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Available online at www.sciencedirect.com
ScienceDirect
Pharmacology & Therapeutics 117 (2008) 313–353
Pharmacology & Therapeutics
www.elsevier.com/locate/pharmthera

Associate editor: P. Foster

Understanding the mechanisms of viral induced asthma: New therapeutic directions

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Abstract

Asthma is a common and debilitating disease that has substantially increased in prevalence in Western Societies in the last 2 decades. Respiratory tract infections by respiratory syncytial virus (RSV) and rhinovirus (RV) are widely implicated as common causes of the induction and exacerbation of asthma. These infections in early life are associated with the induction of wheeze that may progress to the development of asthma. Infections may also promote airway inflammation and enhance T helper type 2 lymphocyte (Th2 cell) responses that result in exacerbations of established asthma. The mechanisms of how RSV and RV induce and exacerbate asthma are currently being elucidated by clinical studies, in vitro work with human cells and animal models of disease. This research has led to many potential therapeutic strategies and, although none are yet part of clinical practise, they show much promise for the prevention and treatment of viral disease and subsequent asthma.

Keywords: Respiratory syncytial virus; Rhinovirus; Induction; Exacerbation; Asthma; Allergy; Treatment; Prevention

Abbreviations: AAD, allergic airways disease; AHR, airway hyperresponsiveness; APC, antigen-presenting cell; ASM, airway smooth muscle; BALF, bronchoalveolar lavage fluid; BEC, bronchoepithelial cell; bFGF, basic fibroblast growth factor; CAM, Cellular adhesion molecules; CCR, CC chemokine receptor; CGRP, Calcitonin gene-related peptide; CRP, C reactive protein; dsRNA, double stranded RNA; ECP, eosinophil cationic protein; ENA-78, Epithelial neutrophil-activating peptide-78; FEV_1, forced expiratory volume; FI, formalin-inactivated; G-CSF and GM-CSF, granulocyte and granulocyte-macrophage colony stimulating factor; ICS, inhaled corticosteroid; IFN, interferon, IFN; IL, interleukin; IP-10, IFN-γ inducible protein-10; LABA, long acting beta agonist; LDH, lactate dehydrogenase; LDLPR, low density lipoprotein receptor; LRT, lower respiratory tract; LT, leukotriene; mAB, monoclonal antibody; MCP, monocyte chemoattractant proteins; mDC, myeloid dendritic cell; MHC, Major histocompatibility; MIP, macrophage inhibitory proteins; MPV, metapneumovirus; NF-kB, nuclear factor (NF)-kB; NK cells, natural killer cells; NK1, neurogenic receptor 1; OR, odds ratio; PAF, platelet-activating factor; PBMC, peripheral blood mononuclear cell; pDC, plasmacytoid dendritic cell; PEF, peak expiratory flow; Penh, enhanced pause; pfu, plaque forming units; PG, Prostaglandin; PKR, protein kinase R; PVM, pneumonia virus of mice; RAD, reactive airway disease; RANTES, Regulated on activation normal T cell expressed and secreted; RR, relative risk; RSV, respiratory syncytial virus; RV, rhinovirus (RV); ssRNA, single stranded RNA; TGF, transforming growth factor; Th, T helper lymphocytes; TLR, Toll-like receptors; TNF, tumor necrosis factor; URT, upper respiratory tract; VEGF, vascular endothelial growth factor; vs, versus; WBC, white blood cell.

Contents

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doi:10.1016/j.pharmthera.2007.11.002

1. Introduction

1.1. Asthma

Asthma is thought to affect at least 300 million people of all ages and ethnic backgrounds worldwide (Global strategy for asthma management and prevention, 1995). Between 1 in 5 and 1 in 10 people are affected in Western societies and the prevalence has doubled since 1980 (Umetsu et al., 2002; AIHW, 2005). It is now considered to be an epidemic and results in a massive economic burden to communities. Exacerbations are typically caused by exposure to environmental factors to which the individual is allergic. Although asthma is clearly recognised as an inflammatory condition, our understanding of the mechanisms of pathogenesis remains rudimentary. Clinically asthma is characterised by airway obstruction, wheezing and episodic breathlessness in association with increased sensitivity of the airways to non-specific stimuli (termed airway hyperresponsiveness (AHR)) (Bousquet et al., 2000). Wheezing is a high-pitched whistling or squeaking, which originates from the chest and is made during breathing (Michel et al., 2006). A predominant feature of disease is the acute-on-chronic infiltration of pro-inflammatory activated CD4+ Th2 cells and eosinophils into the airways, which are critical regulators of pathogenesis (Robinson et al., 1993; Kay, 2005). Typical pathogenic features include: IgE production; airway smooth

Table 1
Important factors released by respiratory epithelium upon viral infection (Dakhama et al., 2005a)

Factor type	Family	Molecule
Cytokines	Interferons	IFN-α, IFN-β, IFN-λ
	Interleukins	IL-1β, IL-6, IL-10, IL-11
	Tumor necrosis factors	TNF-α
Chemokines		IL-8
	Monocyte chemoattractant proteins	MCP-1, MCP-4
	Macrophage inhibitory proteins	MIP-1α, MIP-3α
		Regulated on activation normal T cell expressed and secreted (RANTES)
	Eotaxins	1, 2
		Epithelial neutrophil-activating peptide-78 (ENA-78)
		IFN-γ inducible protein-10 (IP-10)
Major histocompatibility (MHC) molecules	Cellular adhesion molecules	MHC I, MHC II
Adhesion molecules		ICAM-1, VCAM-1, Ep-CAM
Integrins		α1–6, 8, 9
Pattern recognition receptors	Toll-like receptors	β1, 4–6, 8
		TLRs 1–10
Lipid mediators	Prostaglandins	CD14
		PGE_2, PGF_{2x}
	Leukotrienes	Thromboxane B_2
		LTB_4, LTC_4, LTD_4, LTE_4
Growth factors	Growth factors	Epidermal GF
		Platelet derived GF
		Transforming GF (TGF)-α,β
		Basic fibroblast GF (bFGF)
		Insulin-like GF
	Colony stimulating factors	Granulocyte CSF (G-CSF)
		Granulocyte-macrophage CSF (GM-CSF)
Antimicrobial peptides	Defensins	α, β
		Lysozyme
	Collectins	Lactoferrin
		Surfactant protein-A,D
Neuropeptides	Endothelins	Substance P
Mucins		Calcitonin gene-related peptide (CGRP)
Oxygen radicals		
Gases		Nitric oxide

muscle (ASM) and goblet cell hypertrophy/hyperplasia; mucus hypersecretion; eosinophil, neutrophil and mononuclear cell infiltration into submucosal layer of the airways; mast cell and macrophage activation; sloughing of airway epithelial cells; and AHR (Foster et al., 1996; Kumar, 2001; Cohn et al., 2004). Th2 cells and activated inflammatory cells release a range of mediators that damage the mucosal epithelial lining and promote an exaggerated repair response that leads to airway remodelling and chronic disease. Remodelling is the result of structural changes of the epithelium, submucosal layer, ASM and vasculature (angiogenesis) (Bousquet et al., 2000; Vignola et al., 2003). It is thought to be a major contributing factor to the development of AHR, and its progression may lead to fixed airflow obstruction and irreversible loss of lung function (Li & Wilson, 1997; Vignola et al., 2003). Thus, airway inflammation is closely linked to AHR and airflow obstruction and recurring inflammatory insults may result in changes that lead to airway remodelling. The mechanisms responsible for the generation of inflammation and remodelling remain poorly understood but may be induced or exacerbated by respiratory viral infection.

1.2. Viruses and asthma

Respiratory infections by RSV, RV, influenza and parainfluenza and metapneumovirus (MPV) have all been implicated in the development of asthma as well as exacerbations. Infection with RSV and RV are by far the most widely and commonly associated with bronchiolitis and childhood wheeze and the induction and exacerbation of asthma (Papadopoulos et al., 2002a; Xepapadaki et al., 2004). RSV may cause earlier and more severe exacerbations and is more frequently linked to the induction of asthma whereas RV is the most common cause of exacerbations in later life (Johnston et al., 1995; Zhao et al., 2002). Whether an infection induces disease depends on viral (type (E.g. RSV, RV)), host (genetic susceptibility, age, immune responses) and environmental (allergen exposure, season) factors. Initial infection occurs by inhalation and spreads to the lower respiratory tract (LRT). Infection is largely restricted to the respiratory epithelium, which induces the release of a wide range of mediators (Table 1) (Dakhama et al., 2005a) that drive subsequent immune and physiological responses specific for each virus (Fig. 1).

RSV and RV are the most important causes of LRT infections in infants under 2 years, causing bronchiolitis that results in wheezing, and breathing difficulties, which may in severe cases result in hospitalization (Sigurs et al., 2000; Kotaniemi-Syrjanen et al., 2003; Henderson et al., 2005; Sigurs et al., 2005). Asthmatics may be more susceptible to viral infections, which lead to more severe LRT symptoms and are associated with increased hospitalization (Corne et al., 2002). Notably the commonest cause of asthma related-death is respiratory viral infection (McCann & Imani, 2007). Severe bronchiolitis resulting in hospitalization has been shown to be associated in

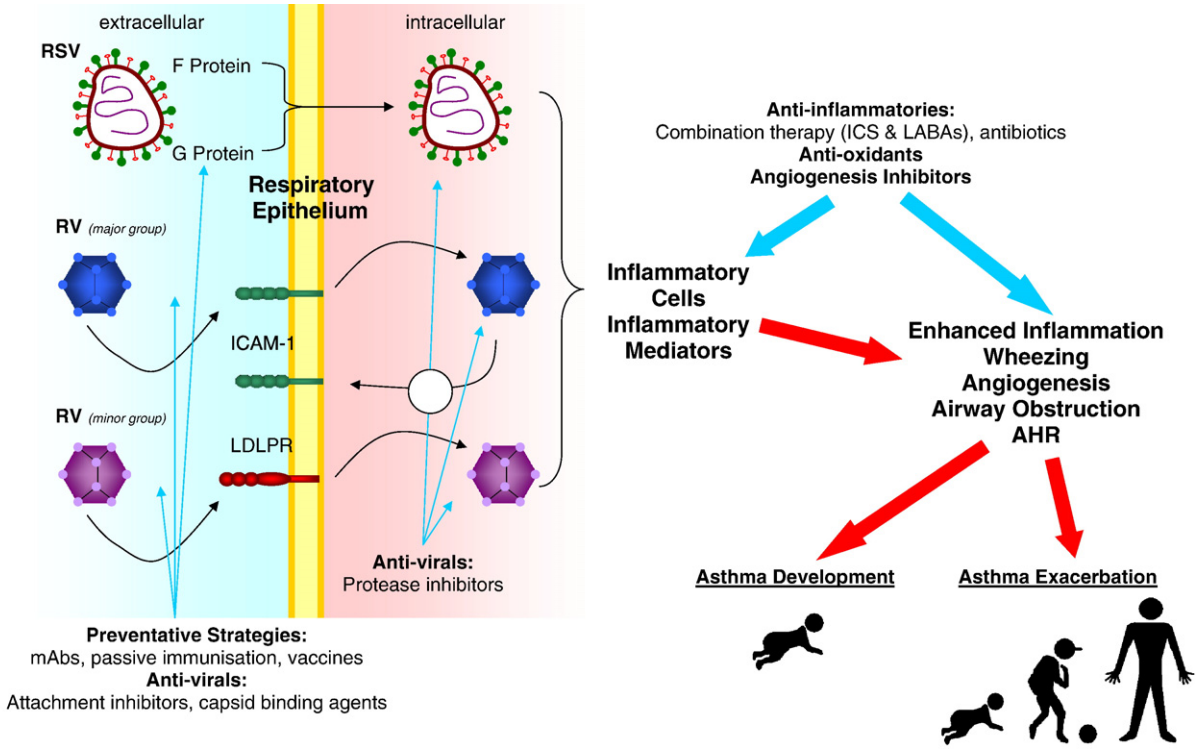

Fig. 1. RSV, RV, asthma and therapy. RSV attaches to and invades the respiratory epithelium through the attachment (G) and fusion (F) proteins. Major and minor group RVs bind to ICAM-1 and LDLPRs on respiratory epithelium, respectively, which induces viral internalisation and upregulation of additional receptors. Upon invasion viral proliferation leads to the induction of inflammatory cells and mediators that enhance allergen penetrance and hallmark features of asthma including inflammation, wheezing, airway obstruction and AHR. This may induce the development and exacerbation of asthma. Various processes in virus-associated asthma may be targeted therapeutically.

some case control studies with a history of recurrent wheeze and a diagnosis of asthma in later childhood (Sigurs et al., 2000, 2005). RSV is the most widely implicated precipitant but recent studies suggest that RV may also be important, particularly after the age of 2 (Heymann et al., 2004). Methods of detection are constantly been improved which will facilitate the elucidation of the role of these viruses in asthma (Pierangeli et al., 2007).

The mechanisms that underpin virus-induced induction or exacerbation of asthma or the factors responsible for predisposing an individual remain poorly understood. It is unclear whether virus-induced bronchiolitis promotes the development of asthma or if those individuals that suffer more severely from infection are also more susceptible to asthma development as a result of genetic susceptibility (including atopy) or aberrant lung function. Understanding the mechanisms of pathogenicity of respiratory viral infections and their association with asthma will be pivotal in the development of prevention and treatment strategies for asthma. Indeed it may be possible to identify at risk individuals and delay infection until later in life and to develop novel therapeutic agents or targets.

In asthma triggers of chronic inflammatory processes (airway inflammation, mucus hypersecretion) are overzealous responses of the asthmatic immune system to normally innocuous antigens or infections and recurrent stimulation leads to airway remodelling. Respiratory viral infections induce immune responses that may have the potential to both initiate and exacerbate asthma.

1.2.1. Induction of asthma

Collectively evidence strongly implicates respiratory viral infections in the development of an asthmatic phenotype in children, although a directly causative role has still not been proven. It is possible that either 1. respiratory infection in early life induces the development of chronic airway inflammation and airway wall remodelling, resulting in persistent wheeze or 2. that some infants have pre-existing Th2 responses that induces susceptibility to virus-induced wheeze (Legg et al., 2003; Stensballe et al., 2006).

1.2.2. Exacerbation of asthma

The evidence for virus-induced asthma exacerbations is stronger than for causation and infections are responsible for the majority of acute exacerbations (80% in children, 50–76% in adults) inducing worsened airflow obstruction and symptoms (Johnston et al., 1995; Wark et al., 2001; Murray et al., 2004; Tan, 2005).

To determine if virus infection directly influences disease in acute asthma exacerbations Wark et al., recruited adults presenting to emergency with acute asthma and determined that 76% of these subjects had evidence of a viral infection (Wark et al., 2002). When compared to subjects with non-infective acute asthma, those with acute asthma and infection had evidence of more severe clinical disease, had a lower mean forced expiratory volume (FEV_1 % predicted), were more likely to be admitted to hospital and had a longer median length of stay. An acute neutrophilic infiltrate was observed in induced sputum, with evidence of neutrophil degranulation, unlike the eosinophilic inflammation associated with non-infective asthma (Wark et al., 2002). In addition lactate dehydrogenase was measured as a marker of asthmatic airway necrosis. Those with virus-associated acute asthma had significantly elevated lactate dehydrogenase activity, which correlated closely with the degree of neutrophil influx and airflow obstruction and was an independent predictor of the severity of the acute illness. These results strongly implicate viral infection as a trigger of exacerbations with increased severity and indicate an important role for viruses in modifying inflammation in acute asthma (Wark et al., 2002).

1.2.3. Asthma phenotypes

There are many different categories and phenotypes of asthma including mild, moderate and severe as well as clinical, allergic and pathophysiologic phenotypes (Wenzel, 2004). Recently Simpson et al., described different inflammatory (neutrophilic, eosinophilic and paucigranulocytic) subtypes of asthma based on the predominant granulocytic cell in induced sputum (Simpson et al., 2006). The precise roles of respiratory viral infection in the development and exacerbation of the different phenotypes of asthma remain largely unknown, however, there is the potential that viral infection may be particularly important in certain phenotypes. Different and customised prevention and treatment strategies may be required for different phenotypes depending on their causes. Targeted anti-viral strategies may be more effective in certain asthma phenotypes for example in severe and neutrophilic asthma, where infectious agents may play a substantial role in pathogenesis (Wark et al., 2002).

1.2.4. Factors inducing susceptibility

Many factors may be involved in susceptibility to virus-induced asthma particularly virus and host factors. Virus infection is the commonest cause of wheeze in children that may lead to the development of asthma (Heymann et al., 2004). The time of year also plays a role with winter the dominant period for viral infections and wheeze (Heymann et al., 2004). Genetic predisposition may be important and many genes are implicated in susceptibility to asthma including those involved in inflammatory responses, IgE regulation, cytokine and chemokine production, airway function and remodelling (Umetsu et al., 2002). Atopy may lead to adverse responses to infection and childhood wheezing is linked to elevated IgE and sensitivity to at least one inhaled allergen. However, the genetics of Western populations that are experiencing the asthma epidemic remain unchanged and the focus of this review will be on other important factors that are associated with RSV- and RV-associated asthma. These include the age of infection and timing of infection relative to allergen exposure, increased innate susceptibility and adaptive immune, cytokine and chemokine responses, as well as environmental conditions and inhaled bacterial endotoxin.

2. RSV and asthma

RSV is a lipid-enveloped single stranded (ss) negative sense RNA pneumovirus and is a member of the Paramyxoviridae family. The virus causes the majority of cases of bronchiolitis in

Table 2
Recent epidemiological studies that link RSV infection with asthma

Study aims	Cohort	Test	Result/conclusion	Reference
To evaluate asthma in children at >13 years with infantile bronchiolitis or pneumonia	Children (127) <2 years hospitalized for bronchiolitis (n=81) or pneumonia (n=46)	Infection, eosinophilia and atopy recorded on admission. Atopic/ asthmatic symptoms at 15 years	Asthma present in 14–23% with bronchiolitis and 12–15% with pneumonia. Early asthma predictive factors were maintained; wheezing, atopic dermatitis, elevated blood eosinophils. Increased risk of asthma for 15 years after infant bronchiolitis and pneumonia.	Hyvarinen et al., 2005
To investigate cytokines, chemokines and ECP in nasopharyngeal secretions of infants =7 months with RSV	Infants with RSV (39), influenza or parainfluenza (9) or controls (50)	Factors in nasopharyngeal secretions	RSV infected infants had higher levels of IL-4, MIP-1β and ECP. IL-4 was higher in RSV infected infants =3 months compared with RSV infected infants =3 months.	Kristjansson et al., 2005
Does severe RSV vs non-RSV viral bronchiolitis in infancy lead to wheezing and reduced lung function at 7 years	Infants (57) hospitalized with acute viral bronchiolitis examined at 7 years vs controls (64)	Epidemiological and clinical data, interview and physical exam	Children hospitalized for bronchiolitis during infancy had reduced lung function and birth weights, more frequent wheezing episodes, and first order family members with asthma. No difference between RSV positive and negative groups.	Fjaerli et al., 2005
Is history, age and passive smoking related to urinary LTE4, wheezing, bronchiolitis and asthma	Infants (33) with bronchiolitis vs controls (25), 1–12 months	Demographic/historical data by parent questionnaire, RSV in nasal secretions and LTE4 in urine	LTE4 is 8 fold higher in infants with bronchiolitis and infected infants <6 months with medical history of eczema or dry cough and/or family history of asthma.	Piedimonte et al., 2005
To investigate the relationship between RSV-bronchiolitis in infancy with atopy and wheeze	Infants (150) <12 months hospitalized for RSV-bronchiolitis	Assessed for wheeze, asthma and atopy (3–7 years)	RSV-bronchiolitis associated with wheeze at 30–42 months (odds ratio (OR) 2.3), 69–81 months (OR 3.5) and asthma at 91 months (OR 2.5) but not atopy (OR 0.7).	Henderson et al., 2005
To evaluate if IgG antibodies to RSV in early life is associated with later asthma	Infants (100) hospitalized for wheezing at <2 years of age	RSV detected by RT-PCR in nasopharyngeal aspirates, and by specific serum IgG	29/100 hospitalized for wheezing had RSV. Hospitalization for wheezing associated with subsequent childhood asthma.	Kotaniemi-Syrjanen et al., 2005
To assess need for vaccine by analysing epidemiological and clinical data from elderly/high-risk adults	Healthy elderly (608) or high-risk adults (heart/lung disease, 540) vs acute cardiopulmonary subjects (1388)	RSV/influenza diagnosed by culture, RT-PCR and serology	Healthy elderly cohort: 3–7% infected with RSV annually High-risk cohort: 4–10% infected with RSV annually Hospitalized cohort: RSV and influenza resulted in similar lengths of stay.	Falsey et al., 2005
To estimate excess morbidity during RSV and influenza infection	Children 1–4 vs 5–14 years	Diagnosis of influenza-like-illness, acute bronchitis, asthma and otitis media	Influenza-like-illness correlated with RSV (40%), influenza virus (60%), acute RSV bronchitis (37%), asthma (9%), otitis media (48%). Children <1 year had highest rates of acute bronchitis. RSV-induced greater illness than influenza, influenza not associated with asthma.	Fleming et al., 2005
To determine if RSV or RV infection and atopic skin prick responses influence severity of asthma exacerbations	Children (50) 4–12 years with acute severe asthma and after 6 weeks and 6 months	Measured; peak expiratory flow (PEF), RSV/RV in nasal aspirates, atopy by skin prick responses	Acute asthmatics had; atopy (74%), RSV (12%), RV (82%); at 6 weeks and 6 months 44% and 25% still had RV, respectively. PEF reduced in asthmatics (no difference; RSV, RV or no virus groups). Severity of PEF reductions linked to persistence of RV.	Kling et al., 2005
To compare clinical features between boys and girls with wheezing triggered by acute RSV bronchiolitis	Boys (90), girls (51) (<6 months)	Systemic; white blood cells (WBCs), eosinophils, serum C reactive protein (CRP) and local inflammation; sputum eosinophils and neutrophils	Blood eosinophilia in acute phase rare in children >6 months. Girls increased WBCs and CRP levels vs boys. Sputum eosinophil scores =2+ only in boys (6/42), neutrophils in both. 28 children with RSV bronchiolitis had subsequent wheeze but not blood or serum eosinophilia during RSV bronchiolitis.	Nagayama et al., 2006a

Does atopy and wheezing, increase relative risk (RR) of hospitalization for RSV in children 0–18 months	RSV hospitalized (2564) vs control (12,816) children followed prospectively (0–18 months)	Chronological analysis of age at hospitalization, wheezing and atopic dermatitis	RR of childhood RSV hospitalization; 1.72 for maternal and 1.23 for paternal asthma, 1.11 for maternal atopic dermatitis, 2.98 for infrequent and 5.90 for recurrent wheeze. Atopic dermatitis associated with increased risk of subsequent RSV hospitalization in infants <6 months old.	Stensballe et al., 2006
To assess effect of gender in recurrent wheezing in children with RSV and asthma	Boys (98) and girls (58) with RSV, 123 <4 years old, 78 had pneumonia and 119 febrile episode	Clinical features and lab data	Deteriorated clinically during acute RSV infection and had elevated serum CRP and reduced blood eosinophils. Girls had higher WBC counts and CRP levels. Blood eosinophils during acute illness higher in boys vs girls aged 2–3 years.	Nagayama et al., 2006b
To compare features of children with LRT metapneumovirus (MPV), RSV and influenza infection and assess if co-infection increases disease severity	Children (516) hospitalized for LRT infection during a 1 year period	Nasal wash specimens tested for metapneumovirus, RSV and influenza	MPV detected in 13% of patients (24% co-infected with other viruses). MPV patients older vs RSV patients. MPV and RSV similarly associated with wheezing and hypoxemia (>influenza). 40% with MPV had atelectasis vs 13% with RSV or influenza. MPV more often associated with pneumonia vs RSV or influenza. MPV more often associated with asthma and less often with bronchiolitis vs RSV.	Wolf et al., 2006
To correlate risk factors and asthma/allergy age 5–6 years with 1st episode of bronchiolitis <12 months and effect of age of 1st bronchiolitis on development of asthma	Children (128) consulted or admitted with 1st bronchiolitis attack in 1st year of life	Retrospective telephone survey based on 2 paediatric hospital emergency registers	72% had family history of allergy, 41% were exposed to tobacco smoke, 81% were hospitalized during 1st bronchiolitis but none put in intensive care. In 12 months before survey, 31% had 1+ wheezing episode, 37% had asthma attack, 25% wheezed after effort, 39% had nocturnal dry cough, 41% had allergic rhinitis, 25% had eczema. 57% of children (47) who had 1+ asthma attack in previous 12 months had family history of asthma.	Sznajder et al., 2005
To determine RSV infection rates in children <5 years with recurrent wheeze or asthma and compare clinical presentation, course and outcome vs age matched asthmatic children with no RSV	Children (73, median age 28 months) recruited from emergency department during peak RSV season	Information on past/present asthma presentations collected, a nasopharyngeal aspirate taken for virus isolation and all children reviewed 1 week after presentation	45% had RSV, 1 had adenovirus. Children <12 months more likely to have RSV (70%). Children with RSV had longer illness before hospital presentation than children with no RSV but were not more likely to be admitted or have longer duration of ongoing symptoms.	Lazzaro et al., 2007
To compare clinical features of MPV, RSV and RV infections in children <3 years presenting to emergency department with acute respiratory illness	Children (931) <3 years admitted for acute respiratory illness over 2 winters	Respiratory viruses detected in nasal washes	3–6% had MPV, 28% RSV and 18% RV. 5 with MPV were co-infected, 2 with RSV, 3 with RV. No difference in the prevalence of bronchiolitis where MPV, RSV or RV were present. Asthma found more often in hospitalized children with MPV and RV than with RSV.	Manoha et al., 2007
To examine effect of different clinical characteristics and treatments on hospitalization of infants for bronchiolitis in outpatient clinic	Infants (320) <2 years presenting with 1st episode of wheezing	Retrospective analysis of medical records	38% of patients with RSV were hospitalized vs 10% without RSV. Children exposed to tobacco smoke hospitalized more often (24%) vs not exposed (12%). Treatment with oral corticosteroids associated with fewer hospitalizations in those with family history of asthma/allergic rhinitis (9.7% vs 24%) and without RSV (2.5% vs 16.7%).	Al-Shawwa and Rao, 2007

early life, which may induce wheeze that may develop into asthma and is also a major precipitant of asthma exacerbations.

Epidemiology — RSV is the most important respiratory pathogen of children under the age of 2 years and primary infections are a common cause of LRT disease (Hall, 1999; Henrickson et al., 2004). The majority of infants are infected during the first year of life, and the incidence of exposure approaches 100% by age 3 (Parrott et al., 1973; Glezen et al., 1986; Hall et al., 1995). These infections are the most frequent cause (50–90%) of bronchiolitis and also induce pneumonia and tracheobronchitis (10–30%) and there are annual epidemics, primarily in infants (Hall, 2001). Around 100,000 children are hospitalized as a result of bronchitis annually in the USA with 50% less than 6 months and 80% less than 1 year of age with an estimated cost of $UD300 million per year (Shay et al., 1999). Hospitalization for bronchiolitis has dramatically increased over the last 20 years (Shay et al., 2001), which may result from changes in childcare practises or a generalized decrease in Th1 immunity in the population. Mortality rates from primary infection are 0.005–0.02% for healthy or 1–3% for hospitalized children (Ruuskanen & Ogra, 1993; Chanock et al., 1957). Most children that contract severe RSV disease have no identifiable risk factors, with the exception of premature birth. Infections also cause severe disease in the elderly and immunocompromised and mild upper RT (URT) symptoms (rhinorrhea, nasal blockage, pharyngitis and cough) can occur at any age (Falsey & Walsh, 1998; Englund et al., 1988). Re-infection is also common, occurring every 2–3 years throughout life usually resulting in mild URT symptoms. Importantly this results from the lack of development of long-term resistance to RSV infection by the immune system (Bont et al., 2002). The majority of symptoms result from the host's immune and inflammatory responses to infection (Openshaw, 1995) and re-infection induces sustained and exacerbated inflammatory reactions.

Pathogenesis — Upon RSV infection and interaction of the virus with the respiratory mucosal surface the viral G protein mediates attachment and the F protein induces the fusion of the viral envelope with the cytoplasmic membrane of the host cell resulting in internalisation (Fig. 1). After invasion the viral ssRNA is released into the cytoplasm and induces the production of viral RNA and proteins that induce inflammatory responses. The RSV proteins and their functions have recently been reviewed by Meyer et al. (2007). The outcome of these inflammatory responses is the development of symptoms of pathogenic infection. Typically, RSV infections in humans are restricted to the mucosal epithelial cells of the URT, causing runny nose, nasal congestion and cough (Hall et al., 1978).

During severe RSV infections, the virus spreads to the LRT resulting in more severe symptoms. In vitro studies of human infection, as well as autopsy samples from infants and children with acute RSV infections, show that viral replication in airway epithelial cells, particularly in the superficial layer of the bronchiolar epithelium, as well as types 1 and 2 pneumocytes. Infection induces the generation of inflammatory mediators and a mononuclear inflammatory response, plugging of the bronchioles with mucus, cellular debris and fibrin strands, as well as necrosis of the bronchiolar epithelium (Piedra et al., 1997; Johnson et al., 2007). The lack of cytopathology during infection implicates inflammatory responses as the pivotal driver of RSV-induced disease (Zhang et al., 2002). Inflammatory cells consist mainly of monocytes, T cells and neutrophils and accumulate around bronchial and pulmonary arterioles, airways and parenchyma and are associated with edema, mucus production, wheezing, airway obstruction and AHR (Johnson et al., 2007). The induction of these disease processes may be involved in the development and exacerbation of asthma.

2.1. Clinical evidence for the association between RSV and asthma

Clinical and epidemiological studies have shown that RSV infections are associated with a rapid increase in the incidence of asthma in paediatric and adult populations worldwide (Wang & Forsyth, 1998; Tan, 2005). These infections are also one of the commonest causes of asthma exacerbations. Recent epidemiological studies that link RSV with asthma are shown in Table 2 and older studies are reviewed in Ogra (2004). RSV infections are the most important risk factor for the development of bronchiolitis leading to recurrent wheezing and respiratory symptoms (decreased lung function, recurrent wheezing, allergic rhinoconjunctivitis). However, it has not yet been conclusively demonstrated that these virus-induced symptoms then progress to the development of asthma. A link between infection, atopy and sensitization to common inhaled allergens (skin prick tests (SPTs), IgE) has also been investigated but the results are inconclusive. Asthmatics may be prone to more severe infections, which may be a prognostic indicator of allergic susceptibility.

2.1.1. Bronchiolitis

Between 50 and 90% of all cases of childhood hospitalizations for bronchiolitis have been attributed to RSV infections (Holberg et al., 1991). Collectively studies show that RSV-induced bronchiolitis and diseases of the small airways often lead to wheezing which frequently progresses to asthma (reviewed in Heymann et al. (2004)).

2.1.2. Wheeze

Many studies have reported that up to 75% of subjects with RSV bronchiolitis suffer from subsequent recurrent wheeze or respiratory symptoms years later (reviewed in Ogra (2004)). An important prospective study by Stein et al., showed that children with more than one LRT RSV infection were 4 times more likely to have frequent wheeze by ages 6 and 11, however the association decreased thereafter and was non-significant by age 13 (Stein et al., 1999). Another smaller prospective study demonstrated that severe RSV bronchiolitis was linked to current wheezing (38%) and reactive airway disease (RAD, 30%) compared to uninfected controls (2% and 3%, respectively) matched for age, sex family, history and environment (Sigurs et al., 2000).

2.1.3. Decreased lung function

Controlled retrospective and prospective studies indicate a link between RSV and bronchial obstruction and decreased lung function, particularly for children with severe RSV disease that results in hospitalization (reviewed in Sigurs (2002b)). Indeed

bronchiolitis is linked to chronic reductions in lung function for at least 10 years after infection (Pullan & Hey, 1982) and children hospitalized with RSV infection before 2 years of age have reduced lung function (but not asthma) 20 years later (Korppi et al., 2004b). Furthermore airway obstruction and AHR is increased in those with RSV bronchiolitis compared to controls (Sigurs, 2002b). A nested case-controlled study also showed that RSV-induced hospitalization correlated with wheezing, LRT infections and asthma during first 4 years of life. The correlation decreased with age and was not significant at 5 years but the association held for increased respiratory symptoms and chronic productive cough at 5–8 years in Alaska native children (Singleton et al., 2003). In the Stein study wheezing subjects were significantly more responsive to bronchodilators, which indicates that reduced lung function results from an abnormality in airway tone (Stein et al., 1999).

2.1.4. Asthma

RSV bronchiolitis, particularly in early life, is strongly linked to the development of asthma. Indeed 50–92% of children with bronchiolitis develop wheezing and asthma 3 (23% versus (vs) 1%), 5, 7.5 (30% vs 3%) or even 10 or more years later (Sly & Hibbert, 1989; Sigurs et al., 1995; Larouch et al., 2000; Sigurs et al., 2000; Ogra, 2004). Recent studies have found that children hospitalized with RSV have an increased risk of recurrent wheeze up to the age of 13 years, independent of atopy and other asthma risk factors, identifying RSV infection as a potential inducer of asthma. Indeed these subjects have significantly more respiratory symptoms, (43% vs 8% for asthma/recurrent wheezing), sensitization to common allergens (50% vs 28% SPT, 45% vs 26% serum IgE), airway obstruction and AHR at age 13 years compared with controls (Sigurs et al., 2005). Other recent reports have also shown that RSV infection is associated with concomitant respiratory symptoms (wheezing) and subsequent asthma at 2 years of age and that high rates of RSV are isolated from children with recurrent wheeze or asthma (Lazzaro et al., 2007; Lee et al., 2007). The association was not confirmed in other longitudinal or cohort studies and may depend on individual airway structure, genetic predisposition and environmental factors (reviewed in Martinez (2003)).

2.1.5. Atopy

There may be a link between atopy, RSV infection and wheezing (Sigurs et al., 2000) and risk factors may include the severity of RSV infection encountered. Atopic children have low Th1 responses in cord blood and elevated Th2 responses compared to non-atopics. Atopic adults also have Th2 responses to allergens whereas non-atopics have low-level Th1 responses (Ogra, 2004). Elevated expression of Th2 responses may promote susceptibility to RSV infection and may also lead to exaggerated airway inflammation and reduced lung function. However, the link between RSV infections and atopy is controversial and was not found in other studies (Stein et al., 1999; Kneyber et al., 2000).

2.1.6. Allergic sensitization

Early RSV infections (first 2 years) may induce allergic sensitization to unrelated antigens in genetically predisposed individuals (Sigurs et al., 1995; Forster et al., 1996). Indeed RSV bronchiolitis in the first year of life leads to increased IgE levels (33% vs 2% in controls) and is the most important risk factor for allergic sensitization and recurrent wheeze (16% vs 4% controls) (Schauer et al., 2002). Furthermore allergic sensitization and asthma are significantly more common in all age groups in children with a prior RSV bronchiolitis (23% vs 2% and 41% vs 22% of controls, respectively) (Sigurs, 2002a). It appears that the severity of infection may be important and severe infection has been associated with the development of allergic sensitization 3, 6 or 7.5 years after hospitalization (Sigurs et al., 1995, 2000). Mild infection, does not appear to be a risk factor for allergic sensitization (Stein et al., 1999; Sigurs et al., 2000). However, the data are conflicting and severe infection has been shown not to induce sensitization (at age 2–10 years) by other investigators (Pullan & Hey, 1982; Carlsen et al., 1987; Noble et al., 1997).

2.1.7. Asthmatic predisposition to more severe infection

Pre-existing Th2 or impaired Th1 responses and asthmatic predisposition may promote susceptibility to acute bronchiolitis and hospitalization for RSV infection (Legg et al., 2003; Stensballe et al., 2006). Furthermore, individuals with damaged airway epithelium or that are otherwise immunologically predisposed may be additionally susceptible to infection enhancing epithelial damage and causing a vicious cycle of disease perpetuation.

2.1.8. RSV infection as an indicator of susceptibility

Although RSV infection has been extensively linked with inducing asthma and reduced lung function it is possible that infection is also a marker of susceptibility to allergic and/or infectious respiratory disease. It has been shown that although RSV infection is the greatest risk factor for wheezing and asthma in subjects with a family history of asthma, asthma does not develop in those without infection or a family history (Sigurs et al., 2000; Sigurs, 2001). This indicates that RSV bronchiolitis is a marker of increased risk of asthma susceptibility. More recently it has been suggested that the host response to infection rather than the nature of the infection itself is the best prognostic indictor for subsequent allergic disease (Everard, 2006a, 2006b).

Atopy may also be a risk factor for susceptibility to more severe RSV infections and exacerbations, which result in higher rates of mortality and hospitalization (Jhawar, 2003). A large nested case-controlled study demonstrated that maternal atopic dermatitis and maternal and paternal asthma (RR 1.72 and 1.23, respectively) were risk factors for RSV hospitalization in infants <1.5 years (Stensballe et al., 2006).

Taken together studies show that RSV infection in early life results in bronchiolitis that leads to wheezing and decreased lung function, which may progress to asthma. These processes may be enhanced in atopic individuals with elevated Th2 responses. Moreover there is evidence, although not conclusive, that severe infection may also promote sensitization to allergens, which may further increase the risk of wheeze and asthma. Asthmatics may be susceptible to more severe infections, which may be used as an indictor of susceptibility to the development of asthma.

2.2. Mechanisms of predisposition and exacerbation shown in humans

Experimental RSV infection of humans has been used to investigate the mechanisms of pathogenesis of infectious disease and the association with asthma. These studies have demonstrated that the virus persists in nasal washes for 5–14 days (Noah & Becker, 2000) and that infection induces bronchiolitis and airway inflammation. This promotes epithelial sloughing, mucus hypersecretion and an increase in viscosity and edema which in turn lead to hyperinflation of the lungs, airflow obstruction, cough and wheeze (Holt & Sly, 2002a). Symptoms may persist and resolution of tissue damage may take several weeks or results in structural remodelling of the airway wall and airway narrowing (Pare et al., 1997; Holt & Sly, 2002a). The mechanisms of RSV pathogenesis that lead to wheezing, AHR, allergy and asthma are still not well understood and many studies have implicated a range of different factors. These include: the age of first infection; type of innate and adaptive responses elicited; mucosal damage and repair involving remodelling (including angiogenesis) and; enhanced neurogenic stimulation leading to ASM spasm and bronchoconstriction.

2.2.1. Age of first infection

According to the hygiene hypothesis (Strachan, 2000), the Th2-biased immune system of the newborn must encounter Th1-inducing agents during childhood in order to develop the ability to mount a Th1 response. However, whether infections in infancy induce beneficial Th1 responses depend upon the type of infection and the mechanisms responsible for these effects have not been characterised. Thus the immaturity of the developing immune system during early life and the nature of immune responses to infections may be significant determining factors in the development of persistent wheeze and asthma.

Whether RSV infection induces immunological and pathological processes that may lead to asthma may depend on the age at which an individual is first infected. The immune phenotype of early life may lead to enhanced viral replication and Th2-dominated inflammation induced by infection or allergen exposure. Increased levels of IL-4 were detected in RSV infected infants less than 3 months old compared to more than 3 months but the converse was true for eotaxin and there was no difference in MIP-1β or eosinophil cationic protein (ECP) (Kristjansson et al., 2005). In mothers infection induced high levels of IFN-γ and low levels of IL-4 whereas newborn infants produced 4–7 times less IFN-γ and higher levels of IL-4 but at age 3 these levels approached those of their mothers (Mbawuike et al., 2001). The immune response in early life may be involved in the induction of more severe LRT pathology in response to primary infection but infection of older children is not as severe and involves largely URT symptoms. An additional pathological consequence is that viral infections in early life may generate pulmonary inflammation during the development of the lung, small and large airways and immune, inflammatory and neuronal programing (reviewed in Gern et al. (2005)). This may result in altered pulmonary structure and immune responses leading to enhanced pro-inflammatory and Th2-biased immune responses that may precipitate deleterious changes in lung structure and function. Furthermore, the small size of neonatal bronchioles determines that they may become obstructed more readily, which may result in reduced clearance and confer enhanced severity of pathogenic infection in this age group. The combination of these events may have long-term effects on lung function, chronic respiratory inflammation, remodelling, alveolarization and epithelial dysfunction (Gern et al., 2005) and consequently may promote the development of asthma.

However, whether RSV infection induces Th1 or Th2 responses in humans in early life is debateable. Another alternative explanation for the association of RSV infection in infancy with asthma is that early life infection induces Th1 memory and therefore CD8+ CTL responses, which have the potential to induce pathologic immune responses to reinfection. RSV infection induces viral specific CD8+ cells in infants and the levels of cytotoxic lymphocytes (CTLs) are inversely proportional to IL-4 responses. CTL responses are directly linked to protection against infection, CTL memory is initiated upon reinfection and MHC I CD8+ levels correlate directly with IFN-γ levels (Mbawuike et al., 2001).

2.2.2. Immune responses

Immune responses induced to RSV enhance viral clearance but are also implicated in disease pathogenesis. Ineffective or aberrant innate and adaptive immune responses against RSV have been widely linked to more severe and recurring infections and the development and exacerbation of asthma in both adults and children (Glezen et al., 1986; Hall et al., 1991). Primary infection, particularly early in life, leads to an incomplete immune response, which does not elicit the development of sustained memory immunity. With respect to allergy RSV infection might only trigger defective immunity in genetically susceptible individuals or that allergic inflammatory and immune responses may promote the influx of virus-specific cells into the airways increasing inflammation and AHR (Schwarze et al., 1999c). Elucidation of the pivotal immune responses that are protective against RSV will lead to a better understanding of the processes that result in bronchiolitis, wheezing and progression to asthma.

2.2.3. Innate host responses

Innate responses to RSV infection have not been widely studied but may play an important role in RSV-induced asthma. RSV infection of the respiratory epithelium induces innate cellular and cytokine responses, which have substantial effects on adaptive T cell development and cell-mediated immune responses.

Neutrophils are the main immune cell in the bronchoalveolar lavage fluid (BALF) of patients with severe RSV LRT disease and increased IL-8 levels, which is a potent chemoattractor of neutrophils, are also a prominent feature. Macrophages internalise and process virus and present antigens to adaptive immune cells and also release IL-12 and IL-10. Monocytes release IL-1, -6, -8, -10, platelet-activating factor

(PAF) and PGE2 on exposure to RSV that further promote pro-inflammatory responses (Schaller et al., 2006).

Eosinophils are also recruited to the airways during primary RSV infection, which may contribute to the development of allergic airways disease (Schwarze et al., 1999a). Infected patients also have more plasmacytoid dendritic cells (pDCs) and myeloid DCs (mDCs) in the RT and reduced numbers of these cells circulating in blood (Gill et al., 2005). This suggests that they are recruited to the lung during infection and may be an important target in RSV vaccine development. Understanding the interplays between these different cells types will be crucial in elucidating the effects of infection on the development of asthma.

IFN-α is an important type I IFN innate cytokine that is released upon viral infection and induces innate and adaptive cellular responses. Blood cultures of asthmatic children and adults release significantly reduced amounts of IFN-α upon RSV infection, which indicates a systemic innate deficiency in asthmatics that may lead to heightened susceptibility to infection (Gehlhar et al., 2006).

2.2.4. Adaptive immunity

2.2.4.1. Th1/Th2 responses. RSV has several T cell epitopes and infection induces CD4+ and CD8+ and Th1 and Th2 adaptive cellular responses as well as pro- and anti-inflammatory cytokines and chemokines in humans both in vivo and in vitro (Meyer et al., 2007). Established infections are primarily cleared by a combination of Th1 and Th2 cell responses and the balance between the two may be crucial in determining the outcome of infection, the severity of RSV-induced disease and predisposition to asthma. Aberrant responses of either of these subsets may induce pathology. Nevertheless most studies suggest that Th1 responses may result in viral clearance and mild symptoms whereas an aberrant bias towards a Th2 phenotype may lead to more intense RSV-induced disease and promote the development of asthma (Psarras et al., 2004). Indeed individuals with elevated Th2 responses are predisposed to reduced viral clearance and more severe disease compared to subjects with only URT infection independently of age or viral load (Roman et al., 1997; Aberle et al., 1999; Legg et al., 2003; Gern et al., 2006). Early life RSV bronchiolitis results in enhanced IL-4 and IFN-γ responses to RSV in later childhood (Pala et al., 2002) and IL-4 is the most important stimulus for Th2 development and IgE production. Th2 responses are enhanced during severe disease that develops upon natural infection of RSV-vaccinated infants (Kim et al., 1969; Kapikian et al., 1969) and severe RSV bronchiolitis correlates with elevated humoral Th2 responses, and may be linked with atopy, increased IL-4:IFN-γ and reduced Th1 (IFN-γ, IL-12 and IL-18) responses. RSV infections may also have persistent immunological effects and induce long-term Th2 memory responses during sensitization to inhaled allergens in childhood (Holt & Sly, 2002a; van Rijt et al., 2005). Other studies have demonstrated that mild bronchiolitis is associated with a shift towards Th1 responses and is usual in most individuals, and also that there is no increase in Th2 responses in severe bronchiolitis (Garofalo et al., 2001). These contrasting results may be attributable to differences in the timing of sampling during infection, the lack of definitive detection of RSV or determination of virus load, age and atopic status of individuals.

2.2.4.2. Cytokines and immunomodulatory molecules. Cytokines of the Th1, Th2 or regulatory type are implicated in RSV-induced asthma and the effect of pro-inflammatory cytokines and chemokines released in response to infection may have particularly important roles.

Th2 (IL-5) cytokines are upregulated in children with acute asthma and children with acute bronchiolitis or IL-5 levels have higher numbers of eosinophils (Martinez, 2003). These responses may play key roles in the progression from bronchiolitis to asthma, however, the importance of eosinophils and IL-5 in RSV-induced asthma has not yet been confirmed.

IL-12 is a Th1 cytokine that promotes the development of Th1 cells, the release of IFN-γ and IL-2 from Th1 and natural killer (NK) cells and suppresses Th2 responses. Levels of IL-12 may be reduced in subjects that are more susceptible to infection. Non-specifically stimulated whole blood cultures from patients with RSV disease released significantly less IL-12 than controls and IL-12 levels inversely correlated with disease severity (Bont et al., 2000b). IL-12 and IFN-γ (and IL-4) responses were suppressed during acute RSV disease but returned to control levels during convalescence and IFN-γ (and IL-4) levels were not different in subsequently wheezing infants (Bont et al., 2000a). Reduced IL-12 levels may lead to elevated susceptibility to Th2 responses to RSV infection and predisposition to asthma.

IL-10 is a regulatory cytokine, which may promote an asthma phenotype by suppressing Th1 cytokine production and antigen presentation promoting enhanced susceptibility to infection and Th2-dominated responses that induce wheezing and pro-asthmatic responses to subsequent antigen challenge (Bont et al., 2000a, 2000b). IL-10 levels did not change during acute RSV disease but increased during convalescence and were significantly higher in subsequent wheezers and those that went on to develop recurrent wheeze and asthma. Thus, the enhancement of IL-10 and inhibition of IL-12 production upon RSV infection may suppress immune function and permit more severe infection and disease progression.

It has also been suggested that RSV infection may change and enhance the profile of pro-inflammatory cytokine and chemokine release that promotes more severe infection and alters the nature of the T cell response promoting allergy and inflammation. Patients with RSV-induced bronchiolitis had elevated levels of MIP-1α (but not RANTES), which correlated with disease severity in nasopharyngeal secretions and RANTES, ICAM-1, IL-4 and -5 and IgE in serum (Sung et al., 2001; Garofalo et al., 2001, 2005). Furthermore treatment of human epithelial cells with TGF-β, which plays a pivotal role in airway remodelling and asthma, increased RSV replication and TNF-α secretion and p38 mitogen-activated protein kinase activation. This may contribute to the elevated inflammatory responses in virus-associated asthma (McCann & Imani, 2007). By contrast RSV-induced wheezing does not correlate with IL-8

levels in nasal lavage fluid but is elevated with influenza or RV infection (Gern et al., 2002).

Thus it is possible that infection induces regulatory mechanisms that suppress immune responses allowing viral replication resulting in increased inflammatory responses. Alternatively infection may induce inflammatory responses that enhance infection and allergic inflammation. This may subsequently promote the induction of regulatory mechanisms to limit inflammation-induced damage of host tissue.

2.2.4.3. Other immune factors. In vivo and in vitro studies have shown that RSV infection of the respiratory epithelium causes the release of other factors that have immune functions including arachidonic acid metabolites and LTs, mediators released by eosinophils and chemokines (reviewed in Ogra (2004)). Cell adhesion and homing molecules such as CD11b, ICAM-1 and E-selectin and antigen-presenting molecules including human leucocyte antigen classes I and II are also upregulated upon infection. Several transcription factors are also activated, which may induce the expression of a range of genes such as NK-IL-6 and nuclear factor (NF)-kB. These factors regulate immunomodulatory mediators (TNF-α, IL-1β, IL-2, -6, -11 and GM-CSF), adhesion molecules (ICAM-1, VCAM-1 and E-selectin) and chemokines (IL-8, MIP-1α, MCP-1, eotaxin and RANTES) (Garofalo et al., 1996; Oh et al., 2002; John et al., 2003; Makela et al., 2003; Schaller et al., 2006). In combination these mediators and molecules induce the influx of inflammatory cells and may contribute to the development of infection-induced inflammatory and immune responses, AHR and asthma.

2.2.5. Antibody responses

Ineffective or aberrant humoral responses may also have a role in RSV-induced asthma. Infection induces increases in B cell numbers (Roman et al., 1997) and the production of serum and mucosal IgM, IgA and IgG antibodies. These are important in protection and not disease but occur at lower levels in infants. Antibody responses to primary infection are ineffective and involve the production of partially neutralising antibodies against the G and F proteins (Psarras et al., 2004) but these responses are reinforced (especially IgG and IgA) upon reinfection. RSV-specific IgE antibodies are also produced (Welliver et al., 1980) in the majority of children and increased amounts and persistence promote the development of wheezing (Ogra, 2004).

2.2.6. Pathological responses and angiogenesis

Increased vascularity (angiogenesis) surrounding the airway wall is associated with chronic and fatal asthma but also with mild-to-moderate asthma in both children and adults (Li & Wilson, 1997; Vrugt et al., 2000; Barbato et al., 2006), suggesting a pathogenetic role at all stages of asthma development. Angiogenesis is regulated by a balance between pro-angiogenic (vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenins, chemokines) and anti-angiogenic (endostatin, canstatin, tumstatin, arresten) factors (Cohen, 2002). Vasodilation of the increased number of blood vessels in response to inflammatory stimuli may lead to edema and inflammation of the bronchial wall (influx of inflammatory cells, including eosinophils and release of mediators), airway narrowing and AHR (Black & Page, 1994; Wilson, 2000; Salvato, 2001; Nomura et al., 2005). Elevated levels of VEGF, bFGF and angiogenins occur in the airways of asthmatics and correlate with increased vascularity, vessel permeability and AHR (Hoshino et al., 2001; Kanazawa et al., 2004; Nomura et al., 2005; Feltis et al., 2006). VEGF influences vascular permeability through the formation of blood vessel fenestrations and vasiculo–vasculo organelles (Dvorak et al., 1996; Esser et al., 1998; Neufeld et al., 1999). VEGF also stimulates endothelial cell proliferation and migration, matrix remodelling, and vasodilation, as well as inhibiting endothelial cell apoptosis and all of these processes are involved in angiogenesis (Neufeld et al., 1999). VEGF also enhances sensitization of the RT to allergens and promotes Th2 inflammation (Lee et al., 2004).

RSV infection may contribute to the development of asthma by inducing the production of VEGF and angiogenesis. VEGF has been detected in nasal washings from RSV infected patients, indicating that infection stimulates VEGF production, which may have a role in disease (Lee et al., 2000). Furthermore, features of RSV bronchiolitis and pneumonia such as submucosal, adventitial, and interstitial edema are due to alterations in blood vessel permeability brought about by VEGF activity (Dvorak et al., 1996; Esser et al., 1998; Neufeld et al., 1999).

The specific role of VEGF in RSV infection has not been intensively investigated. It is released from airway epithelial cells, Th2 cells and mucosal fibroblasts upon infection (Lee et al., 2000) but is also produced by monocytes, macrophages, T cells, keratinocytes, granulocytes, eosinophils and smooth muscle cells (Gaudry et al., 1997; Horiuchi & Weller, 1997; Neufeld et al., 1999).

Thus a novel mechanism may be involved in RSV-induced asthma whereby the induction of VEGF upon infection may lead to the development of angiogenesis that can enhance the inflammatory response. Furthermore, RSV-induced secretion of VEGF may contribute to exacerbations by increasing vascular permeability, and recurring infections may contribute to cycles of VEGF production that promote angiogenesis and remodelling.

2.2.7. Involvement of neural networks

Neurological and immunological interactions that occur as a result of RSV infection have also been linked to the generation of airway inflammation, AHR and RAD in children. Excitatory non-adrenergic non-cholinergic nerves (NANCe) release neurotransmitters including substance P that participate in the early phase of the inflammatory response and also have an immunomodulatory role (Piedimonte, 2002). Sloughing of the epithelium during infection may lead to exposure of neurogenic receptors (NK1), which enhances the pro-inflammatory effects of substance P leading to ASM spasm and bronchoconstriction and contributing to respiratory symptoms.

Nerve growth factor participates in neuronal development and has beneficial effects on inflammation, repair and remodelling. However, it has been suggested that these effects may

become pathogenetic during RSV infection and allergic inflammation and exacerbate inflammation and AHR (Nassenstein et al., 2006).

2.2.8. Co-infections

The effect of co-infection of with RSV and other viruses has been little studied, however, notably the risk of developing bronchiolitis is 5 times greater in infants with co-infection with RV (Papadopoulos et al., 2002a).

2.3. Mechanisms of predisposition shown experimentally

Animal models of infection and allergic airways disease (AAD) have been developed and used extensively to substantially contribute to the understanding of the mechanisms that underpin RSV-induced asthma and exacerbations. In particular rodent (mouse and to a lesser extent rat) and bovine models have been used to elucidate the mechanisms of the associations and to trial therapeutic agents and vaccines. Chimpanzees are permissive to human RSV and are the best animal model but their availability and cost limits all but the most advanced clinical tests (Whitehead et al., 1998).

Using these models important factors have been identified that may play key roles in RSV-induced asthma and include; age of first infection, timing of infection relative to allergen exposure, induction of asthma, endotoxin exposure, innate factors and adaptive immunity, suppression of immunity, angiogenesis, neural networks and latent infections. The importance of the different viral proteins in infection has also been investigated.

2.3.1. Rodent models of primary RSV infection

The use of animal models enables experimental protocols to be conducted that are not possible in humans. In particular the precise investigation of the different ages of infection, combinations of the timing of infection relative to allergic sensitization and collection of invasive tissue samples can only be achieved in animals. Although there are problems with these models of RSV challenge including that RSV does not replicate in mice and does not induce the recruitment of granulocytes that is observed in human disease, such models have been used extensively to gain valuable insights into the mechanisms of RSV-induced disease.

In most mouse models RSV infection induces significant acute respiratory inflammation and changes in lung function. High doses (10^{7-8} plaque forming units (pfu)) induce severe alveolitis and pneumonia and low doses (10^{5-6} pfu) result in bronchiolitis without these effects (Dakhama et al., 2005a).

Five days after infection mice develop acute airway obstruction, which correlates with the progression of inflammation and histopathology that is characterised by intense perivascular and peri-bronchial/bronchiolar influx of monocytes/macrophages and some neutrophils and lymphocytes. These cells also occur in the alveoli during the peak of inflammation but leakage into the airway lumen is absent (Jafri et al., 2004). Infection peaks at days 4–5 and resolves between days 7 and 9 and cytokines and chemokines (IL-8, MIPs and RANTES) are released by the respiratory epithelium and alveolar macrophages in response to infection. After 3 days NK cells influx into the BALF and are replaced between 4 and 8 days by CD4+ and CD8+ cells which return to pre-infection levels at 21 days (Hussell & Openshaw, 1998). CTLs induced in response to infection contribute to extensive peri-bronchiolar and -alveolar inflammation and are associated with disease symptoms (Ostler et al., 2002). IFN-γ is important in viral clearance and contributes to pathology and is primarily released by NK cells with levels peaking at day 4. Only low levels of IL-4 and Il-5 are present, however, IL-4, -5, -10, -12, -13 and IFN-γ are produced in the lungs within the first few days and their relative levels determines the course of disease (Kalina & Gershwin, 2004). IL-10 plays a pivotal role in the development of infection-induced AHR in the absence of allergen exposure (Makela et al., 2002).

Acute infection develops into chronic disease with features of chronic inflammation (histopathology score) and AHR (in terms of enhanced pause (Penh)) after the clearance of virus and up to 22 weeks after infection (Mejias et al., 2005). Penh is a non-invasive method of whole body plethysmography and uses a single exposure to a spasmogen to determine the overall level of AHR in all airways. Jafri et al., used Penh to show that airway obstruction and AHR remained for 42 and 154 days after infection, respectively, and that AHR correlated with chronic inflammation which persisted but not in alveoli (Jafri et al., 2004). This supports the concept that RSV disease may induce long-term respiratory changes in children (Stein et al., 1999; Sigurs et al., 2005). However, these studies need to be confirmed by the measurement of lower airway resistance using invasive methods that precisely measure changes in airway and tissue specific function and employ dose responses to spasmogens. Studies using these methods (measurement of respiratory impedance and airway resistance) have shown a lack of long-term effects of RSV infection on lung function (Dakhama et al., 2005c; Collins et al., 2007).

Infection also induces mucus hypersecretion in the central and peripheral airways in the acute and chronic phases and severe and progressive pneumonia develops with increases in histopathology and chronic inflammatory changes. These processes contribute to airway obstruction (Penh) that develops in the acute phase and progresses but does not correlate with viral load in BALF, and this agrees with observations made in children (Jafri et al., 2004). The intensity of inflammation declines over time but remains around airways and vessels. Neutralising monoclonal antibodies (mAbs) against RSV substantially decrease inflammation and disease severity (Mejias et al., 2004).

2.3.2. Age of first infection

Using mouse models it has been shown that the age of first infection plays a key role in shaping dominant immune responses later in life (reviewed in Hansbro et al. (2004)) and establishes the subsequent pattern of Th cell responses and the nature and severity of ensuing respiratory diseases (Culley et al., 2002; Holt & Sly, 2002a; Horvat et al., 2007).

Primary RSV infection in neonatal mice has the same profile as in adults, however, it is associated with a slower and diminished IFN-γ response and the development and persistence of Th2

cytokine release by CD4+ T cells. These effects are substantial and can reverse the protective effect of mycobacterial exposure on AAD (Li et al., 2006). Neonatal RSV infection results in early TNF-α release and has long-term adverse effects on the respiratory system. These effects include the induction of AHR, peri-vascular and -bronchial inflammation and subepithelial fibrosis, which are exacerbated by subsequent allergen exposure and involve persistent IL-13 expression and mucus hypersecretion (You et al., 2006). The generation of Th2 responses during immunological development has profound modulatory effects on the balance of subsequent Th1/Th2 responses and promote a marked increase in the Th2 phenotype in adulthood (Chen et al., 2001; Walzl et al., 2001; Culley et al., 2002). The neonatal Th2 bias may result from the types of T cells or dendritic cells present, their environment or a combination of these effects (Nelson et al., 1994; Goriely et al., 2001; White et al., 2002; Bartz et al., 2003).

Subsequent re-infection later in life may reinforce aberrant Th2 responses (cytokines (IL-13) and CD4+/CD8+ cells) and alter responses to allergens resulting in enhanced inflammation, mucus hypersecretion, AHR and allergy (enhanced weight loss and Th2 cell and eosinophil recruitment to the airways) (Chen et al., 2001; Walzl et al., 2001; Culley et al., 2002; Dakhama et al., 2005b). This suggests that AAD of adults primed with an infection as neonates is likely to be caused by factors that promote Th2 responses (Culley et al., 2002; Holt & Sly, 2002a). If initial infection is delayed until mice are 3 weeks of age IFN-γ production increases and upon reinfection, although airway inflammation still occurs, there is a subsequent reduction in the severity of disease with no mucus production or AHR (Culley et al., 2002; Dakhama et al., 2005b). Thus delaying the age of infection until later in life may be an effective strategy for the prevention of RSV-associated asthma.

2.3.3. Asthma induction by RSV infection

Animal models have been used to determine if RSV can induce the development of asthma by triggering pro-asthmatic immune responses that lead to variable airflow obstruction and airway inflammation. Chavez-Bueno et al. (2005) demonstrated that RSV induces acute and chronic disease with features of AAD independently of allergic sensitization or genetic background in mice. RSV infection of BALB/c or C57BL/6 mice in the absence of allergic sensitization led to similar levels of acute airway inflammation, airflow obstruction and AHR and the degree of airway inflammation correlated with AHR. The immune response was surprisingly similar between the two strains and was characterised by virus-dependent release of IFN-γ. Importantly infection and inflammatory responses were not short lived. Acute infection developed into persistent infection that correlated with airway inflammation, which were present 77 days after infection. While virus load and airway inflammation were greatest during the acute phase, chronic infection correlated with chronic inflammation and airflow obstruction. Thus RSV infection can initiate acute events that result in airflow obstruction and possibly AHR and these changes can persist beyond the initial inflammatory response.

Other investigators have also shown that RSV infections in early life act synergistically with atopy to drive the development of allergic asthma (Holt & Sly, 2002b; Kusel et al., 2007). However, the immunological processes involved in the induction of allergic sensitization by RSV infection are not likely to be the same as those involved in exacerbation of allergic asthma by RSV.

2.3.4. Timing of infection

The effect of RSV infection on AADs may be critically dependent on the relative timing of infection, allergic sensitization and challenge (Peebles et al., 2001; Barends et al., 2002, 2004). The majority of studies in mice show that RSV augments AAD, however, conversely some investigators suggest that RSV infection prevents atopy. Indeed RSV induces a strong Th1 and IFN-γ mediated response that may modulate responses to allergens (Peebles et al., 2001; Juntti et al., 2003). These contrasting observations can be explained as two competing immune responses are occurring simultaneously, which may subtly differ in different protocols. The development of allergy may depend on the phenotype of the immune response to allergens and RSV at the time of exposure.

2.3.4.1. Allergen exposure prior to infection. Prior exposure of the airways to allergen independently of allergen type predisposes to increased severity of virus-induced AAD in mice and is likely to play a role in humans (Peebles et al., 2001; Makela et al., 2003; Kalina & Gershwin, 2004). Infection enhances Th2 cytokine responses, mucus secreting cell hypertrophy, eosinophil influx into the lung and AHR in response to allergen and increases the severity of AAD. The potency of the Th2 responses elicited overrides the counter-regulatory effects of Th1 responses that are typically induced by infection (Randolph et al., 1999).

2.3.4.2. Infection prior to allergen exposure. Exposure of RSV infected mice or rats to allergens increases inflammation, mucus production and AHR and prolongs RSV replication (Peebles et al., 1999; Kalina & Gershwin, 2004; Hassantoufighi et al., 2007). In particular CD4+ and CD8+, IL-4, -5 and -13, RANTES and MIP-1α inflammatory responses in the lung are enhanced (Lukacs et al., 2001; Barends et al., 2004; Schaller et al., 2006). Blocking IL-13 during infection reduces chemokine expression and AHR and blocking RANTES removes the effects of infection upon later allergen sensitization and challenge (Lukacs et al., 2001). The absence of the CC chemokine receptor 1 (CCR1) in deficient mice leads to reductions in T cells, IL-13, eosinophils, mucus and AHR (John et al., 2005). These observations suggest that chemokines and their receptors play roles in RSV-induced aberrant responses to allergens and may be important in mobilisation of virus- and allergen-specific T cells and allergic inflammation.

It is possible that RSV infection may contribute to the development of allergy by damaging the respiratory mucosa, which exposes APCs and T cells to allergen, which may break tolerance and induce systemic Th2 sensitization. Excessive infection-induced respiratory damage may occur in predisposed individuals as a result of defects in T cell mobilisation, activation or activity.

2.3.4.3. Concomitant allergen exposure and RSV infection. If allergen challenge of sensitized mice occurs concomitant with infection the inflammatory response is again enhanced and leads to Th2 responses (IL-5, -10 and -13) to RSV and promotes chronic infection (Makela et al., 2002). IL-5 production leads to eosinophilia, IL-13 to the release of MCP and further Th2-mediated inflammation but IL-10 does not further enhance AHR. If rats are sensitized to extracts of the common household mould *Aspergillus fumigatus*, which induces eosinophilia and Th2 cytokine release, RSV infection before allergen challenge exacerbates the inflammatory response and AHR that is dependent on viral replication. Infection causes increased expression of MHC II on alveolar macrophages, which may be involved in initiating immune responses to allergens. Persistence of infection is induced and is related to reduced IFN-γ expression again suggesting that allergic sensitization can affect the progression of RSV infection (Kalina & Gershwin, 2004). Treatment of infected *A. fumigatus* challenged animals with recombinant IFN-γ reduced allergic responses and Th1 and Th2 cytokines but not AHR. This suggests that pathological and physiological factors of disease are independent (Hassantoufighi et al., 2007).

Taken together these studies suggest that in general RSV induces increased severity of Th2-mediated AAD and that the development of AAD increases the severity and persistence of RSV infection.

2.3.5. Exposure to endotoxin/pollution

Some reports have suggested that exposure to endotoxin or environmental pollution can affect the progression of RSV infection (Gurkan et al., 2000; Monick et al., 2003). Exposure to such factors alters the relative proportions of cytokines produced upon infection and affects disease progression, however, the cellular and molecular processes involved are not understood.

TLR-4 expression may provide a link between RSV, endotoxin and asthma. TLR-4 expression is not usual in resting airway epithelium and requires high endotoxin exposure to be upregulated. RSV infection induces increased expression of TLR-4 and responsiveness to endotoxin and initiates potent inflammatory responses (Monick et al., 2003). This may be linked to human asthma as endotoxin also induces asthma exacerbations in children with RSV-induced asthma (Park et al., 2001).

2.3.6. Immune responses to RSV infection

Animal models have been used extensively to elucidate the host immune responses that are induced by RSV RT infection and how these responses may contribute to the development and exacerbation of asthma. Infection may inhibit or modulate the activity of both innate and adaptive (particularly T cell) immune responses during the development of disease, which may play a crucial role in the induction of pathology and AAD.

Primary RSV infection induces innate responses that involve eosinophil and neutrophil influx into the lung that results in AHR and a cytokine response that is dominated by IFN-γ (Schwarze et al., 1999b). Eosinophil infiltration is IL-5 but not IL-4 or IFN-γ dependent and is critical for the development of AHR. The innate response (first 3 days) is also characterised by an influx of NK cells producing IFN-γ, which are replaced by adaptive CD4+ and CD8+ cells and the release of IL-12 with low levels of IL-4, -5 and -13 (Openshaw, 1995; Boelen et al., 2000; van Schaik et al., 2000; Openshaw, 2001). T cell responses facilitate viral clearance but also induce host tissue damage and pathology.

2.3.7. Innate responses

Infection induces innate responses involving TLRs, cytokines, chemokines and DCs that ultimately direct the development of adaptive T cell and antibody responses (Durbin & Durbin, 2004; Krishnan et al., 2004).

2.3.7.1. TLRs. RSV may use a variety of host cell factors to bind to and enter cells including TLR-4, CX3R1, heparin and caveolin (Kalina & Gershwin, 2004). Binding to TLR-4 initiates the production of IL-6, -8 and -1β and TNF-α and may be responsible for the initial response to infection. However, the role of TLRs in virus-induced asthma is controversial. Some studies report that the innate immune response to RSV infection in mice is dependent on the expression of CD14 and TLR-4 (Kurt-Jones et al., 2000; Haeberle et al., 2002), which may interact with the viral F protein (Openshaw et al., 2003). Another report argues the opposite saying there is no significant role for TLR-4 in infection (Ehl et al., 2004).

TLR-3 recognises double stranded (ds) RNA and is constitutively expressed on respiratory epithelial cells and DCs. TLR-3 signals independently of myeloid differentiation factor 88 to induce NF-κB activation and the expression of IFN-β Activation of TLR-3 leads to apoptosis and elimination of infected cells and virus. Recently it has been shown that TLR-3 and protein kinase R (PKR) are upregulated in human airway epithelial cells by RSV infection, which enhances epithelial responsiveness by activation of NF-κB and IL-8 and may sensitize these cells to subsequent viral or bacterial infection (Groskreutz et al., 2006).

2.3.7.2. Chemokines. Chemokines are produced by stromal, epithelial and immune cells and regulate immune responses (chemoattraction of leukocytes into the lung), inflammation, mucus production and angiogenesis. Although cellular inflammation is often similar in response to different viral infections the types of chemokines and the levels that are released that drive subsequent immune responses differ substantially (Schaller et al., 2006). Primary RSV infections induce the expression of chemokines belonging to the CXC (IL-8, MIP-2 and IP-10), CC (RANTES, eotaxin, MIP-1α, MCP-1 and T cell activation gene-3) and C (lymphotactin) families in the lung. MIP-1α expression is high and MIP-1α deficient mice have reduced lung inflammation, but RSV titres that are the same as in wild-type mice. Thus RSV-associated lung inflammation may be mediated by early production of inflammatory chemokines (Haeberle et al., 2001).

2.3.7.3. DCs. DCs are the most important antigen-presenting cell (APC) and take up viral antigens, traffic to local lymph nodes and present antigen to naïve T cells causing their differentiation

into effector cells. DCs direct innate and adaptive immune responses to viruses and allergens and are essential for allergic sensitization. Different subsets of DCs exist with different functions. mDCs present viral antigens and promote Th2 cell expansion to a great extent than pDCs, which are more important in the development of tolerance (van Rijt et al., 2005). DC networks are less active in infant animals but can be enhanced and mDCs are increased upon viral infection (Holt & Sly, 2002b; Zuniga et al., 2004). It is possible that the reduction of pDCs by conversion into mDCs during early life viral infection may inhibit the development of tolerance and exacerbate allergic responses.

2.3.8. Adaptive immunity

The outcome of RSV infection may depend on the nature of the adaptive response and the balance of Th1 and Th2 immunity. Primary infection of BALB/c mice induces a mixed Th1/Th2 response with an early burst of IFN-γ release that is important in determining the phenotype of the subsequent response (Boelen et al., 2002; Openshaw & Tregoning, 2005). Other adaptive responses involving CD8+ cells and B cell/antibody responses also play significant roles in responses to RSV.

2.3.8.1. Th1 CD4+ T cell responses. RSV infection typically induces a robust Th1 response with elevated levels of IFN-γ and IL-12 in mice. IFN-γ is the archetypal Th1 cytokine and its release and signalling through the IFN-γ receptor during infection are pivotal in controlling the Th1/Th2 response to infection. These processes are essential in moderating eosinophil migration and IFN-γ and CD8+ cells are crucial for viral clearance. In the absence of IFN-γ a dominant Th2 response induces eosinophil influx of the lung and AHR (Barends et al., 2003). By contrast, the absence of IL-12 and IL-18 has little effect (Boelen et al., 2002). IL-12 does, however, induce Th1 and suppresses Th2 responses, promotes IFN-γ production from NK and CD8+ cells, reduces IL-4 and IL-5 production from CD4+ and CD8+ cells and can prevent but is not essential for inhibiting virus-induced eosinophil influx to the lung (Hussell & Openshaw, 2000). IL-12 does not function through CD4+ or B cells and exacerbates disease in mice sensitized to allergen (Openshaw et al., 2003). The removal of CD4+ or the induction of CD8+ cells also eliminates eosinophil influx (Hussell et al., 1997).

TNF-α is another Th1 cytokine and is over-produced during viral RT infections, which exacerbates inflammation by promoting neutrophil and eosinophil influx. Anti-TNF-α treatment of mice leads to ablation of weight loss and illness without affecting viral clearance and does not induce adverse side-effects, which indicates a potential for use in therapy (Hussell et al., 2001).

2.3.8.2. Th2 responses. Th2 responses are induced by RSV infection in a variety of animal models or in the absence of IFN-γ (Boelen et al., 2002), which may contribute to the development of AAD. These responses are potent and are similar to Th2 responses observed after allergen exposure of allergic individuals (Braciale, 2005). RSV-induced IFN-γ and IL-12 responses do not diminish the Th2 response during the development of RSV-induced allergy although the response is even greater in the absence of IFN-γ (Barends et al., 2003). It is a specific set of T cells, a CD4+Vβ14+ subpopulation, that induces a superantigen type response to RSV and is pivotal in inducing Th2 mediated pathology (Varga et al., 2001). T1/ST2 is a surface receptor of the IL-1 family expressed on Th2 but not Th1 cells. T1/ST2 was present on a subset of CD4+ T cells from mice with RSV-induced eosinophilia and T1/ST2 mAb treatment reduced Th2 but not Th1 pathology (Walzl et al., 2001).

These studies suggest that under some circumstances infection may induce strong Th2 responses but the mechanisms of how this leads to AAD are unknown. IL-4, -5, -10, -11 and -13 are Th2 cytokines that are released during Th2 responses and are likely to be involved in RSV-induced AAD.

In mice, primary RSV infection results in increased IL-4 levels, however, it's importance in the development of Th2 responses and AAD is unclear. IL-4 deficient mice or mice treated with a neutralising anti-IL-4 and immunized with vaccinia virus expressing protein G had no reduction in pulmonary eosinophils or Th2 cytokine secretion. Furthermore infection of IL-4 deficient mice resulted in enhanced numbers of eosinophils in the lung and AHR (Johnson & Graham, 1999; Johnson et al., 2003).

By contrast IL-5 release may be pivotal in RSV-induced Th2 responses and AAD. Infection of mice results in IL-5-mediated AHR and eosinophil influx into the lung in association with strong IFN-γ responses. IL-5 but not IL-4 or IFN-γ is the critical mediator of eosinophil influx, AHR and allergy (Schwarze et al., 1999a). IL-5 deficiency leads to a reduction in pulmonary eosinophils and AHR, which can be reversed by replenishment with IL-5. Treatment with anti-very late antigen-4 prevents the influx of eosinophils into the lubg and AHR in response to RSV infection or IL-5 replenishment.

RSV infection induces the expression of IL-10 in mouse pulmonary T cells (Hussell et al., 1996). The absence of IL-10 inhibits the development of AHR in response to allergen sensitization and challenge. RSV infection overcomes this deficiency and induces eosinophil infiltration of the lung, airway mucus production and AHR, which are associated with increased Th2 responses (Makela et al., 2002).

IL-11 is associated with the development of RSV-induced AHR and may promote the release of other Th2 cytokines (Einarsson et al., 1996).

Although IL-13 is required for the development of mucus hypersecretion and AHR in mouse models of AAD and after secondary RSV infection of infected neonates (Kuperman et al., 2002; Dakhama et al., 2005b) it does not appear to play an important role in the induction of these responses after primary infection (Park et al., 2003). This has not yet been investigated in humans.

It is likely that as well as inducing a Th2 phenotype RSV may take advantage of Th2 responses that predominate in asthmatics that are ineffectual against infection. However, surprisingly RSV clearance can occur under Th2 conditions whereby eosinophils take up RSV and inactivate the virus with ECP (Soukup & Becker, 2003). IL-10 increases FasL expression on macrophages and CD8+ cells and therefore also has an anti-viral effect (Ruan et al., 2001).

2.3.9. CD8+ cells

CD8+ T cells target several RSV proteins and are sufficient to clear RSV from infected mice (Cannon et al., 1988; Cherrie et al., 1992), however, clearance and immunopathology still occurs in CD8-deficient mice (Graham et al., 1991). Adoptive transfer of virus-specific CD8+ CTLs which home to the lung eliminates RSV. These cells induce viral clearance through perforin/granzyme-mediated lysis of virus-infected cells (Aung et al., 2001). However, perforin (which is also produced by NK cells), CD95L and TNF are not necessary but IFN-γ release is crucial for CD8+ T cell-mediated clearance. RSV was eliminated with unchanged kinetics from perforin deficient mice (Aung et al., 2001; Ostler et al., 2002), whereas treatment of mice with neutralising antibody to IFN-γ or transfusion of IFN-γ-deficient effector CTLs abolished virus control and induced CD8+ T cell-mediated pathology (Ostler et al., 2002). By contrast, high dose primary infection in IFN-γ deficient mice led to attenuated immunopathology, but only slightly delayed clearance. This suggests that other cells and molecules can partially substitute for CTL-derived IFN-γ driven virus clearance and further implicates IFN-γ as a pivotal immune factor in RSV-induced immunopathology and CD8+ T cell-mediated control (Ostler et al., 2002).

CD8+ cells may also suppress the development of virus-specific Th2-dominated immune responses and eosinophilia although the mechanism of these effects remains unknown. Hussell et al., showed that early IFN-γ release by CD8+ cells in response to RSV infection of mice resulted in suppression of Th2 responses (Hussell et al., 1997) and the suppression of Th2 responses by CD8+ cells is by other studies (Srikiatkhachorn & Braciale, 1997a). Interestingly the Th2-inducing effects of the RSV G protein can be nullified by incorporating a CD8+ epitope onto the G protein (Srikiatkhachorn & Braciale, 1997a).

2.3.10. CD4+/CD8+ interactions

CD4+ and CD8+ T cells are exposed to presented viral antigens in the lymph nodes of the respiratory tract, which induces differentiation, activation and mobilisation of both effector and memory T cells. Memory CD4+ T cells move to the RSV infected RT and proliferate and differentiate into cytokine releasing effector cells and induce their effects in situ (Varga et al., 2000, 2001). During differentiation the cells are subject to infection-induced modulation and it is likely that the CD8+/CD4+ interaction is occurring concurrently. Similar interactions may take place during allergen provocation in the RT of asthmatics that have the potential to substantially affect T cell phenotype, an effect that may be enhanced upon infection. Thus the complex interactions of CD4+ and CD8+ with infectious stimuli and allergens may be pivotally important in the development of RSV-induced AAD. These processes may be targeted therapeutically to suppress allergen specific Th2 responses in the lung.

2.3.11. Adaptive immune responses to RSV proteins

Differential immune responses are induced by different RSV proteins, which may be important in the development or exacerbation of asthma. Protein G vaccination of mice induces CD4+ but not CD8+ memory populations and leads to reduced NK cell influx and IFN-γ production. This results in the induction of Th2 responses, in the absence of a CTL response, with IL-4 and IL-5 release by Th2 cells which promotes eosinophilia upon subsequent infection (Alwan et al., 1994; Srikiatkhachorn & Braciale, 1997a; Walzl et al., 2001; Openshaw et al., 2003). G protein defective RSV has been used to demonstrate that this protein is crucial in promoting airway inflammation and reduced lung function (Schwarze & Schauer, 2004).

F or M2 protein immunization induces a mixture of NK, Th1 type CD4+ and MHC I CD8+ cells, leading to IFN-γ production and reduced disease (Alwan et al., 1994; Srikiatkhachorn & Braciale, 1997a; Openshaw et al., 2003). NK cells and the IFN-γ they release regulate the induction and proliferation of CD8+ cells, which then clear the virus. Primary RSV infection induces the expansion of activated $M2_{82–90}$ (H-$2k^d$ restricted peptide epitope in RSV M2 protein)-specific CD8+ T cells in lung. This implies that activation and proliferation of M2-specific CD8+ T cell precursors is normal.

Taken together these results suggest that natural infection that induces Th2 responses may be dominated by responses to the G protein exacerbates infectious and allergic disease, whereas responses to the F and M2 proteins may be Th1 mediated and reduce disease.

2.3.12. Antibodies

Antibody responses may have several roles in RSV-associated AAD in mice. RSV infection may induce the development of pro-allergic IgE antibodies and their receptors in the lung which may induce mast cell degranulation and AHR (Dakhama et al., 2004). The activation of anti-viral protein kinase upon infection may lead to isotype switching of B cells to produce IgE (Rager et al., 1998). Other studies have shown that exposure to allergen during acute RSV infection of mice results in the production of antigen specific IgG1 responses, which are characteristic of Th2 immunity (O'Donnell & Openshaw, 1998). Moreover non-neutralising antibody produced in response to formalin-inactivated (FI) virus may induce immune complex formation in the lung (Openshaw et al., 2003), which may be involved in pathogenesis.

Infants have poor T cell independent antibody responses and produce different types of antibodies compared to adults. Early life infection with RSV may fix the nature of antibody production as well as T cell responses to subsequent infection throughout life and promote the development of AHR (Openshaw et al., 2003).

2.3.13. Suppression of immunity

Studies in mice have suggested that RSV may promote its own infection by suppressing host immunity resulting in enhanced inflammation. The RSV G protein may attenuate innate responses by binding to TNF-α (Valarcher & Taylor, 2007) and the induction of cytokine production from monocytes and macrophages through the inhibition of TLR-4-NF-κB mediated signalling (Polack et al., 2005). RSV-specific T cells may play a crucial role in viral clearance and limited evidence suggests that infection may suppress the activation and activity of T cells leading to enhanced viral replication and disease (Harcourt et al., 2006). During suppression the F protein of RSV may down-regulate the activity of T cells and cytokine production including

the release of IFN-γ (Kondo et al., 2004; Schauer et al., 2004). RT infection also suppresses CTL immunity through the rapid loss of virus-specific CD8+ memory cells and IFN-γ release (Chang & Braciale, 2002), which is mirrored in humans by the lack of the induction of immunological memory. This effect may occur during antigen receptor signalling and varies to different extents in different T cell functions (CTL activity, cytokine release). This may result in reduced CTL activity and allow viral persistence but may also enable the maintenance of cytokine responsiveness facilitating further virus- or allergen-associated inflammation promoting enhanced disease. Despite this evidence immune suppression during infection has not yet been observed in humans. These effects may only be present in the lung and are not detectable in the blood (Braciale, 2005) and it is unknown if CD8+ T cells with this phenotype exist in the RT of humans with severe RSV infection.

RSV infection may also attenuate protective immune responses by suppression of type I IFN (IFN-α,β), modulation of DC activity, G protein mimicry of the CX3C chemokine, which may inhibit T cell migration and by producing viral variants that are not recognised by neutralising antibodies (Tripp, 2004; Meyer et al., 2007). The viral elements responsible for dysregulated immune responses are not known.

2.3.14. Latent infection

Persistent RSV, other viral or bacterial infections, which are local or systemic, or underlying chronic lung disease may promote susceptibility to RSV-induced asthma. Emerging evidence from both mouse and human studies suggests that RSV infections may persist at low levels, by mechanisms involving suppression of immunity and avoidance of immune detection and total clearance (Seemungal et al., 2001; Schwarze et al., 2004). This occurs in mice with functional neutralising antibody and CTL responses even though the virus possesses a CD8+ epitope and productive infection can be reactivated by depletion of CD4+ and CD8+ cells (Schwarze et al., 2004).

In mouse models RSV is detectable by culture up to just 7 days after infection but by PCR for up to 77 days independently of genetic background and viral copy number correlates with AHR (Tripp, 2004; Chavez-Bueno et al., 2005). This association has not yet been proven but treatment with neutralising antibody reduces viral numbers and disease severity (Mejias et al., 2005). However, it is possible that viral detection by PCR under these conditions may represent the persistence of viral debris following the administration of high viral inocula and may not be part of the disease process. It remains unknown whether viral persistence occurs in children following bronchiolitis and the development of wheezing and long-term pulmonary abnormalities. If persistent infection does occur chronic inflammation and altered immune (cytokine/chemokine) responses may be induced in individuals with RSV-induced AAD. Thus persistent infection may be important in long-term morbidity and may provide a new therapeutic target.

2.3.15. Angiogenesis

Limited mouse and primate studies of acute and chronic asthma also link VEGF with pathophysiological features of RSV infection and asthma (Lee et al., 2002; Suzaki et al., 2005; Avdalovic et al., 2006; Lee et al., 2006). Over-expression of VEGF in the lungs of mice to levels found in asthma or during RSV infection, induces angiogenesis, oedema, inflammation, vascular remodelling, mucus cell hyperplasia/metaplasia and AHR (Lee et al., 2004).

2.3.16. Involvement of neural networks

Rat models of RSV-induced bronchiolitis have also been developed, where rats rapidly clear the virus in a similar manner to the self-limiting infection of human infants (Piedimonte et al., 1999). This model has been used to investigate the alteration of neural networks by RSV infection and infection of infant rats has enabled the analysis of the effects of early life infection (King et al., 2001). A stronger neurogenic inflammatory response develops in the LRT of infant rats than in adult rats during infection, which may explain why bronchiolitis presents in infants but as an URT infection in older subjects. Induction of inflammation involves the upregulation of NK1 in infected lungs (but not NK2 that is expressed on ASM fibres) that are activated by NANCe nerves and mediate substance P induced immunomodulation and neurogenic and cellular inflammation that may lead to edema and obstruction (King et al., 2001; Piedimonte, 2001; Tripp et al., 2002). NK1 receptors are also upregulated on T cells in bronchus-associated lymphoid tissue in response to infection, which may then be attracted into the airways and release pro-inflammatory cytokines in response to neurogenic stimulation by airborne irritants. Recurrent stimulation may lead to persistent cycles of airway inflammation and obstruction. Inhibition of NK1 or the administration of CGRP prevents RSV-induced AHR and may be potential therapeutic targets (Dakhama et al., 2005c). Neurogenic stimulation by RSV infection also induces the release of LTs from mast cells that induce mucus secretion (Wedde-Beer et al., 2002). Another alternative is that infection-induced damage may expose nerve endings and substance P and neurokinin A may then mediate ASM contraction (Jacoby, 2002). Other investigators have shown that RSV infection of rats and ferrets results in increases in cholinergic mediated contraction of ASM and reduced inhibitory NANCe responses (Larsen & Colasurdo, 1999).

2.3.17. Pneumonia virus of mice

Despite the vast array of literature describing studies of RSV infections in animal models, there is no single model which duplicates the pathological features of disease observed in human infection. The major drawback in studies of RSV-induced disease in mice is that RSV is not a natural mouse pathogen. Symptoms induced upon infection are minimal compared to those observed in human infants, the virus has limited replication and infected animals show few if any signs of respiratory illness (Domachowske et al., 2001). Furthermore, large doses of the virus are required and primary infection is rapidly aborted (Collins et al., 2001). Such discrepancies between human disease and mouse models have severely hampered the investigation of RSV-induced disease.

The pneumonia virus of mice (PVM) is the closest genetic relative of RSV and is a natural mouse pathogen (Easton et al.,

2004). Unlike RSV, PVM infection of mice reproduces many of the acute inflammatory responses described for RSV infection in humans. PVM replicates rapidly inducing inflammation leading to mucus plugging of the airways and overt signs of disease from URT symptoms to fatal pneumonia, which is dependent on the administered dose (Domachowske et al., 2004; Easton et al., 2004). Virus replication is associated with an influx of granulocytes and severe inflammatory bronchiolitis. We have recently established mouse models of RSV-like disease using PVM infection of neonates and adults (Hansbro et al., 2007). Importantly neonatally infected mice also exhibit many of the symptoms observed during RSV disease of human infants (Bonville et al., 1999; Harrison et al., 1999; Rosenberg et al., 2005).

These models are now being utilised to more precisely elucidate the host pathogen relationships that result in RSV-induced asthma. The importance of inflammation in inducing disease has been demonstrated and inflammatory responses remain active after the cessation of viral replication. MIP-1α and its receptor CCR1 play pivotal roles in these inflammatory responses. A recent study using PVM in wild-type and TLR-4 deficient mice showed that there is no difference in the clinical, functional, histological and virological parameters investigated indicating that PVM infection is independent of TLR-4 signalling (Faisca et al., 2006). Anti-viral therapy with ribavirin alone has little effect, however, treatment with ribavirin in combination with the anti-inflammatory agent and the CCR1 antagonist met-RANTES substantially reduces morbidity and mortality following infection (Rosenberg et al., 2005).

2.3.18. Bovine RSV and respiratory dysfunction

Bovine RSV, pathogenesis and vaccine development have recently been reviewed (Meyer et al., 2007; Valarcher & Taylor, 2007). Bovine RSV is closely related to human RSV and is the most common cause of LRT disease and the major single health problem in calves worldwide, particularly in winter (Stott et al., 1980; Valarcher & Taylor, 2007). Indeed 60–70% of epizootic respiratory diseases in the first year of life are attributable to bovine RSV with mortality typically between 2 and 3% but reaches 20% in some outbreaks (Meyer et al., 2007). Models of bovine LRT infection have been developed and used to investigate responses to infection and evaluate bovine vaccines. Bovine RSV is a natural pathogen and pathogenic infection of cattle shares many similarities with RSV infection in humans (Van der Poel et al., 1994). Infection is largely restricted to respiratory epithelial cells but causes little cytotoxicity and pathology is mediated by inflammatory responses to infection (Viuff et al., 2002; Valarcher & Taylor, 2007). These responses also involve innate (neutrophil and macrophage recruitment) and adaptive (pro-inflammatory cytokine and chemokine release) immune responses that result in respiratory damage. The mechanisms of pathology have been investigated using genetic manipulation of the virus and have implicated the G and SH proteins in infection and the F protein and non-structural (NS) proteins in inflammatory responses (reviewed in Valarcher & Taylor (2007)).

2.3.18.1. Pathogenesis. Bovine RSV is transmitted by direct contact, in airborne droplets or is possibly transferred passively by humans (Hall et al., 1980; Mars et al., 1999). Pathogenic infection of the RT is much more common in calves than adults (Stott et al., 1980), which results from a lack of specific immunity in naïve animals. Maternally-derived antibodies afford some protection but primary infection induces the most effective immunity (Kimman et al., 1987). Infectious disease takes 2–5 days to develop and has similar clinical features to humans and may possibly result in persistent infection (Valarcher et al., 2001). Disease of the URT induces mild symptoms of coughing and mucus production. In the LRT bronchiolitis and bronchopneumonia occur in association with edema, wheezing and dyspnea (Verhoeff et al., 1984; Belknap, 1993).

2.3.18.2. Immune responses. Infection of cattle induces inflammation involving the influx of mononuclear cells, neutrophils, CD4+ (of mixed Th1/Th2 phenotype) and CD8+ cells and sometimes eosinophils into peri-bronchial regions along with necrosis and apoptosis of epithelial cells (Viuff et al., 2002; Antonis et al., 2006). CD8+ CTLs have a major role in the clearance of primary infection in calves and lymphocyte proliferation is attenuated by infection in vitro (Keles et al., 1998; Antonis et al., 2006), whereas antibody-mediated responses are important in protection against secondary infection. The lumen of the airways become occluded with mucus and inflammatory debris and remodelling events occur that lead to breathing difficulties (Kimman et al., 1989; Viuff et al., 2002). It is likely that similar immune responses are elicited as in human infection with RSV and involve the induction of NF-kB and the generation of pro-inflammatory cytokines and chemokines. The interaction of viral components (F protein and dsRNA) with TLRs is known to drive the development of the immune response in cattle (reviewed in Valarcher & Taylor (2007)). As in humans and mice there are age-related differences in immune responses to infection in cattle with reduced protective and enhanced inflammatory responses in younger animals. In response to primary infection younger calves have enhanced fever, virus-specific TNF-α, IL-6 and IFN-γ release from PBMCs and reduced peripheral blood mononuclear and B cells and virus-specific IgA and neutralising antibody responses (Grell et al., 2005).

2.3.18.3. The roles of viral components in disease. The immunology and pathogenesis of bovine RSV infection has been intensively studied using reverse genetic engineering of the virus, which is less variable that human RSV and the results may be extrapolated to human disease (Collins et al., 1995). The importance and roles of different bovine RSV proteins in the induction of pathogenesis and immune responses have been elucidated using these genetically manipulated viruses.

The G protein of RSV is the major attachment protein and deletion mutants do not replicate in the absence of the G proteins in vivo, although replication is unaffected in vitro (Karger et al., 2001; Schmidt et al., 2002). Bovine viruses expressing only the secreted segment of the G protein also have 100 fold attenuated replication, which is 10 fold higher than mutants missing the entire G protein (Teng et al., 2001). Mutants expressing the membrane-anchored G protein segment have no impairment of replication in the URT but were attenuated at least 10 fold in the

LRT and did not induce pathogenic responses in the lung (Maher et al., 2004). This suggests that both fragments of the G protein are important in infection and pathogenesis with the secreted portion playing a particularly significant role.

Cleavage of the bovine F protein is required for activation and results in the production of the peptide and tachykinin, virokinin that induces the production of NKs, substance P and other neurogenic pro-inflammatory mediators. Virokinin itself induces ASM contraction and therefore directly contributes to airflow restriction but does not have chemotactic properties (Zimmer et al., 2003). By contrast cleavage of human RSV does not produce tachykinins (Valarcher et al., 2006). Deletion of various parts of the bovine F protein has shown that it is not necessary for proliferation in vivo but deficient mutants promote substantially reduced airway inflammation and eosinophil influx (Valarcher et al., 2006). The F protein is responsible for suppressing lymphocyte proliferation (Schlender et al., 2002), however, there is no effect on the expression of the eosinophil attractants RANTES or MIP-1α in the absence of the F protein suggesting that other mechanisms may be involved in the suppression of eosinophil influx in cattle (Valarcher et al., 2006).

NS proteins, particularly NS2, of bovine RSV downregulates the IFN-α/β response involving the attenuation of IRF-3 and STAT-2 (Schlender et al., 2000), which has subsequently also been shown with human RSV (Spann et al., 2004, 2005; Lo et al., 2005). The lack of NS proteins results in highly attenuated replication and a lack of pathogenesis in vitro and in vivo (Whitehead et al., 1999; Valarcher et al., 2003).

The SH protein is not required for replication of bovine RSV in vitro (Karger et al., 2001) but replication is attenuated in the LRT of chimpanzees and calves in vivo (Whitehead et al., 1999; Valarcher & Taylor, 2007).

2.4. Summary

Epidemiological studies have widely linked RSV infection to the development of bronchiolitis, wheeze, decreased lung function and possibly asthma in childhood. This association is equivocal later in life but may depend on the severity of the infection. There may also be a role for RSV in the induction of sensitization to allergens and allergy may predispose to more severe infection. Enhanced severity of symptoms that result from infection may be a useful prognostic marker for the future development of allergic disease.

Both human and animal studies have investigated the mechanisms of how infection induces pathogenesis and how this is associated with asthma. The age of first infection is crucial in determining clinical outcomes and early infection may promote the development of a pro-allergic phenotype. This indicates that delaying the age of infection until later in life may be an effective therapeutic strategy. Infection induces both innate and adaptive immune responses that promote Th2 immunity and eosinophil influx into the lung that may contribute to the development of AHR. The timing of infection relative to allergen exposure and the immune phenotype of the individual may be significant in determining the effects of infection. An interesting novel concept is that RSV infection may induce the development of angiogenesis, which might exacerbate inflammatory exudation, edema and bronchial obstruction in asthma. Other new hypotheses are that RSV may induce immune suppression allowing viral persistence and latent infections that can be reactivated. Neural networks also have the potential to be involved through the induction of neurogenic inflammation.

3. RV and asthma

RVs are small non-enveloped ssRNA viruses of the Picornaviridae family and to date over 100 different serotypes have been identified (Savolainen et al., 2002).

Epidemiology — RVs are responsible for the majority of cases of URT infectious disease in humans particularly colds and have recently also been implicated as the cause of significant numbers of LRT infections. RV is also the most common respiratory viral pathogen that induces wheeze at all ages (Kusel et al., 2006). A third of all episodes of acute respiratory illness involve wheezing in both children and adults and RV infections are implicated in 3 times as many cases as other viruses. Wheezing may then progress to the development of asthma. The mechanisms of pathogenesis of RV are much less well understood than for RSV.

Pathogenesis — RVs are divided into major and minor classes based on receptor binding properties. Major group RVs bind to ICAM-1 while minor group viruses bind to very low-density lipoprotein receptors (LDLPR) (Fig. 1) (Dreschers et al., 2007). Binding of RV to LDLPRs induces a conformational change in the capsid that is required for viral uptake. Intracellular internalisation occurs through the activation of sphingomyelinase that generates ceramide-rich membrane rafts, which facilitate uptake. RV is not thought to replicate in LRT, however, it is possible that RV can infect the LRT to a certain extent or alternatively that immune responses to URT infection has knock-on effects on inflammation in the LRT. Infection of the URT typically causes coryza and pharyngitis and virus can be detected in fluids from the nasopharynx, tonsils and the middle ear (Holgate, 2006).

3.1. Clinical evidence for the association

3.1.1. RV and asthma exacerbation and induction

Recent epidemiological studies correlating RV with asthma are shown in Table 3. RV is more strongly linked with exacerbations of asthma although it is emerging that RV may also be important in asthma induction. There is a strong association between RV infection and childhood wheeze and exacerbation of asthma that may lead to hospitalization (El-Sahly et al., 2000; Jartti et al., 2006). RV is implicated in wheeze in 80% of children >3 years of age and 45–80% of adults and is responsible for 60% of cases of asthma exacerbations (Johnston et al., 1995; Nicholson et al., 1997; Atmar et al., 1998; Freymuth et al., 1999; Tan, 2005). Interestingly the peak of severe asthma exacerbations in children occurs shortly after their return to school after breaks and in adults one week later

Table 3
Recent epidemiological studies that link RSV infection with asthma

Study design	Cohort	Test	Result/conclusion	Reference
To determine contribution of respiratory viruses to asthma exacerbations in children	2–17 years with asthma exacerbations (case) vs well controlled asthma (control)	Detection of RV, RSV, enterovirus, coronavirus, parainfluenza, influenza, adenoviruses and bocavirus	RV in 60% case vs 18% control. Only RV associated with exacerbations. Symptomatic RV infections contribute to asthma exacerbations in children.	Khetsuriani et al., 2007
To determine relationship of respiratory virus exposure to symptoms and subsequent asthma and atopy at 1 and 2 years	"high-risk" children (455) born into asthmatic/atopic families	Nasal specimens analysed for parainfluenza, RSV, RV and enterovirus. Correlated virus with respiratory symptoms and asthma/atopy	Virus associated with increased odds of cold and cough. Parainfluenza and picornavirus were associated with rhinitis. RSV associated with wheezing. Parainfluenza increased odds of atopy at 1 year. Parainfluenza and RSV associated with asthma at 2 years.	Lee et al., 2007
To assess RV infections in children hospitalized for RT infection in a secondary public school	Children (304) admitted to hospital with fever or RT infection with RV	Viral diagnosis in nasopharyngeal washings	76 had RV (25%), 30% had RSV (71% of these <2 years old). 60% had recurrent wheeze, 24% bronchiolitis, 8% pneumonia, 5% URT infection, 58% fever. RSV/RV frequently isolated. RSV commonest followed by RV in infants, RV commonest in children.	Calvo Rey et al., 2006
To assess viral prevalence and significance in mechanically ventilated patients	Adults ventilated for 48+ hours and admitted to intensive care	Assessment of respiratory samples and tracheobronchial aspirates	45 viruses isolated in 22% of patients. RV most common (42%) Herpes simplex virus-1 (22%), influenza (16%). Confirmed pathogenic role of viruses (particularly RV) in intensive care units.	Daubin et al., 2006
To evaluate RV in bronchial biopsies from infants with recurrent asthma symptoms	Steroid naïve infants (201) 3–26 months	Bronchial biopsies assessed for RV. Lung function using body plethysmography	45% had RV. Abnormal lung function in 86% of infants with RV and 58% of infants without RV. RV frequently found in LRT of infants with recurrent symptoms, most of these had increased airway resistance.	Malmstrom et al., 2006
To investigate role of respiratory infections in stable adult asthma	Asthmatics (103) vs controls (30)	Sputum and oropharyngeal swabs for RV, RSV, enterovirus and adenovirus	RV detected in 7% controls, 9% mild and 16% moderate asthmatics. RV positive asthmatics have greater asthma symptoms and lower FEV_1 than RV negative cases.	Harju et al., 2006
To determine if RV infection and atopic skin prick responses influence severity of asthma exacerbations	Children (50) 4–12 years with acute severe asthma admitted to emergency. Re-assessed; 6 weeks/month	Peak expiratory flow (PEF) measured. RV and RSV detected in nasal aspirates. Atopy diagnosed by skin prick responses to allergen	74% of patients were atopic. On admission 82% had RV, 12% had RSV. After 6 weeks 18/44 had RV. After 6 months 4/16 had RV. PEF reduced in asthmatics (no difference between RV, RSV or no virus groups). Severity of PEF reductions linked to persistence of RV. >40% asthmatic children had RV 6 weeks after acute exacerbation.	Kling et al., 2005
To assess effect of viral RT infections during infancy on development of subsequent wheezing and/or allergic disease in early childhood	Children (285) birth—3 years genetically at high risk of developing allergic respiratory disease	Nasal lavage to evaluate effect of timing, severity and etiology of viral RT infections during infancy on wheezing in the 3rd year of life	Risk factors for 3rd year wheezing; passive smoke, older siblings, allergic food sensitization, moderate/severe respiratory illness without wheezing and 1+ wheezing illness with RSV/RV/other pathogens during infancy. RV induced wheeze in 1st year strongest predictor of subsequent wheeze in 3rd year (63%) vs 20% of other infants.	Lemanske et al., 2005

and this coincides with peaks in RV infections in the spring and fall (Longini et al., 1984). Indeed in the fall RV was present in 29% and 52% of children that were asymptomatic or were having asthma exacerbations, respectively (Johnston et al., 2005).

3.1.2. Importance of RV infections in childhood wheeze and asthma

While RSV may be an important factor in early childhood wheeze, the majority of children become infected with RSV, but most do not go on and develop asthma, suggesting that other factors are important in this process. To investigate this Lemanske et al. (2005) prospectively recruited a cohort of children (285) at risk of asthma based on family history and followed them for 3 years to determine the relationship between viral infections and symptoms of asthma, confirming infective episodes and etiology by PCR on nasopharyngeal aspirates. At 3 years non-infective factors such as exposure to environmental tobacco smoke, the presence of asthma in an older sibling and IgE-mediated food allergies were associated with persistent wheeze. RSV infections were also linked with an increased risk, however the strongest independent predictor of persistent childhood wheeze was an episode of RV infection associated with wheeze (OR = 10, 63%). This suggests that RV infection in

infancy is associated with the development of asthma later in life. Others agree and have shown that RV infection is the primary risk factor for wheezing in children under 3 years but that atopy is more important thereafter (Heymann et al., 2004). Other recent studies have also indicated that recurrent wheezing in infants is promoted by RV and possibly to a greater extent than RSV (Lehtinen et al., 2007). A pathophysiological link between RV and asthma is indicated by the tendency of children infected with RV to be older than those infected with RSV (13 months vs 5 months) and an increased frequency to present with atopic dermatitis and blood eosinophilia (Korppi et al., 2004a). In the study by Korppi et al., children (81) hospitalized for wheezing before the age of 2 were assessed by bronchoscopy and had a prior median symptomatic period of 6 months. Of these children 40% were found to be asthmatics by the age of 3 years, and 90% of these were atopic. Early predictors of asthma were atopic dermatitis, specific IgE and inhalant allergy and RV was common during wheezing (58%). Another recent study of infants (192) under the age of 3 hospitalized with bronchiolitis identified RV and RSV in 21% and 30% of cases, respectively (Jacques et al., 2006).

3.1.3. Asthmatic predisposition to more severe infection

It is unknown if asthmatics are more likely to develop colds, or if they are at a greater risk of more severe colds. Corne et al., attempted to investigate this by recruiting couples, one of whom had asthma and determining if the asthmatics were more at risk of developing colds. They found that those with asthma were no more likely to develop colds (10% vs 9% of controls), but they were substantially more likely to develop LRT symptoms associated with these colds (43% vs 17%). This implies that viral infections in asthmatics are more likely to increase LRT inflammation (Corne et al., 2002). Other investigators have shown that asthmatic infants hospitalized with wheezing have a greater chance of testing positive for RV than non-asthmatics, which suggests that asthma sufferers may also be more susceptible to infection (Xatzipsalti et al., 2005). Furthermore, RV infection may be more long lasting in asthmatics and may persists for up to 6 weeks after hospitalization for acute asthma exacerbation and RV-induced AHR may also last for several weeks (Kling et al., 2005).

3.2. Mechanisms of predisposition and exacerbation shown in humans

3.2.1. In vivo studies

Experimental RV infection of human volunteers has enabled the study of the effects of infection on immune responses, lung function and asthma as well as the role of atopy and timing of infection on disease outcomes (Papadopoulos et al., 2004). Infection upon binding of RV to ICAM-1 (Greve et al., 1989) not only contributes to pathogenesis but also to the induction of allergic inflammation (Canonica et al., 1995). RV also induces the upregulation of ICAM-1 by activation of NF-κB, which promotes further infection (Papi & Johnston, 1999). RV infects the bronchial epithelial layer and some mononuclear cells and fibroblasts (Papadopoulos et al., 2004) and increases airway inflammation, with changes observed in induced sputum and endobronchial biopsy. The bronchial epithelium of asthmatics is particularly susceptible to the cytotoxic effects of viruses (Papadopoulos et al., 2000; Mosser et al., 2005). Initial infection initiates RV-induced exacerbations and cytotoxicity leads to increases in the penetrance and effects of allergens.

3.2.1.1. Immune responses. In both asthmatics and non-atopic non-asthmatics inflammatory changes involving almost all types of inflammatory cells are observed upon RV infection. In mild to moderate asthma, infection induces increased levels of IL-8 and neutrophils in sputum, promotes the influx of eosinophils, mast cells, CD4+ and CD8+ cells into the airway wall and enhances AHR (Fraenkel et al., 1995; Grunberg et al., 1997a; van Benten et al., 2001). de Kluijver et al., showed the inflammation upon infection may be less intense in the non-asthmatic, although these differences were small and no direct comparison with asthmatics was made at the time (de Kluijver et al., 2002). Nevertheless reduced IFN-γ responses in subjects with asthma may promote susceptibility to infection and there is an inverse correlation between IFN-γ levels and RV persistence and symptoms in asthmatics (Gern et al., 2000). This deficiency may result from either the lack of recruitment of appropriate inflammatory cells or a reduced response from these cells.

Other differences in immune responses to RV infection have also been observed that may be involved in increased susceptibility of asthmatics to infection or enhanced inflammation. In subjects with acute RV-induced asthma Wark et al., found that increased serum IP-10 levels but not RANTES or IL-8 was specifically associated with infection and correlated with the degree of airflow obstruction (Wark et al., 2007). These subjects also had less bronchodilatory response (increase in FEV_1) to salbutamol compared to non-infective acute asthma, which correlated with levels of IP-10 in serum. IP-10 is a chemokine that binds to CXCR3 and is important in the recruitment of T cells to infected tissues. Increased levels of IP-10 can activate mast cells and cause their migration. Mast cells that are resident in the ASM are specific for asthma and also express CXCR3. Therefore, RV infection of the bronchial epithelium may lead to an early release of IP-10 that sets off a chain of events that enhance pre-existing asthmatic airway inflammation and promotes the migration and activation of mast cells into the ASM thereby worsening bronchoconstriction and reducing the response to bronchodilators.

Nasal secretions of experimentally infected healthy volunteers contain increased levels of IL-1β, a pro-inflammatory cytokine (Proud et al., 1994), while RV infection of subjects with allergic rhinitis or asthma, or in children with virus-induced asthma, resulted in elevated levels of G-CSF and IL-8 as well as increased blood and nasal neutrophilia (Teran et al., 1997; Gern et al., 2000).

3.2.1.2. Decreased lung function/AHR. Several investigators have demonstrated that RV infection worsens airway inflammation and obstruction and enhances AHR to non-specific bronchoconstrictors in subjects with asthma or atopy. However, despite showing an ability to infect these subjects and recovery

of virus, significant exacerbations of disease are not observed (Grunberg et al., 1997a). While treatment with inhaled corticosteroids (ICSs) effectively reduces AHR and even airway eosinophilia, it has no effect on virus-induced airway inflammation. This suggests that in the acute phase there is a disparity between these features of disease.

Epidemiological studies also link RV infection with reduced lung function. For example, a recent study detected RV in the respiratory epithelium in 45% of infants with recurring respiratory symptoms (201, 3–26 months) which was associated with abnormal lung function (decreased airways conductance, 86%) compared with RV negative infants (58%) (Malmstrom et al., 2006).

It is possible that the serotype of RV involved may be important and some strains such as RV16 are particularly associated with reduced lung function and increased AHR and symptoms of asthma and rhinitis (Peebles & Hartert, 2000).

3.2.1.3. Atopy. It is unknown whether atopy predisposes to infection and infection-induced asthma. To determine whether atopy influences infection and worsening of AHR, Xepadaki et al., studied a group of asthmatic children dividing them by atopy (Xepapadaki et al., 2005). In both groups AHR increased 10 days after a cold and remained elevated for 5–11 weeks. Where the groups differed was that atopic children experienced more symptomatic colds and acute exacerbations, leading to a cumulative affect that resulted in the persistence of AHR. Therefore, increased susceptibility of atopics to infection was demonstrated and recurring inflammatory insults were implicated as an indirect cause of more severe AHR.

3.2.1.4. Timing of infection. As with RSV the timing of infection relative to allergen exposure may also be important in determining the outcome of RV infection in asthma. The severity of asthma exacerbations increases if infection occurs simultaneously with allergen exposure in asthmatics (Green et al., 2002). However, infection after allergen exposure has little effect on AHR (de Kluijver et al., 2003).

3.2.2. In vitro studies

Numerous important studies have used a variety of in vitro cell culture systems to provide valuable insights into the pathogenesis of RV and its association with asthma. Specifically the role of cytotoxicity, immune responses, AHR and remodelling involving angiogenesis have all been implicated. Importantly it has now become clear that viral infection directly influences lower airway inflammation in asthma and that asthmatics may have a defective innate response in bronchoepithelial cells (BECs) to RV. The BEC is the first point of contact between infecting viruses and the host and plays an important early role in initiating innate and adaptive immune responses and inflammation (Bals & Hiemstra, 2004).

3.2.2.1. Cytopathic effect (CPE). There is conflicting evidence surrounding the nature of the CPE of RV infection on epithelial cells. While some studies have found that RV induces considerable CPE albeit with serotype dependent variation (Schroth et al., 1999; Papadopoulos et al., 2000), others report none or very little CPE in vitro or in vivo (Winther et al., 1984, 1990).

3.2.2.2. Immune responses. The magnitude of immune responses as well as differential regulation of different innate and adaptive components has been implicated in the increased susceptibility of asthmatics to RV and in RV-induced asthma and exacerbations. Upon RV infection of asthmatics more intense inflammatory reactions involving mucus production, the influx of neutrophils, eosinophils, activated macrophages, CD4+ and CD8+ T cells into the RT and pro-inflammatory mediator release are associated with more severe and persistent LRT involvement and symptoms and delayed repair (Bossios et al., 2005; Edwards et al., 2006; Inoue et al., 2006). Although there are similarities in the cellular immune responses to RSV and RV infection there are significant differences in the levels and types of cytokines and chemokines released and the cell types that produce them. Ineffective presentation of viral antigens also occurs in asthmatics, which may impair immunity and lead to asthma symptoms (Parry et al., 2000).

3.2.2.3. Pro-inflammatory mediators. Many authors have used epithelial cell culture models to demonstrate that RV induces the release of pro-inflammatory mediators and it is possible that differential regulation of these factors may be important in RV-associated asthma.

RV infection promotes the production of oxygen radicals in the bronchial epithelium and leads to the increased expression of NF-κB and enhanced pro-inflammatory responses (Contoli et al., 2005). The activation of p38 mitogen-activated protein kinase by Rho A has been shown to play an important role in these processes (Dumitru et al., 2006). RV infection of human cell lines relevant to the RT and asthma has demonstrated that the production of many different pro-inflammatory cytokines, chemokines and molecules is induced including; eotaxins 1 and 2, IL-1β, -4, -6, -8, -16, ENA-78, IP-10, GM-CSF, TNF-α, RANTES, MCP-1, MIP-1α and ICAM-1 (Subauste et al., 1995; Grunberg et al., 1997a; Johnston et al., 1998; Yamaya et al., 1999; Gern et al., 2000; Papadopoulos et al., 2000; Suzuki et al., 2001; Papadopoulos et al., 2001; Hosoda et al., 2002; Konno et al., 2002; Gern et al., 2003; Hall et al., 2005; Edwards et al., 2006). Of particular importance is the increased production of IL-6, -8 and -16 and eotaxin, IP-10 and RANTES, which are released upon RV infection of BECs (Papadopoulos et al., 2004). IL-6 and IL-16 participate in the maturation of B and T cells and eosinophil and macrophage chemotaxis, respectively. RANTES and eotaxin are also powerful chemoattractors of eosinophils and drive airway eosinophilic inflammation (Papadopoulos et al., 2004). IL-8 and ENA-78 and RANTES and IP-10 are potent neutrophil and T cell chemoattractants, respectively (Edwards et al., 2006). IL-8 is also linked with reduced lung function in experimental RV infection, suggesting that chemokines may be involved in asthma exacerbations (Grunberg et al., 1997b). Importantly IP-10 and RANTES are the chemokines released in the greatest quantities from BEC infected with RV (Wark unpublished). Moreover, when BECs were treated with dexamethasone IP-10 was only inhibited at

the maximum dose, although RANTES and IL-6 were inhibited at all doses. This is in keeping with experimental findings that moderate doses of ICSs are effective in reducing AHR and infiltration of eosinophils, but do not prevent the accumulation of cytotoxic T cells. Therefore RV infection may induce neutrophil and T cell influx that may exacerbate allergic inflammation and which may be refractive to ICS treatment.

3.2.2.4. IFNs. The release of type I and III IFNs from BECs are important innate responses to infection. They have anti-viral properties and play roles in apoptosis of infected cells and directing adaptive immune responses to clear the virus (Takaoka et al., 2003; Wark et al., 2005).

Asthmatics have recently been shown to be particularly susceptible to the development of RV LRT infections as a result of reduced IFN-α responses. This was shown in vitro by demonstrating that peripheral blood mononuclear cells (PBMCs) from both children and adults with asthma responded to RV inoculation with a reduced release of IFN-α (Corne et al., 2002; Gehlhar et al., 2006).

Wark et al., have established a model of acute RV infection of the airway epithelium, using primary BECs (pBECs) acquired by bronchoscopy and cultured in vitro. Asthmatic pBECs allow a significantly greater replication of RV compared to controls. The innate response of asthmatic pBECs to RV is deficient in the release of IFN-β (Wark et al., 2005), which promotes susceptibility to infection. This defect directly impairs the ability of the infected host cell to undergo early apoptosis and allows increased virus replication and ultimately cytolysis of infected cells. The defect is the result of altered intracellular signalling and not ICAM-1 expression or viral internalisation. The administration of exogenous IFN-β induces apoptosis and suppresses viral replication, which identifies the potential for therapeutic intervention (Wark et al., 2005). As IFN-β is released mostly by BECs, little IFN-β was detected in the BALF of individuals experimentally infected with RV. Further confirmation of the importance of these pathways was provided when microarray analysis of RV-infected differentiated BECs identified 48 genes that were upregulated and most of which are involved in type I IFN pathways. The expression of these genes could be blocked by anti-IFN-β mAB or a dsRNA inhibitor (Chen et al., 2006).

RV infection of pBECs also induces the release of the novel immune mediator and type III interferon, IFN-λ, which also has anti-viral effects on RV replication (Contoli et al., 2006). Again this group demonstrated that asthmatic pBECs had a markedly deficient IFN-λ response to infection with RV compared to healthy volunteers. The importance of the early response to infection was confirmed in vivo by experimental RV infection of volunteers; those with the greatest impairment of early release of IFN-λ to RV had significantly increased virus replication, severity of cold symptoms, airway inflammation and a more severe fall in lung function (Contoli et al., 2006).

It has been suggested that defective innate immune responses result in deficient adaptive Th1 responses. In support of this PBMCs from asthmatics have been shown to have deficient IFN-γ responses to RV (Wark et al., 2005). Others have made similar observations and demonstrated time and dose dependent increases in IFN-γ, IL-12 and IL-10 following RV infection of PBMC (Papadopoulos et al., 2002b). Cells from normal subjects produced higher levels of IFN-γ and IL-12, while those from atopic asthmatics predominantly produced IL-10 (Papadopoulos et al., 2002b).

3.2.2.5. TLRs. TLR-3 recognises RV and is upregulated upon infection, which leads to NF-κB expression (Groskreutz et al., 2006). If TLR-3 is blocked anti-viral responses are suppressed and RV replicates and is released (Hewson et al., 2005). PKR is released and promotes the generation of pro-inflammatory IL-6, -8 and RANTES. Asthmatics may be deficient in TLR-3, which may predispose to reduced immune responses and increased and persistent RV infection that may lead to enhanced viral cytotoxicity (Hewson et al., 2005).

3.2.2.6. Th2 responses. Th2 responses that are typically elevated in asthmatics including those involving IL-4, increase the expression of ICAM-1 on airway epithelium, which promotes increased RV infection (Bianco et al., 1998). ICAM-1 may participate in eosinophil and T cell influx into the LRT in asthma. Elevated Th2 along with reduced Th1 responses may promote susceptibility to RV infection and more frequent viremia in subjects with acute asthma exacerbations (Xatzipsalti et al., 2005).

3.2.2.7. AHR/remodeling. While viral infections initiate lower airway inflammation and asthmatics appear more susceptible these observations do not provide a link that infections increase the severity of AHR or long-term airway remodelling. Infection of pBECs clearly will initiate the release of pro-inflammatory mediators that will enhance the recruitment and trafficking of inflammatory cells to the airways. However, it is unknown whether this will affect remodelling, either directly by influencing ASM or indirectly by affecting other features of remodelling in chronic asthma. To determine if RV infection could directly influence remodelling, in vitro culture models of pBECs and fibroblasts from subjects with mild or moderate asthma and healthy controls have been employed. Fibroblasts have the potential to play a critical role in remodelling and changes in the airway matrix (Holgate et al., 2004). Infection of pBECs and fibroblasts from these subjects does not lead to the release of the known mitogenic factors TGF-β or endothelin-1. Culture of fibroblasts with media taken from pBECs that had been infected with RV also did not induce the release of these mediators. There was also no increase in the expression of the contractile protein alpha smooth muscle actin, which may be expressed if these cells develop a contractile phenotype and there was no change in cellular morphology. These results suggest that RV infection does not induce the release of pro-remodelling factors from these cells. However, exposure of fibroblasts to both RV directly and RV conditioned pBEC media did promote inflammation with the induction of inflammatory mediators, similar to those observed in BECs. This observation agrees with the results of Oliver et al., who demonstrated that asthmatic ASM cells released significantly more IL-6 upon

infection even though the virus does not replicate in these cells (Oliver et al., 2006).

3.2.2.8. Angiogenesis. Limited data suggest that RV may be involved in the generation of angiogenesis and remodeling. Infection induces the release of VEGF and FGF and other fibrosis and angiogenesis inducing factors from primary airway epithelial cells, epithelium and fibroblast cell lines and these factors have also been detected in RV-infected nasal secretions (Ghildyal et al., 2005; De Silva et al., 2006; Psarras et al., 2006; Volonaki et al., 2006). Psarras et al., demonstrated that RV infection of BECs led to the induction of VEGF and that when endothelial cells were treated with the medium of infected BECs they began to form tubules and to proliferate. These effects were inhibited by anti-VEGF and enhanced when the endothelium was exposed to Th2 cytokines (Psarras et al., 2006).

3.3. Mechanisms of predisposition shown experimentally

RV research and the development of therapeutic strategies have been severely hampered by the lack of a small animal model with which to investigate disease pathogenesis and the induction and exacerbation of asthma by RV. Mice do not express ICAM-1 that can be used as a receptor by major group human RVs and these viruses do not establish infections in mice. Minor group RVs, however, can infect airway epithelial cells of mice by binding to LDLPRs (Dreschers et al., 2007). A recent report (Newcomb et al., 2006), describes a mouse model of minor group RV infection with RV1B. Virus persists in mouse lungs and induces neutrophilic inflammation in the airways involving MIP-2 expression whereas major group RV does not. This model has since been used to demonstrate that RV infection induces mucin production in vivo and that mucin production in a model of murine AAD is also increased by RV infection (Bartlett et al., 2007).

3.4. Summary

RVs are the most common cause of common colds and URT illness and have recently been implicated in many LRT episodes. RV infections are also the commonest cause of wheezing and asthma exacerbations, particularly in individuals >3 years of age. RV-induced wheezing may lead to the development of asthma and recent evidence suggests that RV may be the most important risk factor for wheeze and asthma in early life. As is the case with RSV, asthmatics may be more susceptible to RV and are more likely to suffer from more severe RV disease.

In vivo and in vitro studies have been used to demonstrate that RV infection exacerbates airway inflammation, obstruction and AHR and atopic status and timing of infection may have important effects. These studies show that the magnitude and phenotype of the immune response to RV has a major impact on asthmatic outcomes. Importantly in vitro studies have identified defects in innate (type I and III IFNs) and adaptive responses from cells from asthmatics to RV infection. These defects may promote susceptibility to infection and contribute to the association between RV infection and the induction and exacerbation of asthma.

4. Therapeutic strategies

In order to prevent virus-induced asthma and exacerbations we require a better understanding of predisposing factors for viral diseases and their association with the causation and exacerbation of asthma. The development of effective anti-viral agents and the conduct of large-scale prospective and intervention trials are also required to assess the potential of such strategies to prevent these diseases (Wennergren & Kristjansson, 2001).

In asthmatics RSV and RV infection directly enhanced airway inflammation and worsened airflow obstruction. Much of this occurs as a result of the immune responses, which develop rapidly upon infection. For example, responses to naturally occurring colds due to RV infection results in the development of symptoms within 12 h which peak at 2–3 days but have resolved within a week (Gwaltney et al., 1996). The rapid response to infection, the often relatively mild symptoms that occur and brisk resolution determine that it is difficult to devise interventions that will be of substantial clinical benefit. In the case of asthmatics where acute exacerbations are triggered by virus infection the clinical consequences are more severe with increased asthma symptoms, medication use, emergency presentations and hospital admissions (Johnston et al., 1996). Wark et al., have shown that virus-induced acute asthma worsens airway inflammation and the degree of this inflammatory response is directly related to the severity of acute clinical symptoms (Wark et al., 2002). Therefore, effective therapeutic interventions would need to alter the natural history of virus-induced asthma, prevent the worsening of airway inflammation and lead to significant clinical improvement. Potential therapeutic strategies to this problem have been discussed but the approaches with the most promise include: prevention of infection from occurring; anti-viral agents that eliminate infection while reducing the host's immune response; treatments that specifically target enhanced inflammatory responses that are induced during virus-induced asthma exacerbations and supplementation of protective anti-viral responses. Anti-oxidants and angiogenesis inhibitors may also have beneficial effects (Fig. 1).

4.1. Preventative strategies

Research into the inhibition of RSV and RV infection may have important implications for the prevention of LRT infections and the development and exacerbations of asthma. There are no human vaccines available and further research is required to develop effective anti-viral preventative strategies, specifically anti-viral mAbs, passive immunization and vaccines. Although the evidence that anti-viral regimes reduce the risk of developing RAD later in life is scarce, the use of antivirals to delay RSV infection in particular to later in life may reduce subsequent virus-related illnesses including asthma (Culley et al., 2002). Genetic factors are fixed but investigations

of such interventions may be developed to reduce asthma incidence have become areas of intense interest.

4.1.1. Antibodies

mAbs have demonstrated efficacy in preventing RSV infection and inflammation in animal models and a humanised mAb (palivizumab) against the RSV F protein is effective against RSV and wheezing in children and reduces hospitalization in high-risk individuals. Studies in mice show that palivizumab reduces viral load, acute disease and long-term lung function abnormalities (Mejias et al., 2005). In the rat model palivizumab inhibits the invasion of virus particles into the LRT epithelium and the upregulation of the NK1 receptor. This prevents acute neurogenic LRT inflammation and long-term susceptibility to inflammation that is induced by infection (Piedimonte et al., 2000; Piedimonte, 2002). A randomised controlled trial with palivizumab in children achieved a 55% reduction in RSV hospitalization. Reductions were observed in children with (39%) and without (78%) chronic lung disease (IMPACT study, 1998). A recent large prospective case-controlled study of at risk pre-term infants showed that palivizumab also protected against recurrent wheezing (13% vs 26% controls) and physician-diagnosed recurrent wheezing (8% vs 26% controls) independently of confounding variables (Simoes et al., 2007). One drawback is that escape mutants have been detected both in vitro and in vivo (Zhao et al., 2004; Zhao & Sullender, 2005), indicating that preventative strategies should be targeted at multiple epitopes, Because palivizumab is effective in preventing hospitalization in high-risk groups, optimisation of its pharmacokinetic profile, extension of its half-life and increasing its neutralising abilities would produce an even more efficacious preventative strategy for RSV-associated disease. Motavizumab is a derivative of palivizumab and is now in clinical trials. This mAb has a 20 fold increase in neutralising abilities in vitro and its use reduces viral titres by 100 fold and inhibits viral replication in the RT compared to palivizumab in rats (Wu et al., 2007). Further studies are required to establish the application of such mAbs for the prevention of asthma.

Other mAbs have also been used in mice as treatments of features of RSV-induced disease. RSV infection of mice leads to eosinophil and neutrophil influx into the lung and AHR, which are inhibited by anti-IL-5 (Schwarze et al., 2000). Administration of FI-RSV and anti-IL-4 leads to a decrease in disease features and IL-4 production following challenge, but IFN-γ release is increased (Tang & Graham, 1994). T1/ST2 mAb treatment reduced Th2 but not Th1 pathology and may also be a good treatment (Walzl et al., 2001). All of these mAbs require further assessment.

4.1.2. Passive immunization

Currently the only option for preventative treatment of RSV is passive immunization, which has been in use for many years. A randomised placebo controlled trial investigated the applicability of RSV immunoglobulin to prevent RSV-associated hospitalization (PREVENT study, 1997). Treatment reduced hospitalizations for LRT infections but required monthly administration of high levels of fluids and proteins. In another small study, children with chronic lung disease who were treated with immunoglobulin 7–10 years previously had improved lung function, reduced atopy and RAD events (Wenzel et al., 2002). This indicates that prophylaxis may be able to prevent RAD in children. Studies in rats also show that passive transfer of antibodies against RSV induces protection in the LRT but not the URT and that serum titres of $\geq$1:380 are protective whereas titres of $\leq$1:100 are not (Prince et al., 1985).

4.1.3. Vaccines

Infants who are the most susceptible to virus infections are protected by maternal anti-viral antibodies (largely IgG1) that are passively transferred, however these antibodies inhibit the induction of protective responses against primary infection or vaccination. As a result primary or recurrent infections do not induce protective immunity until later in life (Henderson et al., 1979; Glezen et al., 1986). Natural protection against viral disease results from a combination of antibody, cell-mediated and T-helper cell immunity. This combination of responses target the virus before infection of host cells as well as destroying cells that have already been infected with virus. Therefore, potentially an ideal vaccine would need to stimulate humoral, cellular and T-helper responses similar to those induced by natural infection. Vaccine development has been hampered by the requirement for early-life vaccination, the confounding effects of poor neonatal immune responses, the presence of maternal antibodies, the difficulties in balancing immunogenicity and attenuation and the risk of vaccine-induced disease. Mucosal immunization may overcome the immune suppressive effects of maternal antibodies.

4.1.3.1. FI-vaccines. The widely recognised need for an effective RSV vaccine has also been held back by the adverse events following the implementation of the FI-RSV vaccine. This vaccine was developed in the 1960s but not only was it poorly effective it infamously induced more severe RSV-related disease upon natural infection in clinical trials (Kapikian et al., 1969; Kim et al., 1969). The vaccine induced high serum antibody titres that were of low neutralising activity, lymphocytes with an enhanced RSV-specific proliferative response and blood eosinophilia (Openshaw et al., 2001). These observations were repeated in mice given the same vaccine or the G protein of RSV. Enhanced disease involved eosinophilia of the lung (Srikiatkhachorn & Braciale, 1997b; Johnson & Graham, 1999; Tripp et al., 2000) and aberrant CD4+ T cells releasing Th2 cytokines (IL-4, -5, -10 and -13) (Waris et al., 1996; Srikiatkhachorn & Braciale, 1997b; Johnson & Graham, 1999; Tripp et al., 2000). Reduced IL-12 and CD8+ responses were also detected (Waris et al., 1996; Aung et al., 1999). FI-bovine RSV has also been tested in calves and also induces the development of immunopathology, ineffective neutralising antibodies and induced inflammatory responses leading to eosinophil influx into the lung and enhanced IgE production upon subsequent infection (Gershwin et al., 1998; Antonis et al., 2003; Kalina & Gershwin, 2004). As is the case with RSV in mice the FI-bovine RSV did not induce long-term T cell

memory (Antonis et al., 2006). Thus it has been proposed that vaccines did not possess sufficient neutralising antibody or CTL responses and on subsequent infection the virus was not cleared but induced a potent Th2 response that exacerbated disease.

4.1.3.2. Subunit vaccines. Subunit vaccines are not associated with enhancing disease and may have potential applicability. However, the variability of human viruses is an issue that must be taken into account in the development of subunit vaccines. For RSV, vaccination with the F protein confers cross-protective immunity whereas only group-specific immunity is developed when the G protein is used (Sullender et al., 1990; Simard et al., 1997).

Intranasal followed by parenteral immunization with the RSV F protein protects against both upper and lower RT infection in mice but may induce pulmonary pathology (Murphy et al., 1990; Connors et al., 1992; Walsh, 1994). A chimeric parainfluenza viral vaccine expressing the F protein has also been developed and is protective against infectious challenge in primates (Tang et al., 2004). In a variety of different human studies F protein vaccines have now been shown to be immunogenic and safe in children with or without chronic respiratory disease and pregnant women and reduce the prevalence of infections but not LRT infections (Groothuis et al., 1998; Munoz et al., 2003; Piedra et al., 2003; Simoes & Carbonell-Estrany, 2003; Meyer et al., 2007). Large-scale trials are now required to confirm efficacy.

Protein G-based subunit vaccines have also been developed but may initially induce detrimental Th2 responses. Immune responses involving IL-4 and IL-13 activity following immunization with vaccinia virus expressing RSV-protein G or FI-RSV must be attenuated to modulate protein G-specific responses resulting in severe RSV-induced disease. However, inhibition of IL-4 or -13 alone has minimal impact on disease (Johnson et al., 2003). The co-administration of cytokines or Th1-inducing adjuvants may abolish initial Th2 responses (Neuzil et al., 1997).

Other novel subunit vaccines in development include the N protein, other chimeric F and G fusion proteins, synthetic peptides, recombinant proteins, recombinant vaccinia and parainfluenza viruses expressing other viral components and DNA vaccines (reviewed in Meyer et al. (2007)).

Subunit vaccines require co-administration with adjuvants to enhance immunogenicity and induce the most desirable immune response. Currently *Quillaja* saponi or its component fractions or CpG oligodeoxynucleotides administered with whole or subunit vaccines induce potent neutralising antibodies and desirable immune responses in mice and reduce disease and infection in calves. This occurs even in the presence of maternal antibodies and particularly when presented as immunostimulating complexes (Meyer et al., 2007). However the applicability of these approaches to humans remains unknown.

4.1.3.3. Live attenuated vaccines. Natural infection does not predispose to enhanced disease upon subsequent infection. Therefore, experimental live attenuated viruses are being developed for RSV (Karron et al., 2005) for intranasal administration during the first days of life but the immunology underlying their activity is poorly understood. Intranasal delivery would induce systemic and local responses and has the potential to promote protective responses in both the upper and lower airways. Infants (6–9 months) have limited immune responses to viral glycoproteins and only highly attenuated virus strains with minimal adverse side-effects can be used in this age group which are particularly susceptible to vaccine-related illnesses. These agents do however induce protective immunity even in the presence of passively acquired antibodies in mice and chimpanzees, which is dependent on CD4+ and CD8+ responses (Crowe et al., 2001). Several cold passaged, temperature sensitive attenuated viruses have been developed that protect against challenge in chimpanzees and induce protective local and systemic immunity in children (Crowe et al., 1995; Karron et al., 1997). These viruses have since been genetically engineered so that they are sufficiently attenuated for use in infants, although their efficacy against infection has not yet been determined (Karron et al., 2005). Genetic engineering is also currently in use to delete non-essential viral genes to produce other live attenuated vaccines or to insert genes to enhance immune responses, for example the insertion of GM-CSF to increase the production of pDCs and macrophages (reviewed in Mayer et al. (2007)). The assessment of attenuated viruses that induce potent protective immune responses with negligible side-effects requires extensive time consuming and expensive clinical trials. Novel prime-boost vaccination strategies with live attenuated and subunit vaccines could be used in combination to further enhance protective immunity.

4.1.3.4. Bovine vaccines. The same problems that apply to human RSV vaccines may also occur in the development of vaccines for calves in the farming industry, but the level of risk of trials is more acceptable. Vaccine development is also less complex as bovine RSV is less variable than human RSV and there is only 1 major antigenic group (Prozzi et al., 1997). This has led to the commercialization of several vaccines, which are protective against infection in calves and their development may have applicability to human vaccines. Further, cattle may be used as the animal model in preclinical human vaccine studies. However, different bovine models have been used with different concentrations of viral inocula and routes of inoculation with passaged virus that does not induce severe disease. Thus results are often not comparable or significant between experimental and control groups (Mayer et al., 2007).

Inactivated bovine RSV vaccines have been available for many years and reduce the prevalence and severity of subsequent infection without enhancing disease (West et al., 1999). Nevertheless, the longevity of induced protection and efficacy in the background of maternal antibodies could be improved. These vaccines are co-administered with CD8+ and Th1 stimulating adjuvants in order to dampen Th2 responses that may be induced by the vaccine and lead to protective Th1 responses (Ellis et al., 2005). Live attenuated bovine RSV vaccines have been developed by passaging virus in cell culture and have recently been commercialized (Vangeel et al., 2006). Unlike FI-vaccines, live attenuated vaccines induce a primed memory T cell response (Antonis et al., 2006) and reduce

pulmonary inflammation upon subsequent infection (Miao et al., 2004). They are administered parenterally, have equivalent efficacy as inactivated vaccines and induce protective immunity in calves (Harmeyer et al., 2006; Ellis et al., 2007). These vaccines are now used in quadrivalent therapies to protect against a number of bovine viruses (Salt et al., 2007). Although effective in cattle inactivated or attenuated bovine RSV are not suitable as vaccines for human RSV as they do not induce protection in chimpanzees (Buchholz et al., 2000).

Thus there are currently no safe and effective human vaccines for RSV or RV and it is yet to be determined if delay or prevention of infection with neutralising antibodies or vaccination can reduce wheezing, long-term lung function abnormalities and asthma. Nevertheless there are many promising approaches and candidates are being used to develop such vaccines, some of which are in human clinical trials to prevent infection, which if successful may be used in attempts to inhibit the development of asthma. The balance of Th1/Th2 immunity to infection determines the severity of disease where Th1 responses mediate clearance and recovery but Th2 responses induce eosinophilia and more severe symptoms. Strategies to augment Th1 responses during vaccination in early life may be useful in preventing the development of virus-induced asthma. Recent efforts have concentrated on the development of the combination of subunit vaccines with Th1-inducing adjuvants and on the development of live attenuated vaccines. The most promising approaches for developing vaccines in young infants are the use of live attenuated vaccines and recombinant viruses expressing components of target viruses.

4.2. Specific anti-viral therapies

There are no effective treatments for RSV/RV-induced disease and currently the optimal therapy for bronchiolitis involves intensive fluid and nutritional support, while the immune response of the subject clears the infection (Jhawar, 2003). In order to maximize the chances of successful treatment an anti-viral agent would need to be taken early during the course of infection in order to modulate the development of the host's immune response. It would have to be highly effective at controlling infection and the virus would need to have no or at least a limited ability to develop resistance. Finally the agent must be acceptable to patients in terms of ease of administration, have a reasonable medication frequency and limited side-effects. Currently the agents of most interest are those that inhibit viral attachment, including those that bind to viral capsids, and inhibitors of viral proteases, which are required for viral replication.

4.2.1. Attachment inhibitors

To cause infection all respiratory viruses need to enter the host's BECs and replicate. Ninety percent of RVs use ICAM-1 as a receptor to enter cells. A soluble ICAM-1 molecule (Tremacamra) has been developed and tested as a competitive binding inhibitor in 4 randomised controlled trials of experimental RV (RV39) colds, used as either a dry powder or nasal spray (Turner et al., 1999). Treatment results in small reductions in symptoms, virus replication and the development of clinical colds. There were no serious adverse events, but medication had to be taken six times a day and was only effective if used within 12 h of infection. It has not been used as a therapy for acute asthma.

The RV outer capsid consists of 4 viral proteins (VP1-4) (Rossmann et al., 1985). Several agents have been devised that bind to VP1 and prevent virus attachment to the host cell. The first agents that were produced, the oxazolinyl isoaxoles, have small clinical effects (Otto et al., 1985) but unacceptable side-effects (Diana et al., 1985). Further developments led to Pleconaril, an agent with broad spectrum activity against RV as an oral preparation to be taken twice daily (Hayden et al., 2003). Pleconaril has been assessed in two Phase II clinical trials to determine its efficacy against natural colds (Hayden et al., 2003). Subjects began treatment 1–1.5 days after cold symptoms commenced. Those taking Pleconaril had reduced cold symptoms and duration of colds (0.5–1.5 days) with few side-effects. An application in the US for FDA approval for use in clinical colds was unsuccessful as it was considered that there was a lack of substantial clinical benefit and concerns about the development of resistant RV mutants. At this stage there have been no clinical trials specifically assessing the efficacy of Pleconaril in acute asthma.

4.2.2. Viral protease inhibitors

RV relies upon proteases to cleave the viral polyprotein for replication to occur. Rupintrivir is a 3C protease inhibitor that targets this process (Dragovich et al., 2002) and has high-level anti-viral activity against all RVs (Dragovich et al., 2002; Binford et al., 2005). Unfortunately in a natural infection study Ruprintrivir failed to improve symptoms or reduce viral load and clinical development was ceased (Patick et al., 2005).

4.3. Anti-inflammatory treatments

Anti-inflammatory treatments have therapeutic potential in virus-associated diseases including asthma by moderating inflammatory responses in response to infection.

4.3.1. Corticosteroids

Long-term treatment of asthma with ICSs controls airway inflammation (Juniper et al., 1990), improves lung function and symptoms and reduces the risk of acute exacerbations (Adams et al., 2005). While ICSs are effective at controlling chronic asthma they do not completely prevent acute exacerbations even in those who achieve good control and are compliant with therapy, with the majority of episodes triggered by viral infection (Reddel et al., 1999). In an attempt to prevent the recurrence of virus-induced wheeze, infants were treated intermittently with ICS or placebo when these symptoms occurred (Bisgaard et al., 2006). Treatment had no clinical effect on acute episodes of wheeze and did not influence whether children went on to develop persistent wheeze (Bisgaard et al., 2006). Another study showed that treatment of acute asthma by increasing ICS dose also did not prevent worsening of disease (FitzGerald et al., 2004). The incomplete effect of ICS in controlling virus-induced asthma was demonstrated best in adult asthmatics using experimental RV infection (Grunberg et al., 2001). Treatment reduced AHR and

airway inflammation prior to infection but had no effect on the development of inflammatory changes that were associated with infection (Grunberg et al., 2001). In a systematic review glucocorticoids were suggested to have little effect in RSV disease (Black, 2003) and they also enhance viral replication and mortality in PVM infection (Rosenberg et al., 2005).

Oral corticosteroids have been used to treat acute viral bronchiolitis in children. However, meta-analysis of seven randomised controlled trials found that there was no effect on length of stay or clinical symptoms and complications (Patel et al., 2004).

4.3.2. ICSs and long acting beta agonists (LABAs)

In asthmatics with persistent symptoms despite ICS use, treatment with combination therapy with ICSs and LABAs effectively controls chronic symptoms and reduces the frequency of exacerbations (Walters et al., 2003). It has recently been shown that early treatment of symptoms of worsening asthma with ICS/LABA, where the LABA has a rapid onset of action and is used to relieve symptoms, does prevent deterioration leading to severe exacerbations (O'Byrne et al., 2005). While these studies did not identify the cause of the acute triggers it is likely that the majority of these were related to virus infection and the combination of ICS/LABAs appear more effective than ICSs alone in preventing exacerbations. In vitro studies demonstrate that treatment of airway epithelial cells with ICS/LABAs has synergistic and additive effects in reducing the release of inflammatory mediators (Edwards et al., 2006). These observations may explain the efficacy of combination therapy and indicate an important role for their use. This may be particularly relevant to children where their value in controlling chronic asthma is not well defined but where AHR is known to persist following viral infection (Xepapadaki et al., 2005). It remains unknown what effect ICS/LABA treatments have on specifically preventing or treating virus-induced asthma.

4.4. Macrolide and ketolide antibiotics

It has recently been recognised that macrolide antibiotics in addition to their anti-microbial action have important immune modulating effects and reduce the release of inflammatory mediators from airway epithelial cells (Takizawa et al., 1997). Epithelial cells infected with RV and treated with erythromycin, showed reduced expression of ICAM-1, IL-6 and -8 (Jang et al., 2006). Initial clinical studies were, however, not promising; using experimental RV infection, treatment of healthy subjects with clarithromycin had little or no effect on the development of cold symptoms or nasal inflammation (Abisheganaden et al., 2000). However, unlike colds where the host inflammatory response in the airways is relatively mild, in conditions characterised by more intense neutrophilic inflammation, such as cystic fibrosis, macrolides appear to be effective (Ferrara et al., 2005), which has encouraged their use in asthma. In a small randomised controlled trial of infants with RSV bronchiolitis treatment with clarithromycin reduced systemic inflammation acutely and led to few wheezing episodes in the following 6 months (Tahan et al., 2007). In a large multicentre study of adults (278) with asthma, subjects were randomised to receive the ketolide, telithromycin (800 mg/d) or placebo (Johnston, 2006). Those treated with telithromycin had a greater reduction in asthma symptom scores, but there was no difference between the groups in terms of improvement in PEF rate. The trial did not document the presence of virus infection but the results suggest the effect is due to an ability to reduce inflammation in acute asthma. Further studies are required to determine if there is a specific effect on virus-induced acute asthma.

4.5. IFNs

Type I IFNs are known to play crucial roles in defence against viruses by inducing anti-viral proteins such as PKR and RNase L in infected cells, apoptosis preventing virus replication and adaptive immune responses to infection (Malmgaard, 2004). The recent observations by Wark et al., that asthmatic BECs are more susceptible to infection with RV, which involves a deficient IFN-β response, suggests that this may be an important therapeutic target to limit the affects of virus-associated acute asthma (Wark et al., 2005). Several trials have also assessed the role of IFN-α2 treatment (1.5×10^6–4.5×10^7 IU/d intranasally) in experimental RV colds in healthy volunteers (Scott et al., 1982; Hayden & Gwaltney, 1984). These studies consistently showed a dose dependent improvement in symptom scores and reduced virus shedding, but treatment was associated with nasal bleeding and a lymphocytic nasal infiltrate. A similar response was observed with recombinant IFN-β (Sperber et al., 1988). When used to treat natural colds IFN-α2 reduced the frequency of colds but had at best modest effects on symptoms (Monto et al., 1986), while serine IFN-β failed to reduce colds or improve symptoms. These modest improvements provide a proof of concept but the clinical benefit is difficult to justify in healthy individuals with mild colds. However, as is the case with the use of macrolides in asthma where the clinical effects of colds are much greater (Corne et al., 2002), efficacy may be more evident. A single case series exists for the use of IFN-α (3×10^6 IU/d) to treat 10 stable but corticosteroid resistant asthmatics (Simon et al., 2003). Treatment resulted in improved lung function and allowed a reduction in oral corticosteroid use. However, side-effects were also common, in the first 4 weeks all subjects experienced a 'flu-like' illness, 9 had nausea and 5 developed headaches. By 5–10 months side-effects were less though 1 subject developed leukopenia severe enough to necessitate temporarily ceasing treatment. The benefits of type I IFNs now need to be assessed in the context of treatment of virus-associated acute asthma. Important issues will need to be resolved including the optimum delivery of medication, whether administration should be to the nose or the airway, how soon treatment needs to commence following the development of cold symptoms and how this may influence airway inflammation and AHR in acute asthma.

4.6. Anti-oxidants

Oxidative stress may plan an important role in asthma but it is unknown whether it is important in virus-induced disease. The anti-oxidant butylated hydroxyanisole has been used in a mouse model of RSV lung infection to reduce RSV-induced

oxidative stress, disease symptoms, weight loss and AHR. The effects were mediated by suppression of neutrophil infiltration and cytokine and chemokine release in the lung (Castro et al., 2006). Thus anti-oxidants may prevent RSV-induced inflammation and have long-term beneficial effects, which may ameliorate the consequences of infection in asthma.

4.7. Angiogenesis inhibitors

The use of VEGF and angiogenesis inhibitors may have the potential to elucidate the specific roles of these factors in asthma and identify their potential as therapeutic targets.

4.8. Summary

No efficacious vaccines yet exist for the prevention of RSV or RV infections in humans. Effective bovine RSV vaccines are available and the formulation of these vaccines may be applicable to the development of human vaccines. Combination therapy with ICSs and LABAs effectively controls persistent symptoms and numbers of exacerbations. This is the current best treatment option but does not treat the cause of the disease and cannot be used to prevent the development of disease in the first instance. A variety of novel options for the prevention of virus-induced asthma need to be fully assessed for their efficacy and applicability.

5. Conclusions

RSV and RV infection of respiratory epithelium may play an important role in the development and exacerbation of asthma, however the pivotal mechanisms underpinning these relationships remain unresolved. Infection is associated with persistent wheeze and decreases in lower lung function and worsens airway inflammation and airflow obstruction acutely in asthma, with other factors contributing to severity. Asthmatics are more predisposed to Th2 responses, may be more susceptible to infection and experience more severe LRT symptoms upon infection. The development of more severe disease may identify those people more at risk of developing wheeze and asthma. Recurring infections may lead to a cycle of inflammation and repair that worsens airway remodelling.

RSV infections are strongly linked to both development and exacerbation of asthma. Early-life RSV infections, particularly those that induce severe disease, induce recurrent wheeze and bronchial obstruction and predispose to RAD and potentially asthma that persists into later life. It is possible that persistent infection affects the developing lung and immune system and predisposes to recurring RSV infections, AHR, reduced lung function and respiratory symptoms. Another alternative is that children with reduced lung function, genetic susceptibility or aberrant immune responses may be at increased risk of infection and that RSV disease is a marker of susceptibility to increased respiratory symptoms. Both of these processes may occur under different circumstances. The association with RSV is inconclusive for allergic sensitization, which may involve IgE-mediated allergy and randomised intervention studies are required to confirm this link. The mechanisms involved in RSV-induced asthma include; age of first infection, the timing of infection relative to allergic sensitization, the nature of innate and adaptive immune responses induced upon infection, latent infections and the potential involvement of pathogenetic processes such as angiogenesis and neurogenic inflammation. A combination of human studies and mouse models has been widely used to elucidate the mechanisms of how RSV infection are linked with asthma and novel models of PVM infection may be particularly useful in the future.

RVs are widely recognised as the commonest cause of clinically significant RT infections and are strongly implicated in the exacerbation of asthma and more recently in the induction of asthma. The mechanisms of these associations have been investigated in human studies both in vivo and in vitro and have been shown to involve deficient immune responses, which may be different to those associated with RSV, that may be influenced by atopy and reduced lung function, AHR and remodelling. Nevertheless more work is needed to elucidate role of RV in LRT diseases. It remains unknown if RV induces the development of wheeze and asthma or if asthmatics are more susceptible to RV infection. Recently a novel mouse model has been described which may be used to substantially contribute to our understanding of RV and RV-associated asthma pathogenesis.

Despite the scope of the problem caused by respiratory viruses in acute asthma no specific vaccines or treatments exist that have been shown to modify the clinical outcome of RSV or RV infection. The optimal approach would be to develop safe and effective prevention strategies, such as efficacious vaccines, which induce immune responses that prevent viral infection. In the absence of a vaccine the different processes involved in the generation of virus-associated asthma and exacerbations could be targeted by specific treatments for individual patients. Several therapeutic options are available and more are emerging. Studies should focus on how established treatments such as with ICS/LABA may modify virus-associated acute asthma and assess novel anti-inflammatory strategies including macrolides or the use of IFNs either alone or in combination to influence the course of disease. The benefits of these strategies now need to be assessed in the context of treatment of virus-associated acute asthma. Important issues will need to be resolved including the optimum delivery of medication, including whether this should be intranasally or directly to the airway. It will be necessary to determine how soon treatment needs to commence following the development of cold symptoms and how this may influence airway inflammation and AHR in acute asthma.

In summary, although RSV and RV have been implicated in initiating inflammation and asthma, whether previous infections modulate the immunological response or damage the airway epithelium, and promote the progression to chronic disease, or susceptibility to later development of exacerbations, remains unknown. Asthmatics may have impaired anti-viral innate responses and defective interactions between innate and adaptive immune responses may promote infection and enhance allergic inflammation and AHR or decrease lung function. Further studies

are required to elucidate the links between infection, immune responses and susceptibility to chronic respiratory diseases and why some individuals but not others develop persistent wheeze and asthma. It is likely that there is a primary deficiency in the respiratory epithelium that predisposes to infection and asthma, which may be exacerbated by recurring exposure to environmental insults. There is a widely recognised need for further understanding of the mechanisms that induce disease and the development of effective vaccines and treatments.

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