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SERIES "HOT TOPICS IN PAEDIATRIC ASTHMA"
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Childhood asthma and infection: virus-induced exacerbations as determinants and modifiers

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ABSTRACT: Respiratory infections have been implicated in the origin and exacerbation of asthma in a variety of ways; however, systemisation of this knowledge in a way helpful for disease management remains suboptimal.

Several conceptual issues need to be taken into account: the fact that the effects of an infection may vary according to genetic background, the current immune status of the host, and parallel environmental stimuli, in addition to the particular infectious agent itself. Moreover, childhood is a very special period because of the continuous processes taking place, such as neural, immune and respiratory maturation.

Epidemiological studies have convincingly demonstrated that the majority of asthma exacerbations, in both adults and children, follow viral upper respiratory tract infections. Asthma exacerbations are still often unresponsive to current asthma treatment, and new therapeutic approaches are required.

This review presents current knowledge on the associations between infection and exacerbation of established asthma with respect to definitions, epidemiology, mechanisms and treatment.

KEYWORDS: Childhood asthma, exacerbation, infections, virus

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Although there is increased awareness of the importance of information on the role of infections in the initiation, clinical presentation and possibly persistence of asthma and related reactive airway syndromes, systemisation of this knowledge in a way helpful for disease management remains suboptimal. Several conceptual issues have to be taken into account: the fact that the effects of an infection may vary according to genetic background, the current immune status of the host, and parallel environmental stimuli, in addition to the particular infectious agent itself. Interestingly, these effects can be nonlinear, and even showing completely opposite results at different exposure levels [1]. Different aspects of the disease, such as control, severity, exacerbation and persistence, may not be pathophysiologically identical and, therefore, each outcome should be addressed individually. Furthermore, the nature and limits of asthma are disputed, often at a philosophical level (is there true asthma, in the Platonic sense?) Childhood is a particularly challenging period, due to the fast and continuous maturation processes involving, among others, the immune, endocrine, neural and respiratory systems. From a clinical perspective, variable patterns or phenotypes may be the result of different underlying mechanisms; however, these remain to be characterised in detail. In the present article, the current evidence is reviewed regarding the associations between infection and exacerbations of established asthma, an area with a relative abundance of data and somewhat better understanding. Effects of infection on disease initiation will be presented in a subsequent article in the present series.

DEFINITIONS

Although a considerable proportion of asthma morbidity, mortality and health costs can be

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attributed to exacerbations, objective criteria for defining such events are lacking, and it is sometimes difficult to differentiate an exacerbation from poor asthma control [2–5]. The lack of a universally accepted definition of an exacerbation creates difficulties in the interpretation of clinical research and trial results, with the exception of severe asthma, exacerbations being defined fairly obviously [6].

The Global Initiative for Asthma guidelines define exacerbations as episodes of progressive shortness of breath, cough, wheezing or chest tightness, or a combination of these symptoms [7]. Exacerbations have also been categorised as mild, moderate, severe or life-threatening. Variability in peak flow or forced expiratory volume in 1 s and other lung measurements have been suggested to define exacerbations, although with poor precision [8]. Several scoring systems, with respect to severity, combining a number of physical signs and symptoms have often proven inaccurate when used in children [9]. A working definition of an exacerbation that has been applied in several trials of pharmacological therapies has been a worsening of asthma of sufficient severity to require intervention from a medical professional or self-administration of oral corticosteroids [10]. A joint task force of the American Thoracic Society and European Respiratory Society has recently defined asthma exacerbations as events characterised by a change from the patient's previous status [4]. Severe exacerbations were defined as events that require urgent action to prevent serious outcomes, such as hospitalisation or death, whereas moderate exacerbations as events troublesome to the patient that prompt a change in treatment and are outside the patient's usual range of day-to-day asthma variation; however, such an exacerbation is difficult to differentiate from poor asthma control requiring additional treatment, as stated previously. Mild exacerbations were not defined because such events can be indistinguishable from loss of asthma control [4]. From a paediatric perspective, these definitions are more difficult to use, taking into account the dependence on parental reporting, as well as the variability among childhood age groups in both pathophysiology and medical practice.

Although several studies have shown that asthma exacerbations are strongly associated with respiratory tract infections, and the term virus-induced exacerbation is not uncommon, only a small number of such studies were prospective [11, 12], and even fewer have simultaneously assessed other potential factors that may contribute to an exacerbation [13]. Furthermore, respiratory infections do not always result in an exacerbation, and there is little evidence that treating or preventing the infection may cure or prevent an exacerbation. Therefore, a causal relationship in the sense of fulfilment of Koch's postulates is difficult to establish. However, in the PRACTicing ALLergology (PRACTALL) consensus report, evident post-infectious asthma activity was used to phenotype children under the term virus-induced asthma [14]. More recently, a European Respiratory Society task force defined episodic viral-induced wheeze in preschool children as a distinct phenotype of asthma-related symptoms [15]. Whether or not repeated episodes of post-infectious wheeze in very young children should be considered asthma remains the subject of debate. However, the same pattern frequently appears in older children with diagnosed asthma, either as predominantly virus-associated episodes or within a multi-trigger syndrome, strongly associated with atopy [16].

The obvious need for delineation of different patterns, or phenotypes, to improve definitions, and consequently clinical practice, is particularly hampered in childhood due to the considerable overlap between patterns and the rapidly evolving natural history. Nevertheless, the clinical experience of acute asthma worsening preceded by a common cold is overwhelming in paediatrics, and confirmed by many epidemiological studies mentioned below. Therefore, although the limits of virus-associated asthma exacerbations in children will continue to be scrutinised and debated, the majority of such events would be clinically indisputable.

EPIDEMIOLOGY

The development of sensitive diagnostic PCR-based techniques permitted demonstration of an already clinically suspected association between common viral respiratory infection and asthma exacerbations. Respiratory viruses have been identified in 80–85% of exacerbations resulting in reduced peak expiratory flow and wheezing in school-aged children, and 45–80% of adults, with rhinoviruses (RVs) being the most frequently detected (60% of cases of asthma exacerbation) [11, 17, 18]. More recent studies have suggested that RV, beside being the most prevalent, is also the only virus significantly associated with exacerbations in children (odds ratio 6.8) [19]. Interestingly, the peak of severe exacerbations in children occurs shortly after their return to school following breaks, and 1 week later in adults, and this coincides with peaks in RV infections in the autumn and, to a lesser extent, spring [20]. In the aforementioned study, the prevalence of virus identification, predominantly RV, was 61% in subjects presenting with an asthma exacerbation compared to 40% in asthmatic controls. There are suggestions that concomitant exposure to allergens (*e.g.* animal dander) at school could contribute to loss of asthma control [21]. It should be noted, however, that the proportion of hospitalisations for asthma requiring admission to an intensive care unit remains constant relative to total admissions [22]. Other respiratory viruses responsible for exacerbations display different seasonal variation; respiratory syncytial virus (RSV) and influenza have been associated with increased asthma exacerbations during the winter months [23].

INFECTION AMONG FACTORS PRECIPITATING ASTHMA EXACERBATIONS

Environmental factors, including pollutants, allergen exposure, psychological stress and weather conditions, may increase the risk of developing an acute asthma exacerbation in both children and adults [24–30]. The multiplicity of precipitating factors points towards a multifactorial cause, as well as potential interactions, which have been shown for some combinations of factors, such as pollutants [31, 32]. The risk of hospitalisation due to asthma exacerbation is synergistically increased in the presence of viral infection, allergen sensitisation and exposure to respiratory allergens [33]. Moreover, studies using sophisticated statistical methods have emphasised the importance of psychological events in asthma control, showing that stressful life events have immediate and delayed effects in significantly increasing the risk of an asthma exacerbation [34]. Nevertheless, no studies have been able to

assess all candidate-precipitating factors simultaneously in order to evaluate their relative importance and interactions. The classical notion that asthma exacerbations in atopic patients are solely related to allergen exposure with subsequent inflammation has been challenged [35].

Respiratory viral infections are the factor most strongly associated with precipitation of acute asthma exacerbations in various populations [36–38]. RVs are the predominant aetiological agents associated with exacerbations; however, other viruses, such as coronavirus, influenza virus, adenovirus, RSV and parainfluenza virus, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* have been also identified, although to a lesser extent [39]. Among the more recently identified viruses, human metapneumovirus has been associated with wheezing episodes, mostly in younger children, whereas bocavirus claims ~5% of asthma exacerbations [40–42]. Nevertheless, RV remains by far the major player in asthma exacerbations; it has also been associated with bronchiolitis, although clearly distinct from asthma-related diseases, and pneumonia [43, 44]. Recent studies have suggested that RV wheezing illnesses in infancy and early childhood are among the major predictors of the subsequent development of asthma at the age of 3 and 6 yrs in high-risk children [45, 46]. RV serotypes were traditionally classified into two groups based on viral protein (VP) 1 and VP4/VP2 sequences, RV-A comprising 74 different serotypes and RV-B 25 serotypes. More recently, using PCR nucleotide sequence homology and phylogenetic analysis, a third group of RVs, RV-C, has been described, including at least nine serotypes [47]. Febrile wheeze and asthma exacerbation were the commonest presentation (76%) of RV-C infection, whereas others have shown strong associations of this novel RV with bronchitis, bronchiolitis and pneumonia [48, 49]. In a prospective study of preschool children hospitalised with acute respiratory illness or fever, RV-C infections accounted for 46% of RV-positive samples and were significantly associated with an asthma diagnosis at hospital discharge compared to patients with RV-A (55 *versus* 36%, respectively) [50]. This result has now been duplicated in various settings, suggesting that this novel group might cause a substantial burden in paediatric asthma [51–53].

M. pneumoniae and *C. pneumoniae* are found more frequently in the airways of patients with asthma than in healthy controls; their role in exacerbations is less clear [54]. In the majority of exacerbation studies looking for a wide array of pathogens, the relative proportion of atypical bacteria is rather low. However, *M. pneumoniae* was identified in 20% of exacerbations in asthmatic children requiring hospitalisation, and in 50% of children experiencing their first asthmatic attack [55]. More recently, atypical infection has been associated with more severe asthma exacerbations, as shown by spirometry and symptom severity scores [56]. In support of the hypothesis that asthmatic airways are more susceptible to *M. pneumoniae* infection, Toll-like receptor 2 expression and interleukin (IL)-6 production are downregulated by *M. pneumoniae* infection during ongoing ovalbumin-induced allergic inflammation [57]. Moreover, airway epithelial cells from asthmatic subjects show increased production of mucin 5 subtypes A and C (the major airway mucin) when infected with *M. pneumoniae* compared to normal controls [58]. These data suggest that pre-existing inflammation might lead to decreased clearance of *M. pneumoniae*, thus increasing airway inflammation. However, whether or not this may result in symptoms, exacerbations or simply airway colonisation remains to be elucidated.

MECHANISMS OF VIRUS-INDUCED EXACERBATION

A wide range of mechanisms has been implicated in the progression from a viral respiratory infection to an acute exacerbation of asthma. These have recently been reviewed in detail [59]. The major and minor group of RVs bind to intercellular adhesion molecule (ICAM)-1 and very low-density lipoprotein receptors on the respiratory epithelium, respectively, which induces viral internalisation and upregulation of additional receptors [60]. The bronchial epithelium is the site of viral replication [61] and participates in the initiation of antiviral responses [62, 63], innate and adaptive immune responses and inflammation (fig. 1) [64]. RVs are also able to pass into the bloodstream, causing viraemia, more frequently during acute and severe asthma exacerbations [65].

RV infection activates inflammatory pathways by increasing levels of neutrophils, eosinophils, CD4+ cells, CD8+ cells and mast cells through increased mRNA expression and translation of IL-6, IL-8, IL-16, eotaxin, interferon (IFN)-γ-inducible protein (IP) 10, CC chemokine ligand 5 (RANTES) and other pro-inflammatory cytokines and chemokines [66]. Moreover, viruses generate systemic as well as neural responses, further inducing inflammation and hyperresponsiveness.

Virus-induced asthma exacerbations are mainly characterised by neutrophilic inflammation [67]. Evidence of neutrophilic degranulation and increased lactate dehydrogenase levels are independent predictors of severity, and increased levels of the potent neutrophil chemokine IL-8 are found in exacerbations [68, 69]. It has been suggested that neutrophil activation and degranulation are associated with clinical severity in virus-induced asthma, probably *via* neutrophil protease-induced mucus secretion by airway gland serous cells [68, 70]. RV infection also results in the release of IP-10, a chemokine involved in T-cell recruitment and mast cell activation. Increased serum IP-10 levels in atopic asthmatics were strongly associated with severe airway obstruction and a reduced bronchodilator response to β_2-agonists. Finally, increased serum IP-10 levels, in combination with low TNF-α levels, were predictive of a virus-induced cause as the exacerbation trigger [71].

Until recently, a major obstacle to research into the mechanisms of RV infection has been the lack of an animal model, since major RVs would not bind mouse ICAM-1 receptors [72]. In 2008, three novel mouse models for both major and minor serotypes of RV, presenting features similar to those observed in RV infection in humans, were developed [73]. Transgenic BALB/c mice with a mouse/human ICAM-1 chimera were successfully infected with the major group virus RV16. These mouse models are characterised by several relevant asthma-related outcomes, such as airway neutrophilic and lymphocytic inflammation, mucus secretion and the induction of mediators also induced by human RVs. Moreover, RV1B, a minor receptor subtype, was capable of binding the mouse receptor and inducing successful infection *in vivo*; increased airway inflammation and hyperresponsiveness has also been

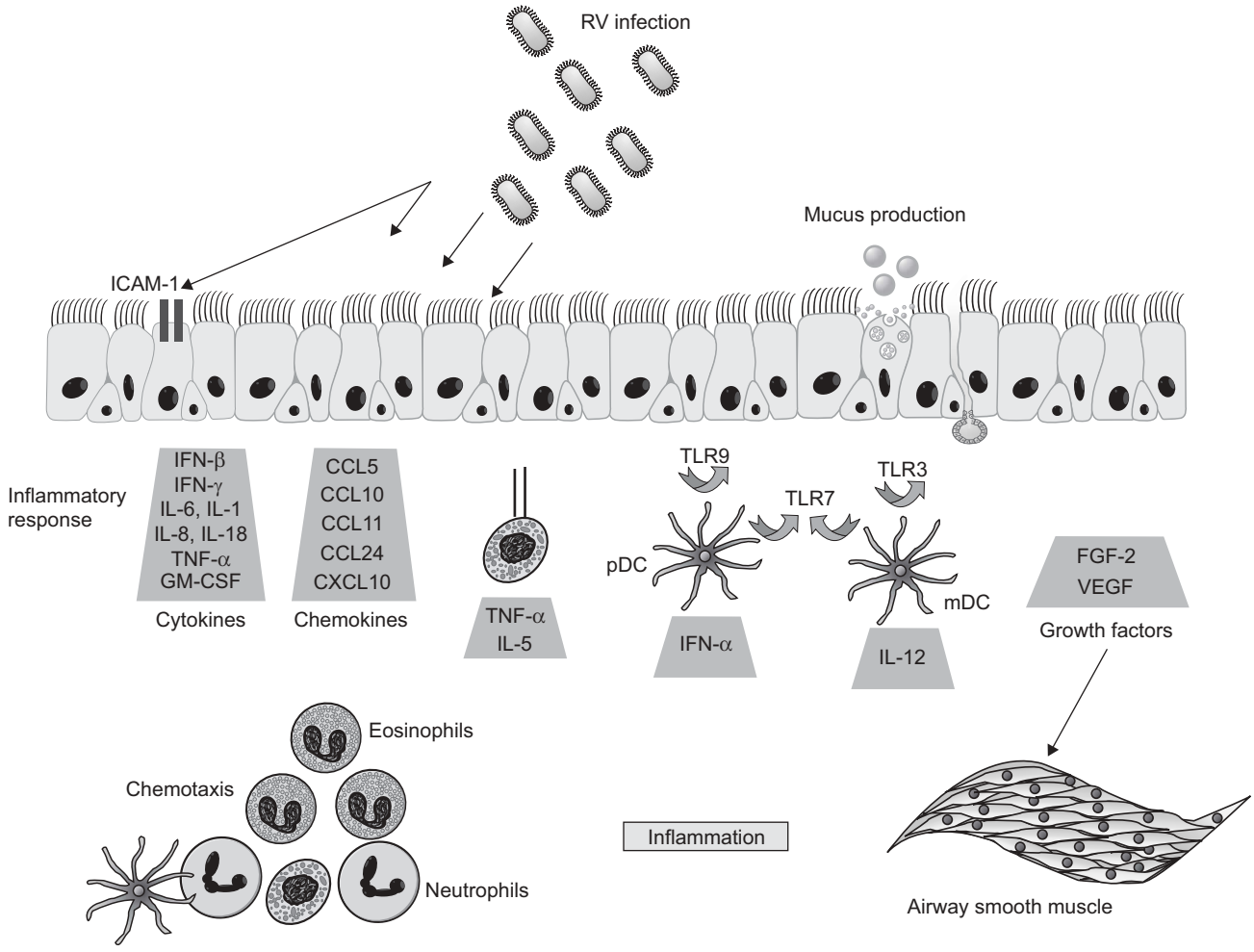

FIGURE 1. Overview of the mechanisms involved in rhinovirus (RV)-induced asthma exacerbations. A variety of mediators produced by epithelial and immune cells following a viral infection result in an inflammatory response, including chemotaxis of neutrophils and eosinophils. An antiviral response is also mounted by both epithelial and dendritic cells, producing interferons (IFNs). In parallel, a number of epithelial-derived growth factors may contribute to airway remodelling. ICAM: intercellular adhesion molecule; IL: interleukin; TNF-α: tumour necrosis factor-α; GM-CSF: granulocyte-macrophage colony-stimulating factor; CCL: CC chemokine ligand; CCL5: RANTES; CXCL: CXC chemokine ligand; CL11: eotaxin; CCL24: eotaxin-2; CXCL10: IFN-γ-inducible protein 10; TLR: Toll-like receptor; pDC: plasmacytoid dendritic cell; mDC: myeloid dendritic cell; FGF: fibroblast growth factor; VEGF: vascular endothelial growth factor.

documented. Phosphatidylinositol 3-kinase/Akt kinase signalling was required for maximal RV1B-induced neutrophilic inflammation, probably *via* its essential role in virus internalisation [74].

Innate immune responses, and in particular IFNs, seem to play a major role in RV-induced asthma exacerbations. Type-I IFNs (α/β) are important components of the innate immune response, inducing an antiviral state in infected and nearby cells, *via* induction of apoptosis and proteins with direct antiviral activity [75, 76]. More recently, a novel class of IFNs has been discovered, named type-III IFNs, including IFN-λ1 and IFN-λ2/3 [77], with a potentially immunoprotective role in the lower airways [78].

In vitro studies have shown that asthmatic bronchial cells are profoundly deficient at producing RV-induced IFN-β, whereas exogenous IFN-β induced apoptosis of RV-infected asthmatic bronchial epithelial cells (BECs) and restoration of innate immunity by inhibition of RV replication, as observed in normal BECs [79].

It has been suggested that defective innate immune responses result in deficient adaptive type-1 T-helper cell (Th) responses. In support of this, peripheral blood mononuclear cells (PBMCs) from asthmatics have been shown to exhibit a deficient IFN-γ response to RV; moreover, RV-stimulated PBMCs from atopic asthmatics resulted in reduced production of IFN-γ and increased IL-4 and IL-10 compared to normal controls [79, 80]. An inverse correlation between IFN-γ production and viral load and/or symptoms score has been documented [81]. In the same context, PBMCs from asthmatic patients were reported to produce less IFN-α2 than PBMCs from normal controls in response to RSV and Newcastle virus infection, also implicating IFN-α in the pathogenesis of asthma exacerbations [82].

With respect to type-III IFNs, it has been documented that RV induces IFN-λ production in a suboptimal way, in both

primary BECs and macrophages from atopic asthmatic individuals. The deficient type-III IFN production strongly correlated with viral replication *in vitro*. Subsequent experimental infection with RV-16 resulted in deficient induction of IFN-λ in bronchoalveolar lavage cells from asthmatics; such induction was strongly inversely associated with severity of clinical illness, reductions in lung function and increased airway hyperresponsiveness [83].

Both type-I and type-III IFNs are induced by RV and influenza infection in BECs. IFN-λ appears to be the most sustained and abundant IFN subtype induced in the lung by RV infections, whereas, in PBMCs, all IFNs were induced to a similar degree. It has been suggested that IFN-λ could serve as a candidate therapy for asthma exacerbations, and possibly other viral diseases in which BECs are the major site of virus replication [84].

Despite the importance of viral infections in asthma exacerbation pathogenesis, a combination of interacting factors is likely to be involved. Recent studies have emphasised the importance of atopy and allergic inflammation in the induction and perpetuation of virus-induced respiratory diseases [85, 86]. Allergens and respiratory viruses act synergistically in the expression of asthma symptoms in adults and children [13, 33]. RV-induced epithelial cytotoxicity could increase the penetration and effects of allergens, leading to further induction of inflammation [87, 88]. Synergy between virus and mite (*Dermatophagoides pteronyssinus* antigen 1)-induced inflammation may also occur through combined nuclear factor-κB activation [89]. In primary BECs, RV infection results in efficient viral replication, leading to cell lysis, which is greater in cells from atopic asthmatics than from healthy controls. Such a response could be attributed to the impaired induction of early apoptosis found in the asthmatic cells, probably due to reduced levels of IFN-β [79]. In line with these observations, it has been shown that, under the influence of an atopic immune response, the epithelial inflammatory response to RV becomes suboptimal with respect to cytokines and chemokines, such as IL-6, IL-8 and RANTES. Such a response is associated with inadequate viral clearance and increased RV-induced cytotoxicity [90]. However, the timing of the infection might play a significant role, since simultaneous infection and allergen exposure significantly increase the severity of asthma exacerbations in asthmatics, whereas allergen exposure prior to infection has minimal or no effect on symptoms or airway hyperresponsivess [33, 91].

In allergen-sensitised and -challenged mice, RV infection exacerbates neutrophilic, eosinophilic and lymphocytic airway inflammation and hyperresponsiveness, mucus secretion and production of both Th1 and Th2 cytokines. Such responses are consistent with the notion that allergic inflammation substantially exacerbates RV-induced inflammation [73].

From another perspective, RV infection has been shown to promote endothelial cell proliferation and differentiation though induction of angiogenetic factors, such as vascular endothelial growth factor (VEGF), possibly contributing to airway remodelling in asthma; this response was further augmented in an atopic host environment [92]. Similar responses were observed for other profibrotic factors, such as transforming growth factor-β [90] and fibroblast growth factor 2 [93]. These data indicate a mechanism through which viral respiratory infections may promote airway remodelling and the chronic asthma phenotype in atopic asthmatic individuals.

TREATMENT

Despite the impact of virus-induced asthma exacerbations, no specific antiviral treatment able to modify the clinical outcome of the viral infection exists. Pharmacological interventions shown to be relatively successful at preventing asthma exacerbations include inhaled corticosteroids and their combination with long lasting β_2-agonists (mostly in adults), leukotriene modifiers and anti-immunoglobulin E therapy [8, 94–97]. Corticosteroids suppress eosinophilic inflammation through various pathways [98, 99]. However, the inability of corticosteroids to modulate several other aspects of the immune response to respiratory viruses that lead to asthma exacerbations make them not as effective at preventing or treating virus-induced asthma exacerbations in different age groups [100]. Leukotriene receptor antagonists, used either prophylactically or therapeutically, exhibit a modest effect on virus-induced asthma exacerbations in children [94, 101, 102]. In conclusion, treating virus-induced asthma exacerbations is far from satisfactory, despite current knowledge regarding the underlying pathogenesis [103].

The antigenic diversity of RVs makes the development of a vaccine rather unlikely. Modulation of the host response to virus infection is a potential approach to therapy; taking into account the impaired IFN production in asthmatics, IFN supplementation could be a targeted treatment for asthma exacerbations [6]. Experimental data from *in vitro* models have shown that the administration of exogenous IFN-β induced apoptosis and suppressed viral replication, suggesting the potential for therapeutic intervention [79]. Attempts with intranasal IFNs (IFN-α2 and IFN-β-serine) have not found their way to the clinic to date, due to either reduced efficacy or severe side-effects [104, 105]. Pleconaril is an agent with broad-spectrum activity against RVs, which has been developed as an oral preparation to be taken twice daily. Although modest efficacy against natural colds has been verified in phase-II clinical trials, concerns have been raised about the development of resistant RV mutants [106, 107]. At this stage, there are no clinical data regarding the efficacy of pleconaril in acute asthma exacerbations.

Macrolides and ketolides, beside their antimicrobial action, possess important immune-modulating effects [108–110]. A multicentric double-blind randomised placebo-controlled study assessed the efficacy of oral telithromycin for 10 days, as a supplement to standard treatment for adults with an acute asthma exacerbation. Significant improvements in both symptoms and lung function were shown in the treatment group, and the effect did not seem to depend crucially upon the presence of atypical bacteria [111]. Further work-up is essential in order to identify patient groups likely to benefit from similar treatment approaches among children [112]. Such a study of clarithromycin in acute exacerbations of asthma in school-age children is underway (data not shown). With respect to RSV-induced lower respiratory tract diseases, recent data indicate lack of efficacy of azithromycin in hospitalised children aged $<$24 months [113].

A variety of novel options for the prevention and treatment of virus-induced asthma, such as antioxidants, VEGF and angiogenesis inhibitors, and more, need to be further assessed for their efficacy and applicability.

CONCLUSIONS

Viral infections are the major precipitant of asthma exacerbations, leading to a complex of inflammatory processes. Although knowledge of the mechanism underlying infection-induced asthma exacerbations has increased substantially since the late 1990s, a great deal of further work is still clearly warranted. Moreover, the interactions between viruses, other pathogens, air pollution, and allergen sensitisation and exposure are incompletely understood. Identification of the mechanisms involved in the pathogenesis of asthma exacerbations should facilitate development of future treatments tailored to the underlying cause.

STATEMENT OF INTEREST

None declared.

REFERENCES

1 Simpson A, Custovic A. Prevention of allergic sensitization by environmental control. *Curr Allergy Asthma Rep* 2009; 9: 363–369.
2 Johnston SL. Overview of virus-induced airway disease. Proc Am Thorac Soc 2005; 2: 150–156.
3 Taylor DR, Bateman ED, Boulet LP, *et al.* A new perspective on concepts of asthma severity and control. *Eur Respir J* 2008; 32: 545–554.
4 Reddel HK, Taylor DR, Bateman ED, *et al.* An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations. Standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180: 59–99.
5 Reddel H, Ware S, Marks G, *et al.* Differences between asthma exacerbations and poor asthma control. *Lancet* 1999; 353: 364–369.
6 Sykes A, Seemungal T. Recent advances in exacerbations of asthma. *Thorax* 2008; 63: 758–760.
7 National Heart, Lung, and Blood Institute, National Asthma Education and Prevention Program, Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. Full Report 2007. Bethesda, US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, 2007.
8 Pauwels RA, Lofdahl CG, Postma DS, *et al.* Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 1997; 337: 1405–1411.
9 Chipps BE, Murphy KR. Assessment and treatment of acute asthma in children. *J Pediatr* 2005; 147: 288–294.
10 Sears MR. Epidemiology of asthma exacerbations. *J Allergy Clin Immunol* 2008; 122: 662–668.
11 Johnston SL, Pattemore PK, Sanderson G, *et al.* Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; 310: 1225–1229.
12 Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; 307: 982–986.
13 Murray CS, Poletti G, Kebadze T, *et al.* Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006; 61: 376–382.
14 Bacharier LB, Boner A, Carlsen KH, *et al.* Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. *Allergy* 2008; 63: 5–34.
15 Brand PL, Baraldi E, Bisgaard H, *et al.* Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008; 32: 1096–1110.
16 Illi S, von Mutius E, Lau S, *et al.* Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006; 368: 763–770.
17 Tan WC. Viruses in asthma exacerbations. *Curr Opin Pulm Med* 2005; 11: 21–26.
18 Atmar RL, Guy E, Guntupalli KK, *et al.* Respiratory tract viral infections in inner-city asthmatic adults. *Arch Intern Med* 1998; 158: 2453–2459.
19 Khetsuriani N, Kazerouni NN, Erdman DD, *et al.* Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol* 2007; 119: 314–321.
20 Johnston NW, Johnston SL, Norman GR, *et al.* The September epidemic of asthma hospitalization: school children as disease vectors. *J Allergy Clin Immunol* 2006; 117: 557–562.
21 Almqvist C, Wickman M, Perfetti L, *et al.* Worsening of asthma in children allergic to cats, after indirect exposure to cat at school. *Am J Respir Crit Care Med* 2001; 163: 694–698.
22 Pendergraft TB, Stanford RH, Beasley R, *et al.* Seasonal variation in asthma-related hospital and intensive care unit admissions. *J Asthma* 2005; 42: 265–271.
23 Bartlett NW, McLean GR, Chang YS, *et al.* Genetics and epidemiology: asthma and infection. *Curr Opin Allergy Clin Immunol* 2009; 9: 395–400.
24 Miller BD, Wood BL, Lim J, *et al.* Depressed children with asthma evidence increased airway resistance: ''vagal bias'' as a mechanism? *J Allergy Clin Immunol* 2009; 124: 66–73.e10.
25 Abe T, Tokuda Y, Ohde S, *et al.* The relationship of short-term air pollution and weather to ED visits for asthma in Japan. *Am J Emerg Med* 2009; 27: 153–159.
26 Eggleston PA. Complex interactions of pollutant and allergen exposures and their impact on people with asthma. *Pediatrics* 2009; 123: Suppl. 3, S160–S167.
27 Mohr LB, Luo S, Mathias E, *et al.* Influence of season and temperature on the relationship of elemental carbon air pollution to pediatric asthma emergency room visits. *J Asthma* 2008; 45: 936–943.
28 Covar RA, Szefler SJ, Zeiger RS, *et al.* Factors associated with asthma exacerbations during a long-term clinical trial of controller medications in children. *J Allergy Clin Immunol* 2008; 122: 741–747.e4.
29 Dales RE, Cakmak S, Judek S, *et al.* Tree pollen and hospitalization for asthma in urban Canada. *Int Arch Allergy Immunol* 2008; 146: 241–247.
30 Heguy L, Garneau M, Goldberg MS, *et al.* Associations between grass and weed pollen and emergency department visits for asthma among children in Montreal. *Environ Res* 2008; 106: 203–211.
31 Peden DB. Pollutants and asthma: role of air toxics. *Environ Health Perspect* 2002; 110: Suppl. 4, 565–568.
32 Chauhan AJ, Inskip HM, Linaker CH, *et al.* Personal exposure to nitrogen dioxide (NO_2) and the severity of virus-induced asthma in children. *Lancet* 2003; 361: 1939–1944.
33 Green RM, Custovic A, Sanderson G, *et al.* Synergism between allergens and viruses and risk of hospital admission with asthma: case–control study. *BMJ* 2002; 324: 763.
34 Sandberg S, Jarvenpaa S, Penttinen A, *et al.* Asthma exacerbations in children immediately following stressful life events: a Cox's hierarchical regression. *Thorax* 2004; 59: 1046–1051.
35 Singh AM, Busse WW. Asthma exacerbations. 2: aetiology. *Thorax* 2006; 61: 809–816.
36 Matthew J, Pinto Pereira LM, Pappas TE, *et al.* Distribution and seasonality of rhinovirus and other respiratory viruses in a cross-section of asthmatic children in Trinidad, West Indies. *Ital J Pediatr* 2009; 35: 16.

37 Cheuk DK, Tang IW, Chan KH, *et al.* Rhinovirus infection in hospitalized children in Hong Kong: a prospective study. *Pediatr Infect Dis J* 2007; 26: 995–1000.
38 Calvo C, Garcia-Garcia ML, Blanco C, *et al.* Role of rhinovirus in hospitalized infants with respiratory tract infections in Spain. *Pediatr Infect Dis J* 2007; 26: 904–908.
39 Xepapadaki P, Papadopoulos NG. Viral infections and allergies. *Immunobiology* 2007; 212: 453–459.
40 Williams JV, Harris PA, Tollefson SJ, *et al.* Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004; 350: 443–450.
41 Foulongne V, Guyon G, Rodiere M, *et al.* Human metapneumovirus infection in young children hospitalized with respiratory tract disease. *Pediatr Infect Dis J* 2006; 25: 354–359.
42 Vallet C, Pons-Catalano C, Mandelcwajg A, *et al.* Human bocavirus: a cause of severe asthma exacerbation in children. *J Pediatr* 2009; 155: 286–288.
43 Papadopoulos NG. Do rhinoviruses cause pneumonia in children? *Paediatr Respir Rev* 2004; 5: Suppl. A, S191–S195.
44 Papadopoulos NG, Moustaki M, Tsolia M, *et al.* Association of rhinovirus infection with increased disease severity in acute bronchiolitis. *Am J Respir Crit Care Med* 2002; 165: 1285–1289.
45 Jackson DJ, Gangnon RE, Evans MD, *et al.* Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008; 178: 667–672.
46 Lemanske RF Jr, Jackson DJ, Gangnon RE, *et al.* Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005; 116: 571–577.
47 Lee WM, Kiesner C, Pappas T, *et al.* A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. *PLoS One* 2007; 2: e966.
48 Renwick N, Schweiger B, Kapoor V, *et al.* A recently identified rhinovirus genotype is associated with severe respiratory-tract infection in children in Germany. *J Infect Dis* 2007; 196: 1754–1760.
49 Lau SK, Yip CC, Tsoi HW, *et al.* Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. *J Clin Microbiol* 2007; 45: 3655–3664.
50 Miller EK, Edwards KM, Weinberg GA, *et al.* A novel group of rhinoviruses is associated with asthma hospitalizations. *J Allergy Clin Immunol* 2009; 123: 98–104.e1.
51 Linsuwanon P, Payungporn S, Samransamruajkit R, *et al.* High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. *J Infect* 2009; 59: 115–121.
52 Peltola V, Jartti T, Putto-Laurila A, *et al.* Rhinovirus infections in children: a retrospective and prospective hospital-based study. *J Med Virol* 2009; 81: 1831–1838.
53 Lau SK, Yip CC, Lin AW, *et al.* Clinical and molecular epidemiology of human rhinovirus C in children and adults in Hong Kong reveals a possible distinct human rhinovirus C subgroup. *J Infect Dis* 2009; 200: 1096–1103.
54 Xepapadaki P, Papadopoulos NG. Atypical bacteria and macrolides in asthma. *Allergy Asthma Clin Immunol* 2008; 4: 111–116.
55 Biscardi S, Lorrot M, Marc E, *et al. Mycoplasma pneumoniae* and asthma in children. *Clin Infect Dis* 2004; 38: 1341–1346.
56 Cosentini R, Tarsia P, Canetta C, *et al.* Severe asthma exacerbation: role of acute *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* infection. *Respir Res* 2008; 9: 48.
57 Wu Q, Martin RJ, Lafasto S, *et al.* Toll-like receptor 2 down-regulation in established mouse allergic lungs contributes to decreased mycoplasma clearance. *Am J Respir Crit Care Med* 2008; 177: 720–729.
58 Kraft M, Adler KB, Ingram JL, *et al. Mycoplasma pneumoniae* induces airway epithelial cell expression of MUC5AC in asthma. *Eur Respir J* 2008; 31: 43–46.
59 Papadopoulos NG, Xepapadaki P, Mallia P, *et al.* Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA^{2}LEN and InterAirways document. *Allergy* 2007; 62: 457–470.
60 Dreschers S, Dumitru CA, Adams C, *et al.* The cold case: are rhinoviruses perfectly adapted pathogens? *Cell Mol Life Sci* 2007; 64: 181–191.
61 Papadopoulos NG, Bates PJ, Bardin PG, *et al.* Rhinoviruses infect the lower airways. *J Infect Dis* 2000; 181: 1875–1884.
62 Papadopoulos NG, Papi A, Meyer J, *et al.* Rhinovirus infection up-regulates eotaxin and eotaxin-2 expression in bronchial epithelial cells. *Clin Exp Allergy* 2001; 31: 1060–1066.
63 Schroth MK, Grimm E, Frindt P, *et al.* Rhinovirus replication causes RANTES production in primary bronchial epithelial cells. *Am J Respir Cell Mol Biol* 1999; 20: 1220–1228.
64 Kelly JT, Busse WW. Host immune responses to rhinovirus: mechanisms in asthma. *J Allergy Clin Immunol* 2008; 122: 671–682.
65 Xatzipsalti M, Kyrana S, Tsolia M, *et al.* Rhinovirus viremia in children with respiratory infections. *Am J Respir Crit Care Med* 2005; 172: 1037–1040.
66 Papadopoulos NG, Papi A, Psarras S, *et al.* Mechanisms of rhinovirus-induced asthma. *Paediatr Respir Rev* 2004; 5: 255–260.
67 Wiehler S, Proud D. Interleukin-17A modulates human airway epithelial responses to human rhinovirus infection. *Am J Physiol Lung Cell Mol Physiol* 2007; 293: L505–L515.
68 Wark PA, Johnston SL, Moric I, *et al.* Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. *Eur Respir J* 2002; 19: 68–75.
69 Pizzichini MM, Pizzichini E, Efthimiadis A, *et al.* Asthma and natural colds. Inflammatory indices in induced sputum: a feasibility study. *Am J Respir Crit Care Med* 1998; 158: 1178–1184.
70 Fraenkel DJ, Bardin PG, Sanderson G, *et al.* Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995; 151: 879–886.
71 Wark PA, Bucchieri F, Johnston SL, *et al.* IFN-γ-induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations. *J Allergy Clin Immunol* 2007; 120: 586–593.
72 Tuthill TJ, Papadopoulos NG, Jourdan P, *et al.* Mouse respiratory epithelial cells support efficient replication of human rhinovirus. *J Gen Virol* 2003; 84: 2829–2836.
73 Bartlett NW, Walton RP, Edwards MR, *et al.* Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med* 2008; 14: 199–204.
74 Newcomb DC, Sajjan US, Nagarkar DR, *et al.* Human rhinovirus 1B exposure induces phosphatidylinositol 3-kinase-dependent airway inflammation in mice. *Am J Respir Crit Care Med* 2008; 177: 1111–1121.
75 Johnston SL. Innate immunity in the pathogenesis of virus-induced asthma exacerbations. *Proc Am Thorac Soc* 2007; 4: 267–270.
76 Takaoka A, Hayakawa S, Yanai H, *et al.* Integration of interferon-α/β signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424: 516–523.
77 Kotenko SV, Gallagher G, Baurin VV, *et al.* IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex. *Nat Immunol* 2003; 4: 69–77.
78 Bullens DM, Decraene A, Dilissen E, *et al.* Type III IFN-λ mRNA expression in sputum of adult and school-aged asthmatics. *Clin Exp Allergy* 2008; 38: 1459–1467.
79 Wark PA, Johnston SL, Bucchieri F, *et al.* Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; 201: 937–947.
80 Papadopoulos NG, Stanciu LA, Papi A, *et al.* A defective type 1 response to rhinovirus in atopic asthma. *Thorax* 2002; 57: 328–332.
81 Gern JE, Vrtis R, Grindle KA, *et al.* Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *Am J Respir Crit Care Med* 2000; 162: 2226–2231.

82 Gehlhar K, Bilitewski C, Reinitz-Rademacher K, *et al.* Impaired virus-induced interferon-α2 release in adult asthmatic patients. *Clin Exp Allergy* 2006; 36: 331–337.

83 Contoli M, Message SD, Laza-Stanca V, *et al.* Role of deficient type III interferon-λ production in asthma exacerbations. *Nat Med* 2006; 12: 1023–1026.

84 Khaitov MR, Laza-Stanca V, Edwards MR, *et al.* Respiratory virus induction of alpha-, beta- and lambda-interferons in bronchial epithelial cells and peripheral blood mononuclear cells. *Allergy* 2009; 64: 375–386.

85 Xepapadaki P, Papadopoulos NG, Bossios A, *et al.* Duration of postviral airway hyperresponsiveness in children with asthma: effect of atopy. *J Allergy Clin Immunol* 2005; 116: 299–304.

86 Heymann PW, Platts-Mills TA, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. *Pediatr Infect Dis J* 2005; 24: S217–S222.

87 Bossios A, Psarras S, Gourgiotis D, *et al.* Rhinovirus infection induces cytotoxicity and delays wound healing in bronchial epithelial cells. *Respir Res* 2005; 6: 114.

88 Hansbro NG, Horvat JC, Wark PA, *et al.* Understanding the mechanisms of viral induced asthma: new therapeutic directions. *Pharmacol Ther* 2008; 117: 313–353.

89 Bossios A, Gourgiotis D, Skevaki CL, *et al.* Rhinovirus infection and house dust mite exposure synergize in inducing bronchial epithelial cell interleukin-8 release. *Clin Exp Allergy* 2008; 38: 1615–1626.

90 Xatzipsalti M, Psarros F, Konstantinou G, *et al.* Modulation of the epithelial inflammatory response to rhinovirus in an atopic environment. *Clin Exp Allergy* 2008; 38: 466–472.

91 de Kluijver J, Grunberg K, Sont JK, *et al.* Rhinovirus infection in nonasthmatic subjects: effects on intrapulmonary airways. *Eur Respir J* 2002; 20: 274–279.

92 Psarras S, Volonaki E, Skevaki CL, *et al.* Vascular endothelial growth factor-mediated induction of angiogenesis by human rhinoviruses. *J Allergy Clin Immunol* 2006; 117: 291–297.

93 Skevaki C, Psarras S, Volonaki E, *et al.* Human rhinovirus induction of fibroblast production by bronchial epithelial cells mediates stroma cell proliferation: association with airway remodelling in asthma. *Allergy* 2008; 63: Suppl. 88, 59–60.

94 Johnston NW, Mandhane PJ, Dai J, *et al.* Attenuation of the September epidemic of asthma exacerbations in children: a randomized, controlled trial of montelukast added to usual therapy. *Pediatrics* 2007; 120: e702–e712.

95 Sorkness CA, Lemanske RF Jr, Mauger DT, *et al.* Long-term comparison of 3 controller regimens for mild–moderate persistent childhood asthma: the Pediatric Asthma Controller Trial. *J Allergy Clin Immunol* 2007; 119: 64–72.

96 Rees PJ. Review: omalizumab reduces exacerbation and steroid use in chronic asthma. *Evid Based Med* 2007; 12: 11.

97 Papi A, Canonica GW, Maestrelli P, *et al.* Rescue use of beclomethasone and albuterol in a single inhaler for mild asthma. *N Engl J Med* 2007; 356: 2040–2052.

98 Edwards MR, Haas J, Panettieri RA Jr, *et al.* Corticosteroids and β_2 agonists differentially regulate rhinovirus-induced interleukin-6 *via* distinct cis-acting elements. *J Biol Chem* 2007; 282: 15366–15375.

99 Pelaia G, Vatrella A, Cuda G, *et al.* Molecular mechanisms of corticosteroid actions in chronic inflammatory airway diseases. *Life Sci* 2003; 72: 1549–1561.

100 Bisgaard H, Hermansen MN, Loland L, *et al.* Intermittent inhaled corticosteroids in infants with episodic wheezing. *N Engl J Med* 2006; 354: 1998–2005.

101 Bisgaard H, Zielen S, Garcia-Garcia ML, *et al.* Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. *Am J Respir Crit Care Med* 2005; 171: 315–322.

102 Robertson CF, Price D, Henry R, *et al.* Short-course montelukast for intermittent asthma in children: a randomized controlled trial. *Am J Respir Crit Care Med* 2007; 175: 323–329.

103 Bacharier LB, Phillips BR, Zeiger RS, *et al.* Episodic use of an inhaled corticosteroid or leukotriene receptor antagonist in preschool children with moderate-to-severe intermittent wheezing. *J Allergy Clin Immunol* 2008; 122: 1127–1135.e8.

104 Sperber SJ, Levine PA, Sorrentino JV, *et al.* Ineffectiveness of recombinant interferon-β serine nasal drops for prophylaxis of natural colds. *J Infect Dis* 1989; 160: 700–705.

105 Farr BM, Gwaltney JM Jr, Adams KF, *et al.* Intranasal interferon-α2 for prevention of natural rhinovirus colds. *Antimicrob Agents Chemother* 1984; 26: 31–34.

106 Hayden FG, Herrington DT, Coats TL, *et al.* Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. *Clin Infect Dis* 2003; 36: 1523–1532.

107 Pevear DC, Hayden FG, Demenczuk TM, *et al.* Relationship of pleconaril susceptibility and clinical outcomes in treatment of common colds caused by rhinoviruses. *Antimicrob Agents Chemother* 2005; 49: 4492–4499.

108 Gotfried MH. Macrolides for the treatment of chronic sinusitis, asthma, and COPD. *Chest* 2004; 125: 52S–60S.

109 Shinkai M, Rubin BK. Macrolides and airway inflammation in children. *Paediatr Respir Rev* 2005; 6: 227–235.

110 Johnston SL, Martin RJ. *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*: a role in asthma pathogenesis? *Am J Respir Crit Care Med* 2005; 172: 1078–1089.

111 Johnston SL, Blasi F, Black PN, *et al.* The effect of telithromycin in acute exacerbations of asthma. *N Engl J Med* 2006; 354: 1589–1600.

112 Little FF. Treating acute asthma with antibiotics – not quite yet. *N Engl J Med* 2006; 354: 1632–1634.

113 Kneyber MC, van Woensel JB, Uijtendaal E, *et al.* Azithromycin does not improve disease course in hospitalized infants with respiratory syncytial virus (RSV) lower respiratory tract disease: a randomized equivalence trial. *Pediatr Pulmonol* 2008; 43: 142–149.