

Indeed, bronchial biopsies have demonstrated increases in CD4<sup>+</sup> and CD8<sup>+</sup> T cells within the epithelium and submucosa of both healthy subjects and asthmatic patients following experimental RV infection (59). IFN- $\gamma$ , together with type I interferons (IFN- $\alpha$  and IFN- $\beta$ ), play an important role in establishing a paracrine antiviral state. Whereas cytotoxic CD8<sup>+</sup> T cells are a crucial player in protective cell-mediated immunity in response to respiratory viruses, a dysregulated CD8<sup>+</sup> T-cell response in the context of a viral infection may place individuals with asthma at risk of severe asthma exacerbations, and even asthma death (82, 83).

The balance between Th1 and Th2 cytokine production can be crucial to viral clearance. It is well established that increased viral replication occurs under the influence of Th2 cytokines (84). Gern et al. found that the IFN- $\gamma$ /IL-5 sputum mRNA ratio during infection was inversely related to peak cold symptom scores and time to virus clearance, suggesting that a strong Th1 response in the airway plays an important role in limiting the cold severity and viral replication (55). Exposure of PBMCs from normal and atopic asthmatic individuals to RV leads to the production of high levels of IFN- $\gamma$ . Nevertheless, the levels are significantly lower in atopic asthmatic individuals; furthermore IL-4 production is

upregulated only in these individuals, leading to considerable reduction of the IFN- $\gamma$ /IL-4 ratio (85). In the same *in vitro* model, reduced expression of costimulatory molecules B7.1 and B7.2 and increased expression of the suppressor molecule CTLA-4 in CD4 T cells were observed in RV-exposed cells of atopic asthmatic individuals, suggesting that virus presentation may be suboptimal in these subjects (86).

The antiviral B cell immune response, as evidenced by immunoglobulin production, follows a tight time schedule (87). Mucosal IgA may be detected at day 3 after experimental virus infection, serum IgM at days 5–6 and IgG at days 7–8; all immunoglobulins increase in amount and avidity during the ensuing 2–3 weeks; their role seems to be less in direct virus eradication and more in the prevention of re-infection. However, it is not clear whether B-cell responses to respiratory virus infection are modified in the context of asthma.

#### Monocytes–macrophages

Alveolar macrophages make up the majority of cells seen in BAL from healthy individuals and asthmatic patients (88, 89). These residential macrophages are ideally placed for early phagocytosis and destruction of virus particles; moreover, human monocytes and macrophages express the major RV receptor ICAM-1. Entry of RV into monocytes leads to activation and production of TNF- $\alpha$  and IL-8 (90, 91). An earlier study reported that RV RNA synthesis was not detected in macrophages (90); however, recent study indicates that limited replication does take place in monocyte-derived macrophages (92), suggesting that macrophages may serve as permissive host cells during infection *in vivo*.

#### Mediators

Mediators produced by structural and immune cells upon a viral infection have regulatory and effector functions shaping the inflammatory response, both locally and systemically (Fig. 4). The epithelium alone is able to produce a broad range of mediators, including IL-1, IL-6, IL-8 (CXCL8), IL-11, TNF $\alpha$ , GM-CSF, eotaxins (CCL10, 11, 24), RANTES (CCL5) and IP10 (CXCL10) (6, 93–101).

Interleukin-8 (IL-8) and granulocyte colony-stimulating factor (G-CSF) have been detected in nasal lavage and sputum after RV infection and correlate with nasal, sputum and blood neutrophils (55). One study has reported that IL-8 is increased in nasal lavage after RV infection in asthmatics but not in normals (102), although there is evidence of IL-8 upregulation during a natural RV infection in healthy students (M. Mäkelä, personal communication). In subjects with allergic rhinitis, RV infection increases production of the eosinophil chemokine eotaxin (103), together with eosino-

phil peroxidase and myeloperoxidase in nasal lavage (104). In normal subjects, experimental RV infection upregulates enzymes in the 5-lipoxygenase and cyclooxygenase pathways in the bronchial mucosa (105). Conversely RV infection increases exhaled nitric oxide (NO) levels in asthmatics and this is inversely correlated with the change in PC<sub>20</sub>, suggesting that NO may have a protective effect against virus-induced bronchial hyper-reactivity (106). Overexpression of human  $\beta$ -defensin 2 in RV-infected epithelium suggests a potential mechanism linking the innate and specific immunity to the virus (107).

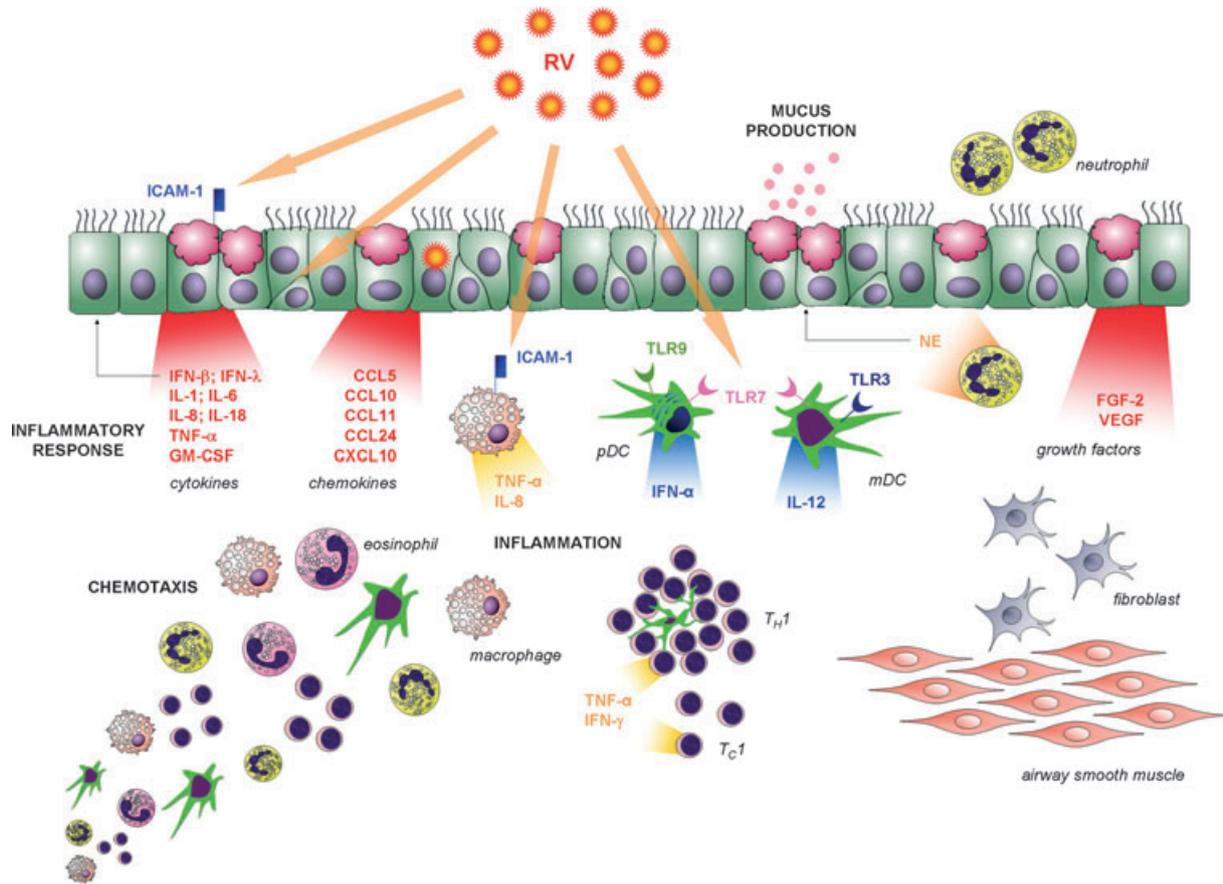
Although most of the above mediators are also found at elevated levels in allergic rhinitis and asthma (108), they are not unique to these diseases. Furthermore, many other processes (e.g. exposure to environmental smoke or diesel exhaust particles, wounding) that target or affect the integrity of the epithelium induce a similar response (109–112). Nevertheless, it is clear that through such mediators, viruses are able to potentiate inflammation as well as aggravate an underlying allergic response (11, 94, 113–116). Influx and activation of recruited or already present effector cells such as mast cells, eosinophils or neutrophils would lead to increased release of toxic components-like histamine, major basic protein, eosinophil cationic protein, eosinophil peroxidase and myeloperoxidase (117, 118). Tissue damage through these proteins may in itself contribute to the exacerbation. Furthermore, effector molecules, such as kinins (119) affect neural pathways inducing bronchoconstriction, as described below.

Viral infections are also able to induce systemic effects mediated by circulating cytokines, indicated by changes in leucocyte numbers following the infection. It is possible that through systemic effects upper airway infection may interfere with the bronchial allergic inflammation, as has been shown for allergen-induced inflammation through IL-5 (120). Nevertheless, the major inducible mediators after a respiratory viral infection are interferons (8). Interferon-mediated innate responses to viral infection are differentially regulated in normal or atopic asthmatic individuals and these differences may prove to be crucial in the development of acute exacerbations (38, 39, 85).

#### Neural mechanisms

Airway patency depends highly on smooth muscle constriction, mucosal oedema and mucus secretion, controlled by the adrenergic, cholinergic and the non-adrenergic noncholinergic (NANC) neural pathways.

Postviral alterations in neural control of the airways have been shown mainly in animal models, either through dysfunction of prejunctional M2-muscarinic inhibitory receptors, which normally inhibit acetylcholine release both directly and indirectly through production of IFN- $\gamma$



**Figure 4.** Upon a viral infection, epithelial and immune cells produce a wide variety of mediators, including interferons, proinflammatory cytokines, chemokines and pro-fibrogenic growth factors, shaping the inflammatory response. Abbreviations: CCL, CC chemokine ligand; CCL5, RANTES (Regulated on Activation Normal T-cell Expressed and Secreted); CCL11, eotaxin; CCL24, eotaxin-2; CXCL, CXC chemokine ligand; CXCL10, IP-10 (IFN- $\gamma$ -inducible protein 10); FGF-2, fibroblast growth factor-2; GM-CSF, granulocyte macrophage-colony stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL, interleukin; mDC, myeloid dendritic cell; NE, neutrophil elastase; pDC, plasmacytoid dendritic cell; RV, rhinovirus; T<sub>C</sub>1, cytotoxic CD8<sup>+</sup> T-lymphocyte type 1; T<sub>H</sub>1, T-helper 1 CD4<sup>+</sup> T lymphocyte; TLR, Toll-like receptor; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ ; VEGF, vascular endothelial growth factor.

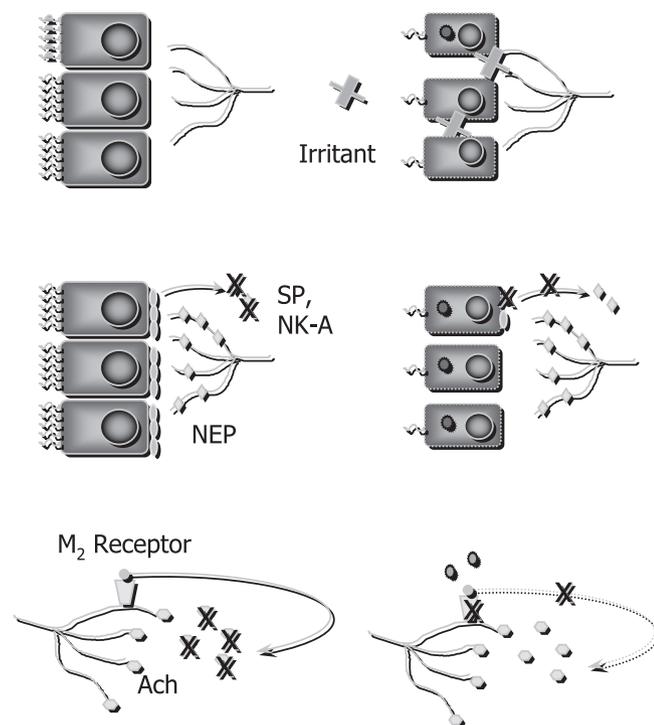
(121), or through the release of bronchoconstricting neuropeptides (Fig. 5; 122–124).

Through the absence of the physical barrier, environmental irritants may directly stimulate unmyelinated sensory nerves. Released kinins may directly induce contraction of smooth muscle cells or lead to a parasympathetically induced bronchoconstriction. This effect may be aggravated by major basic protein that interferes with an M2 receptor-mediated autoinhibitory feedback loop that would normally keep this possible constriction in check. Enhanced neuronal activity will also lead to histamine release from mast cells that can be found in close proximity to nerve synapses. In addition, RSV infection induces changes in the mast cell-nerve synapses resulting in NK1 receptor upregulation and exacerbation of SP-induced bronchoconstriction and SM contraction (125, 126). Furthermore, it resulted in the release of

cysLTs, which in turn amplified the release of tachykinins and potentiated their effects.

Increased NANC excitatory and decreased inhibitory responses have been noted after viral infections (56, 127). Parainfluenza and influenza viruses are cytotoxic to the epithelium, causing loss of neutral endopeptidase (NEP), the major tachykinin metabolizing enzyme, activity and hence loss of tachykinin-mediated control and consequent potentiation of their activities (121, 128). This is also the case for histamine methyltransferases. These enzymes would normally reduce tissue levels of histamine and kinins (129) and so dampen a given response.

In humans, normalization of the airway responses to challenges after atropine treatment suggests that sensory reflexes are abnormal after viral infections (130). Furthermore, effects of viral infection on the adrenergic



**Figure 5.** Nervous system involvement in virus-induced exacerbations of asthma. (Top) Virus-induced epithelial damage may permit irritant and allergen penetration and contact with nerve endings. (Middle) Epithelial damage may also affect the metabolism of neuropeptides, such as substance P and neurokinin-A, which are implicated in cell activation in asthma. (Bottom) Viruses may damage the M2 muscarinic receptor that regulates cholinergic responses by a negative feedback loop, leading to increased cholinergic responsiveness.

receptor have been suggested (131, 132). However, the above pathways have not been further elucidated.

### Molecular pathways

A considerable number of genes are upregulated upon viral infection of epithelial, immune or other cell types. Early events at the epithelial level, studied by use of a gene chip, include upregulation of many interferon-related genes (8). Subsequently, proinflammatory mediator genes are induced. Activation can be initiated by either receptor contact (e.g. ICAM-1) or virus replication, through the production of dsRNA and a dsRNA-dependent protein kinase pathway, as well as an IFN- $\beta$ -mediated JAK-STAT pathway (8). None of these pathways is able to comprehensively explain all the observed effects at a cellular level, suggesting that multiple pathways are involved in a complex network. In this respect, poly-IC, a synthetic dsRNA analogue and TLR3 agonist, is not able to completely reproduce RV infection-mediated effects, although these are in part also mediated by TLR3 (78). From another perspective,

dsRNA is also a potent stimulus of nitric oxide synthase 2 (NOS2) expression (133).

The ability of viral infections to activate transcription factors, notably NF- $\kappa$ B, seems to be a central event in the inflammatory response of the epithelium to infection. Adhesion molecules such as ICAM-1 and VCAM-1 (33, 34) or proinflammatory mediators including IL-1, IL-6, IL-8, IL-11 and GM-CSF have NF- $\kappa$ B sites in their promoter regions and have been shown to be upregulated through virus induction of this transcription factor (134). Nevertheless, NF- $\kappa$ B-independent pathways are also involved (135). Additional transcription factors such as AP-1 and GATA may be involved (33, 34). In human lymphocytes, NF- $\kappa$ B as well as NF-AT2 activation regulates IL-4 production, inducing a Th2 response after exposure to dsRNA. Transcriptional activation could be either direct, or mediated by other virus-induced events such as oxidative stress (136).

Upstream events required for cytokine transcription include phosphatidylinositol (PI)-3 kinase activation, required for IL-8 production, relating at least partly to viral endocytosis (137). Activation of p38 MAP kinase also seems to be a key event, as its blockage resulted in extensive inhibition of RV-induced production of multiple cytokines (138). Stimulation of p38 is mediated by the small G-protein RhoA through membrane rafts (139).

Genetic polymorphisms affecting the course or susceptibility to respiratory syncytial virus bronchiolitis are gaining attention (140, 141). As there are differences between acute bronchiolitis and asthma (142), further such studies in respect to asthma exacerbations are needed.

### Interactions between viral infections and other factors

#### Viral infections, atopy and allergens

It is still uncertain if and to what extent asthmatic patients are more susceptible than normal individuals to airway infections. In a recent longitudinal study RV infections were similarly common in healthy and asthmatics; however, the degree of lower respiratory tract symptoms induced by the infection was more severe and prolonged in the asthmatic group (13). It had previously been suggested that the degree of antibody-mediated protection from RV infection is suboptimal in atopic subjects (143). Interestingly, the degree of worsening of asthma seems to be more pronounced in children with high IgE levels *vs* those with lower (144). Moreover, the odds ratio for wheezing is greater in children with RSV infection if they have evidence of atopy, supporting the theory of detrimental interaction between viruses and an allergic status in the infected patient (145). Nevertheless, it is now clear that the atopic/asthmatic condition may influence the outcome of viral infections independent of the presence of allergen (4, 85). On the other hand, epidemiological studies have shown that sensitized

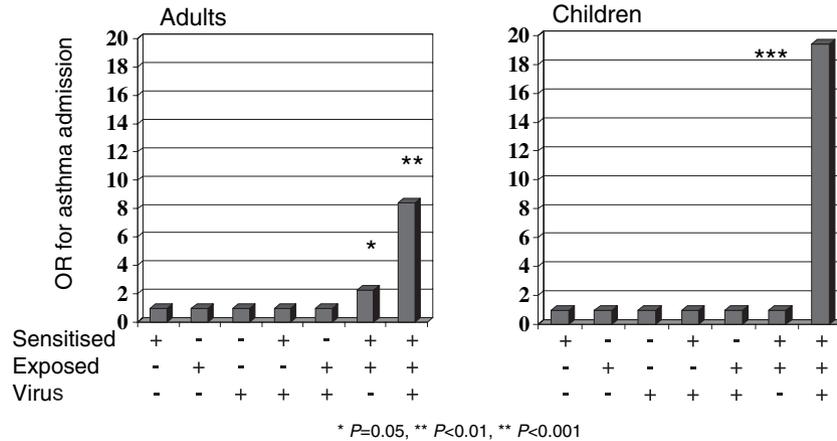


Figure 6. Synergistic effect of allergic sensitization, allergen exposure and viral infection in increasing the risk of hospitalization for asthma in children. Combined exposure and viral infection in sensitized children increased almost 20-fold such risk, while individual risk factor had weaker effects [adapted from Refs (146, 147)].

allergic patients suffering from a viral infection have a very pronounced risk of developing a severe asthma worsening if they were concomitantly exposed to the relevant allergen, with an odds ratio of over 8 in adults (146) and almost 20 in children (147; Fig. 6). It is well established that when patients with mild rhinitis and/or asthma are experimentally infected with RV, a concomitant allergen challenge causes greater recruitment of eosinophils, more pronounced histamine release, enhanced bronchial hyperresponsiveness as well as increased risk of developing late asthmatic responses (25). Interestingly, when patients were exposed to RV after allergen exposure, no additive or synergistic effect on several inflammatory parameters was observed (148, 149). It seems therefore that timing of exposure may be important in a synergistic outcome, supported by recent *in vitro* (150) and animal findings (151).

#### Viral infections and air pollutants

Interactions between viral infection and air pollution have also been demonstrated. A recent study in Kenya monitoring exposure to indoor air pollution and health status of individuals showed a relationship between indoor pollution by biomass combustion and acute respiratory infections in adults and children (152). NO<sub>2</sub> has received attention, as it can be emitted from both gas cooking appliances and motor vehicle exhausts, thus representing both an indoor and an outdoor pollutant. The Air Pollution on Health: European Approach (APHEA) project with data from 15 European cities has shown that an increase of 50 µg/m<sup>3</sup> NO<sub>2</sub> for 1 h was associated with a 2.6% increase in asthma admissions (153). Children exposed to increased levels of NO<sub>2</sub> in school and home showed a significant increase in sore throat, colds and absences from school (154). When personal NO<sub>2</sub> exposure and virus presence were assessed

in a cohort of asthmatic children, high NO<sub>2</sub> exposure was associated with higher infection rates and increased severity of virus-induced asthma symptoms (155). Furthermore, diesel particle exposure increases susceptibility to RSV infection (156) and upregulate RV receptors ICAM-1 and LDL (157).

Smokers have an increased risk for more frequent common colds with longer duration, as shown in epidemiological (158) and experimental infection studies (159). In children, environmental tobacco smoke (ETS) increases the risk of wheezing with colds (160), and asthma hospitalizations (161), possibly by an additive effect on atopic inflammation (162).

#### Conclusion and need for further research

Knowledge of the mechanisms of virus-induced asthma exacerbations has increased substantially in the last few years, although significant gaps still exist. Detailed studies of the molecular pathways that underlie virus-induced inflammation may help identify new therapeutic targets. While information on different aspects of the antiviral response is increasing, integration of the diverse elements and their interactions into a global model is still missing. In this respect, the role of neural elements in humans requires further attention. Further studies are also needed in order to differentiate between the response of normal and asthmatic individuals to viruses and to clarify the mechanisms that lead to increased severity and/or preclude resolution of infection in the latter. Furthermore, differentiation between atopic and nonatopic responses in asthma is still required, as the majority of studies have focused on atopic asthma. Finally, interactions between multiple triggers and their mechanisms, as well as the impact of virus-induced inflammation on subsequent tissue remodelling are only starting to be unravelled.

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