

DR. KATARINA STENBERG HAMMAR (Orcid ID : 0000-0002-5185-8280)

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Rhinovirus and pre-school wheeze

Review

Katarina Stenberg-Hammar^{1,2}, Gunilla Hedlin^{1,2}, Cilla Söderhäll^{1,3}

¹Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden;

²Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden;

³Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden;

For all: Centre of Allergy Research, Karolinska Institutet, Stockholm, Sweden;

Corresponding author mail id : katarina.stenberg-hammar@sll.se

ABSTRACT

Rhinovirus (RV) known as the common cold virus generally only causes a mild upper respiratory infection, but severe lower respiratory symptoms have been associated with RV infections especially in asthmatic individuals. Wheezing is a symptom of airway obstruction, and preschool children wheezing with RV have been associated with increased risk of asthma at school age. There are however conflicting opinions as to whether there are differences in response to RV infection or if wheezing with RV reveals a preexisting impairment that promotes asthma mainly in predisposed children. The advent of molecular diagnostics to detect respiratory viruses has led to new insights into the role of RV infections. This review

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will discuss recent information concerning the role of RV as an important respiratory pathogen related to early onset wheeze and exacerbation of established asthma in preschool children.

INTRODUCTION

Rhinovirus infection can range from asymptomatic, mild symptoms from the upper respiratory tract to severe symptoms from the lower respiratory tract (1), and has been identified as an important contributor to wheezing illness in preschool children (2). RV is associated both with the initial development of airway inflammation (3), as well as exacerbating pre-existing airway diseases such as asthma (4). There is a debate whether the number of respiratory episodes in the first years of life is associated with later asthma development independent of the viral trigger (5), if some species or types of RV are more asthma genic (6, 7) able to evoke distinct host immune responses and initiate asthma, or if wheezing with RV only reveal a preexisting impairment and promote asthma mainly in predisposed children (8). In this review, specific host factors that could contribute to the initiation of wheeze and asthma in preschool children as well as specific characteristics of RV infections that contribute to the severity of illness will be discussed.

PRE-SCHOOL WHEEZE

Definition and treatment of wheeze in pre-school children

Wheeze, a high-pitched whistling sound while breathing, as a symptom of airway obstruction is highly prevalent in preschool children aged 5 years or younger (9). The underlying process of wheeze includes inflammation of the airways, mucus production and reversible tightening of the smooth muscles in the airway walls. The vibration of opposing walls in the narrowed

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airways, causing dyspnea and cough, produces the whistling sound. Almost 50% of all children have reported at least one episode of wheeze before the age of 6 years (10). Viral infections are the most frequent cause behind the wheezing symptom. In recent reports on preschool children presenting at the hospital with acute wheeze, RV was detected in almost 70% of them (11, 12). Despite its high prevalence, there is a lack of evidence and mixed results regarding the pharmacological treatment (13). For symptomatic relief, inhaled short-acting β 2-agonists by spacer are recommended, inhaled corticosteroids (ICS) and leukotriene receptor antagonists may be considered daily or intermittently (13). In preschool children where persistent asthma is suspected, daily ICS has been proven superior to intermittent ICS with regard to lung function, airway inflammation, asthma control and reliever use (14).

Environmental, hereditary and host factors related to risk of wheeze

Well-known environmental and hereditary risk factors for non-atopic wheeze include maternal smoking during pregnancy, limited breastfeeding time, childcare attendance after the age of one year and allergy/asthma in mother and/or father (15). Young age is a known host factor that contribute to viral induced wheezing illness. The prevalence of wheezing is highest during the first year of life (18–32% reported) and then drops to around 10% in the second and third year of life (16). In addition, small airways (17), early sensitization and specific characteristics of the immune system such as low levels of IFN-gamma (9, 18) have been identified as a risk factor for wheezing illness. Also low levels of vitamin D, important for the modulation of the immune system (19) and lung function (20) have been associated with increased susceptibility to infection and the development of asthma and atopy (21). Children with wheeze have shown lower levels of 25-OH-vitamin D compared to healthy controls in several studies, independent of atopy status (20-22).

In a longitudinal birth cohort investigating the relationship between childhood wheeze and atopy/sensitization to food or aero-allergens, 37% of children without allergic sensitization had wheezing illness, related to maternal asthma, parental smoking, and respiratory tract infections (23). In the group of children with atopy, 65% reported wheezing episodes and this phenotype was associated with male sex, early eczema, family history of eczema, and early tobacco exposure. In children with non-atopic asthma, three risk factors were consistent: family history of asthma/rhinitis/eczema, dampness/mold in the household, and lower respiratory tract infections during early childhood (15).

Wheezing phenotypes

Traditionally, in retrospective birth cohorts children with wheeze have been classified into four phenotypes: non-wheezers, early transient wheezers, late-onset wheezers, and persistent wheezers (Fig. 1) (24). Savenije, et al suggested two additional phenotypes: intermediate-onset wheeze with onset of wheeze after 2 years of age, and prolonged early wheeze (Fig. 1) (24, 25). The intermediate-onset, late-onset, and persistent wheezing phenotypes have the strongest associations with atopy, while the transient and prolonged early wheeze are associated with reduced lung function (25). However, in clinical practice, the retrospective wheezing classifications are not applicable in finding a pre-asthma wheezing phenotype since there is a large overlap between subgroups, and patients may also shift from one phenotype to another over time (26). In contrast to the retrospective birth-cohort studies, Lukkarinen et al. suggest a focus on the first wheezing episode which could predict later asthma phenotypes: Sensitization, eczema and rhinovirus in combination at the first severe wheezing episode predict atopic asthma at school-age, whereas respiratory syncytial virus (RSV)-/RV-negative etiology, age younger than 12 months and parental smoking predict non-atopic asthma (27).

One meta-analysis on children with non-atopic wheeze and the relationship with RV infection and asthma, suggests an association between RV-induced wheezing in the first 3 years of life and the subsequent development of wheezing/asthma. (Fig. 2) (28).

RHINOVIRUSES

Rhinoviruses (RV) are non-enveloped single stranded RNA viruses belonging to the genus Enterovirus, family *Picornaviridae*. They are the most common cause of both upper and lower respiratory tract infection in all ages worldwide. To date, there are three recognized species: RV-A, RV-B, and RV-C. RV-A and RV-B, discovered already during the 1950's, were initially classified based on antigenic properties and the term "serotype" was used for different RV variants (29). The third species, RV-C, has been refractive to growth in standard tissue culture, and it was not until 2006 that the RV-C was identified with molecular diagnostics (30). Since then almost 60 new RV-C types have been discovered (31). Today, the classification scheme for RV is based on genome arrangement, capsid properties and conserved sequences and type number is used for different RV variants and follows the species letter (A, B or C) (32). RV-A and RV-B are also classified in major or minor groups depending on the receptor used to bind to human respiratory epithelial cells. Major group RVs (both RV-A and RV-B) bind to the intercellular adhesion molecule 1 (ICAM-1) receptor and minor group (only RV-A) bind to the low-density lipoprotein receptor (LDL-R) (29, 33). The receptor for RV-C has recently been identified as the cadherin related family-member 3, CDHR3 (34). Most RV grow best in temperatures between 33–35°C (as in the upper respiratory tract), but some grow equally well at 37°C (as in the lower respiratory tract) and might be the cause of more severe respiratory symptoms involved in asthma exacerbations, as well as acute bronchiolitis and wheeze in young children (35, 36).

Epidemiology of RV

RV infections are very common in the general population, especially during spring and fall. The incidence is highest in young children (37, 38) and over 90% of children have antibodies against RV at the age of two years (39). In a study on healthy children <2 years old investigated weekly during November until February, the presence of RV was identified with a mean number of 3.7 +/- 2.3 infections per child (40). Most of the RV infections (64%) remained asymptomatic or caused only mild symptoms (40). In a birth cohort including infants with wheeze, RV infections were most frequent in the Spring and Autumn but were more likely to cause severe illnesses in Winter (December to February) (37), indicating other factors than viral load to be of importance in the initiation of RV wheezing illness. In children seeking hospital with lower respiratory tract infections and wheeze, both RV-A and RV-C have been found to be over-represented (2, 37).

RV pathophysiology

The pathophysiology of RV infection has some unique characteristics and the molecular and cellular mechanisms are not fully understood, and research results are sometimes contradicting (41). The primary site of inoculation is the nasal mucosa and to a lesser degree ciliated epithelial cells of the airways (41). The RV infection involves only a small portion of the epithelium causing histamine release and nasal discharge. The virus does not cause any destruction of airway epithelial cells, but disrupts the tight junctions between cells destroying the epithelial barrier (42). Gaps within epithelial layers allow cytokines, immune cells and further viruses and bacteria to penetrate deeper into the airways, causing a massive upregulation of inflammatory mediators in the host. RV infections are often described as a “cytokine disease” (43) causing a neutrophilic inflammatory response, increased vascular permeability and mucus hypersecretion (44). Symptoms are believed to be mediated by the

innate immune response to RV, or enhance an already ongoing IgE-dependent immune response (44) suggesting the symptoms depend on an exaggerated host immune response, not the pathology of the RV (41). It has earlier been suggested that increased viral loads lead to airway inflammation and asthma development (45) but lately it has been proven that the viral titers in asthmatic and non-asthmatic children are the same (4). The viral load drops after 3-4 days, with no difference between healthy and asthmatic individuals, but the symptoms persist longer in the asthmatics (4).

Immune response to RV

The clearance of a RV infection is dependent on both rapid innate immune response as well as the humoral and cellular immune systems (41). (Fig. 2)

Innate immune response

The innate immune response involving type I and type III interferons (IFNs) has a direct restriction of virus replication and plays an important role in cytotoxic and natural killer cell differentiation (41). In both children with non-atopic viral induced wheeze and asthmatic individuals, impaired virus-induced IFN expression has been reported (46). Neutrophils appear rapidly in the sinuses and lungs as well as in peripheral blood as a response to growth factors (such as G-CSF, GM-CSF) and chemokines such as CXCL8, CXCL 5, CXCL 10 and RANTES) (41, 44). An increase in eosinophil levels has also been observed, with an ability to both enhance by-standing allergic reactions as well as promote virus eradication (41, 44).

Humoral immune response

The antiviral effector mechanism of the adaptive immune system involves the formation of RV-specific neutralizing antibodies: mucosal RV specific IgA after three days and RV specific serum antibodies (IgG) within a week (41). Antibodies contribute to viral clearance by direct virus neutralization and promote a more rapid and effective cellular response but are thought to

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be most important for preventing RV infections (41). Antibody production due to RV infections occurs on average in only 50% of infected individuals (47). It is not clear whether or not an increase in RV-specific antibodies is an indicator for risk of asthma later in life, but it is known that asthmatic children have higher antibody response to RV than non-asthmatic children (48). Recently, it was shown that antibody response to RV in our study on children with acute wheeze was associated with longer time with respiratory symptoms irrespective of RV species detected (12). The response to RV-C infection with the formation of specific IgG antibodies has however been reported to be weaker than against RV-A in children both with and without asthma (48).

Cellular immune response

If the innate immune response cannot eradicate the RV infection, memory T cells are capable of a quick response (44, 49). RV-specific T cells can be activated by either serotype-specific or shared viral epitopes from different RV serotypes contributing to antiviral activity and airway inflammation (50). Rhinovirus belonging to the major-group RV has been shown to directly infect and activate human T-cells without intervention of antigen presenting cells (51). There is an ongoing debate, if it is a pre-existing deficient immune response to RV or an exaggerated immune response that cause pre-school children to wheeze with RV. However it is noted that other viruses than RV infecting pre-school children do not cause similar symptoms (52). Also, speaking against the deficient anti-viral response theory are studies that show no difference in viral load between RV infected children with or without asthma (4, 53) as well as similar IFN levels between the groups (53, 54).

RV species and the severity of respiratory illness

To compare the pathogenicity of 160 different RV types is a challenge, since PCR detection in nasal aspiration are frequently positive for RV also in asymptomatic children. In addition, underestimation of the presence of RV-C could be suspected in earlier studies before the introduction of molecular diagnostics, since they do not grow in conventional cultures. In hospital based studies in children with wheeze RV-A or RV-C are a more common finding than RV-B (55). RV-B is associated with milder disease, which might be a reason why RV-B is detected at a lower frequency (55).

In our recent study on children with asthma exacerbations recruited at the emergency department where the majority were admitted to stay at the hospital, RV-C was present in the majority of children and associated with more severe asthma than other RV species or other viruses (12). This is in agreement with a study by Cox et al (56) on children with acute wheeze, where RV-C was the dominating RV species (67.5%), and associated with increased risk of recurrent wheezing episodes with hospital admissions not found with any other RV species or any other virus in their cohort (56). Detection of RV-A and/or RV-C in children with their first wheezing episode has also been associated with increased risk for recurrence and initiation of ICS medication, not seen in children infected with other viruses (57).

There are however several recent studies where no association between RV type and severity of respiratory infection has been found (58), and therefore clinical significance of RV-C is still debated.

RV belonging to the minor-group (12 serotypes of RV-A using the LDL- receptor) has been shown to be more immunogenic than RV belonging to the major group (both RV-A and RV-B using the ICAM-1 receptor) (59). Schuler et al. (33) report that RV infections caused by minor and major RV serotypes cause dissimilar inflammatory response such as different cytokine release, transcription factor phosphorylation, and activation from macrophages,

suggesting that the receptor play an important role in creating the inflammatory response in the human airway upon exposure to RV (33).

RV infections in relation to allergy and asthma

The inception of asthma in childhood has been associated with two major factors: incidence of early sensitization to aeroallergens and incidence of viral respiratory infection, especially RV, during the first 3 years of life (60). In a prospective birth cohort, the prevalence of RV infection during the first year and parental atopy was not related, indicating that the genetic risk of allergy does not seem to increase the risk of infections with RV (38). However, Jartti et al found a positive link between allergic sensitization and rhinovirus associated wheeze in hospitalized wheezing children (median age 1.6 years) not found with other virus (61). Children with atopy wheezing with RV before the age of 3 years are those most likely to develop persistent wheezing at age 6 years (62).

Non-atopic children with multi-trigger wheezing have shown the same airway pathology compared to children with atopic asthma (63) indicating airway remodeling despite having an allergic eosinophil driven inflammation early in life (64).

The close connection between allergic sensitization and wheezing with RV infections have been shown in birth cohorts (3). Infection with RV is associated with disruption of tight junctions of the airway epithelia, and thereby a reduced barrier function (41). Dysregulated epithelial barrier function may promote airway inflammation and reduce tolerance to allergens (65). Sensitized individuals have sustained airway inflammation initiated by innate antiviral immunity after an RV infection (66). In asthma, reduced barrier function could be a primary defect or a result of airway inflammation (65). The recently identified receptor for RV-C, CDHR3 is localized on epithelial cells within intercellular junctions, protected from the exposure to the airway lumen (67). One hypothesis is that loss of tight junctions between

epithelial cells and reduced airway barrier function exposes CDHR3 and increase the susceptibility to RV-C infections in asthmatic individuals (67).

BACTERIAL CO-INFECTIONS WITH RV

The contribution of bacterial colonization to the severity of respiratory symptoms is being discussed. Some studies have reported an aberrant immune response to bacteria in infancy in children with later asthma development (68), and both bacteria and viruses have been independently shown to be associated with wheeze (69). Bacterial colonization (with one or several of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) in the hypopharynx during the first months after birth in a birth cohort with heredity for asthma/atopy were associated with asthma within the first 5 years of life (70). Chronic bacterial colonization of the lower airways has also been suggested as a possible explanation why some children with persistent wheeze are refractory to asthma therapy (71).

In a study where healthy children aged 4-12 years provided nasal samples over a period of 5 consecutive weeks in September, detection of RV increased the risk of detecting bacteria within the same sample or that obtained the following week (72). In particular, detecting *M. catarrhalis* alongside RV increased the likelihood of experiencing a cold and/or asthma symptoms compared with isolated detection of RV (72). It has been shown that RV induce impairment of the antibacterial host defense, and could be one important factor in the pathogenesis of wheeze and exacerbation of asthma (73).

PREVENTION AND TREATMENT OF RV INFECTION

To avoid an RV infection, prevention strategies such as hand cleaning is one of the oldest recommended advices, but rubbing with ethanol-containing disinfectants seem to be less effective than hand washing with soap and water (74). At present, no antiviral agents are available to treat RV infections. Also the genetic diversity of the RV species represents a major obstacle toward vaccine development. There are however promising murine studies showing cross-reactive neutralizing antibodies to be effective against multiple RV serotypes that can be generated by capsid peptide immunization (75, 76). In addition, there is some evidence that T cells can be cross-reactive for multiple RV serotypes and induction of RV specific T cells is suggested as an alternative strategy (75). Anti-IgE (Omalizumab) has been shown effective in preventing seasonal peaks of exacerbations in the Autumn and Spring when used in addition to standard treatment of asthma (77). IFN- β inhalation to improve antiviral responses has been tried (78). It did not reduce asthma symptoms but showed an ability to prevent colds to lead to asthma exacerbations in patients with more severe asthma (78).

GENETIC VARIANTS ASSOCIATED TO WHEEZE AND ASTHMA

A large number of genetic studies have attempted to find genetic variants associated with the risk of childhood wheeze and asthma. In many cases, susceptibility loci are discovered by genome-wide association studies (GWAS) where frequencies of genetic variants throughout the human genome are compared between cases and controls. The first GWAS for childhood asthma was published in 2007, describing the asthma related locus 17q21 including among others the *ORMDL3* and *GSDMB* genes. *ORMDL3* (ORMDL sphingolipid biosynthesis regulator 3) is a member of a gene family that encodes transmembrane proteins anchored in

the endoplasmic reticulum and is involved in regulation of sphingolipid metabolism (79). The exact function of *GSDMB* (gasdermin-B) is unknown, but the protein is most likely involved in epithelial development. The 17q21 locus showed a strong association with early onset asthma (79) and is now one of the most consistently associated genetic risk factors for early onset asthma (80). Children with risk alleles at the 17q21 locus who wheeze during RV illnesses have been associated with a greatly increased likelihood of asthma development but not with allergic sensitization (81, 82). Interestingly, genetic variants in this locus have been associated with RV wheezing illnesses in early life, but not with RSV wheezing illnesses (82).

In 2014, Bonnelykke et al reported the identification of five loci in a GWAS of a specific asthma phenotype characterized by recurrent, severe exacerbations occurring between 2 and 6 years of age (83). Four of these loci were previously known as asthma susceptibility loci, *GSDMB* close to *ORMDL3*, *IL33*, *RAD50* and *IL1RL1*, but also a new asthma susceptibility gene, *CDHR3* (encoding cadherin-related family member 3), which is highly expressed in airway epithelium was identified (83). The protein CDHR3 (cadherine-related family 3) was later reported as the RV-C receptor, enhancing virus binding and replication (34).

CONCLUSION

The inception of asthma in childhood has been associated with two major factors: incidence of early sensitization to aeroallergens and incidence of viral respiratory infection, especially RV, during the first 3 years of life. Conflicting results whether children with wheeze or asthma have an immune deficiency or exaggerated immune response to RV have been reported. There is an ongoing debate if the number of respiratory episodes in the first years of life is associated with later asthma development independent of the viral trigger, or if young children wheezing with RV-A or RV-C have increased risk for asthma at school age compared to children wheezing with other viruses.

Taken together, children wheezing due to RV infection constitute a heterogeneous group, with different environmental, immunological and genetic predispositions. They share a vulnerability to infection especially against RV and are therefore at high risk of developing asthma during later childhood. Careful evaluation of the initial wheezing episodes and identification of the causing virus can be of importance to foresee future risk of recurrent wheeze and asthma development, and to personalize treatment for the individual child.

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Fig. 1

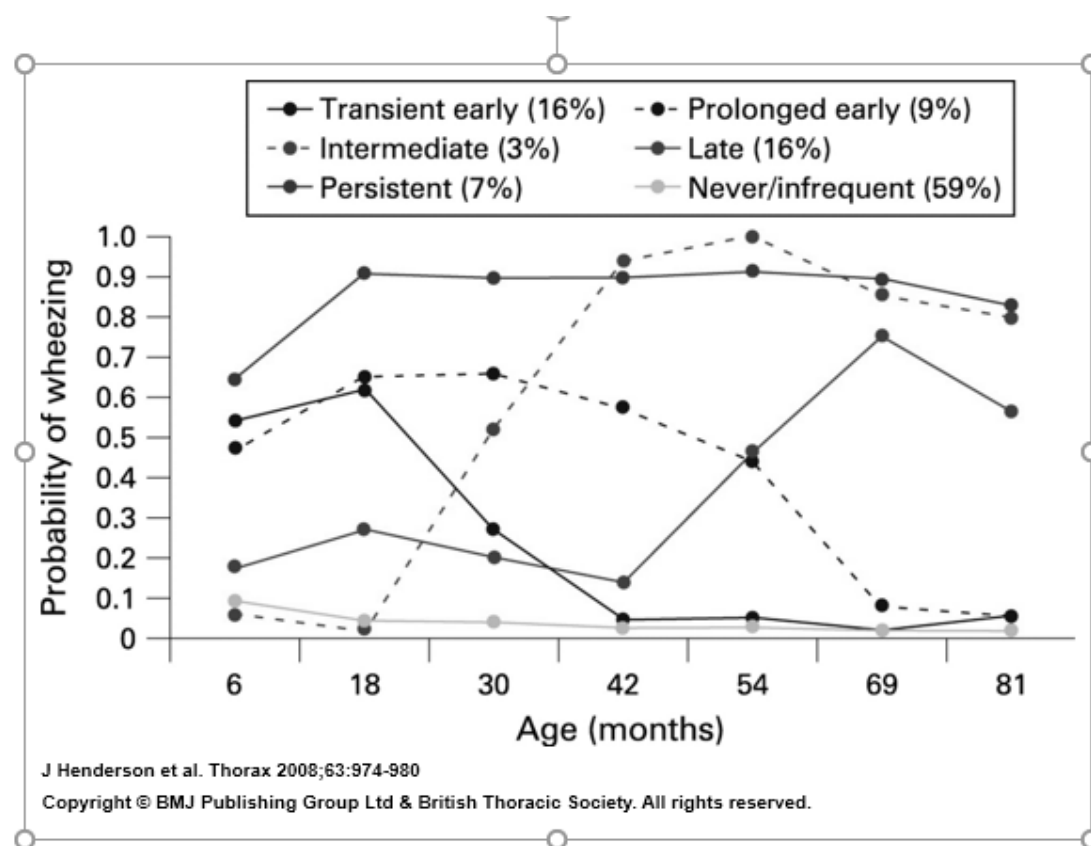


Fig. 2.

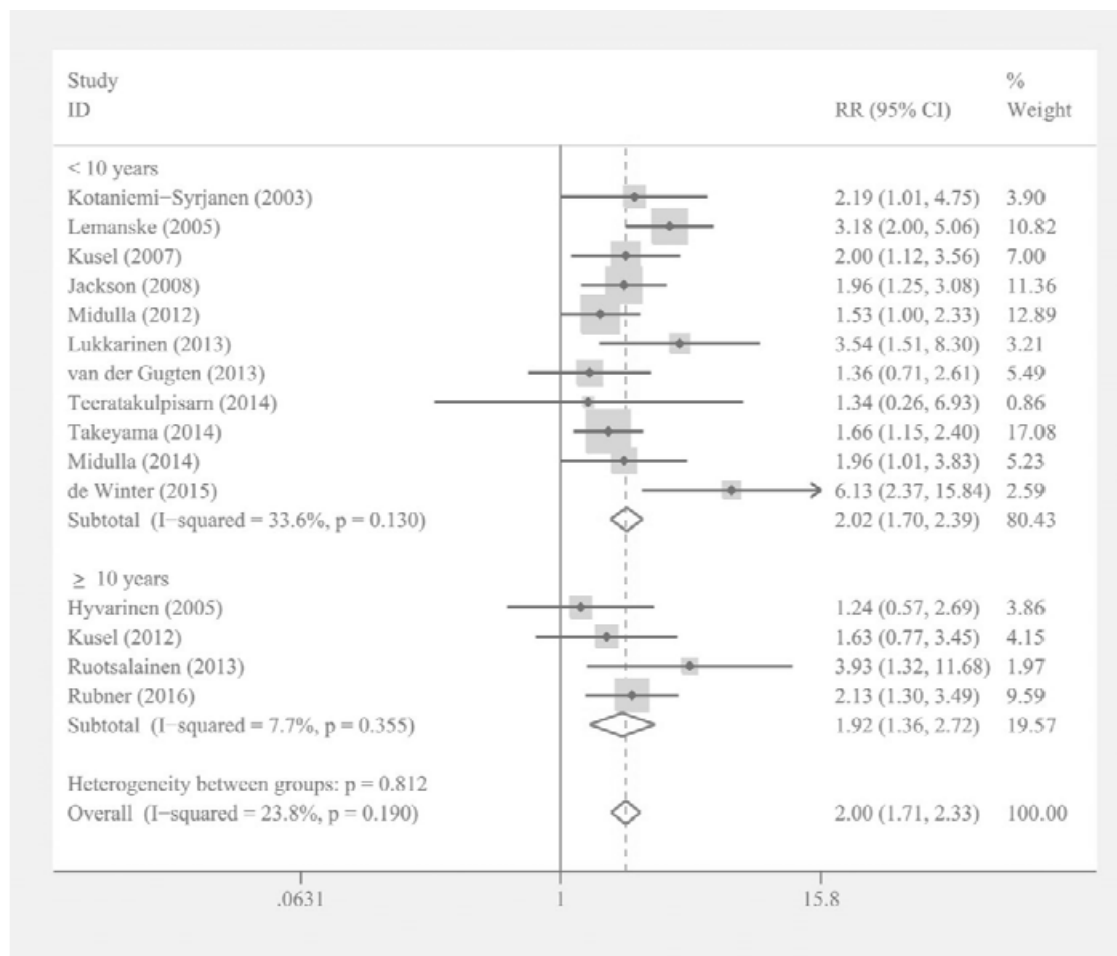
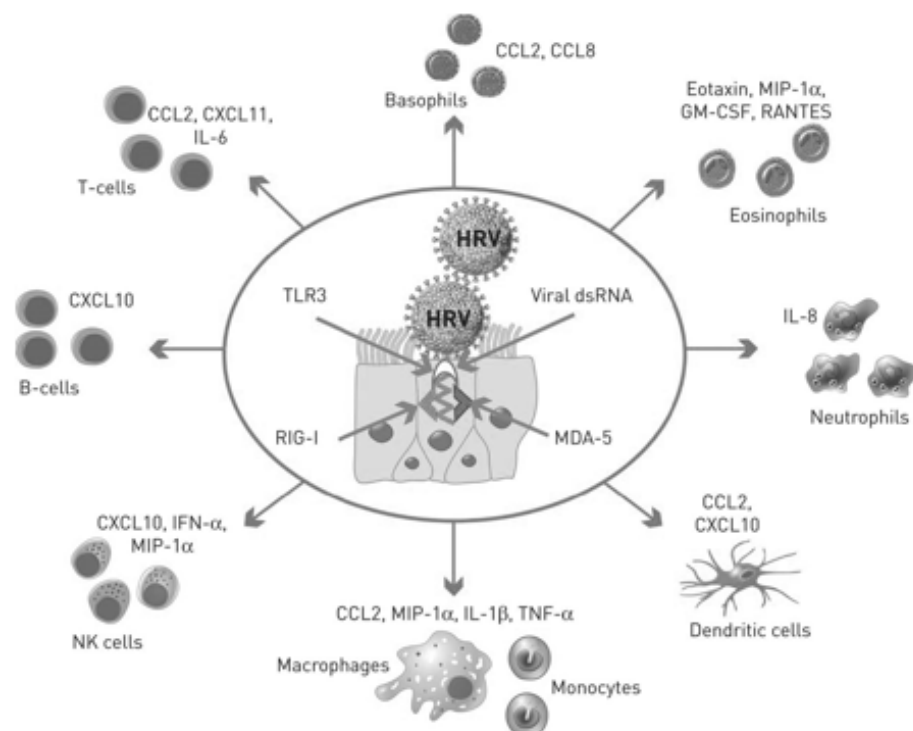


Fig. 3

Response of airway epithelial cells to human rhinovirus (HRV) infection.



Giovanni A. Rossi, and Andrew A. Colin *Eur Respir J* 2015;45:774-789

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Figure Legends

Fig. 1. Prevalence of wheezing at each time point from birth to school age for each of the six wheezing phenotypes identified (n=6265 children) (24).

Fig. 2. Forest plot of the association between early life RV wheezing illness and later onset of wheezing/asthma, stratified by age at follow-up (28) .

Fig. 3. RV infection and response of airway epithelial cells. RV double-stranded (ds)RNA is recognized by airway epithelial cells *via* Toll-like receptor (TLR)-3, which upregulates other pattern recognition receptors, *e.g.* retinoic acid-inducible gene (RIG)-I and melanoma differentiation-associated gene (MDA)-5 (84). The airway bronchial epithelial cells produce cytokines and chemokines that promote recruitment and activation of T- and B-cells, natural killer (NK) cells, macrophages and monocytes, dendritic cells, eosinophils, basophils and neutrophils (shown with their respective chemokines), facilitating clearance of the RV but also amplifying any pre-existing inflammation and contribute to disease exacerbation (84).

IL: interleukin; IFN: interferon; MIP: macrophage inflammatory protein; TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; RANTES: regulated on activation, normal T-cells expressed and secreted (84).