Published in final edited form as:

Curr Opin Pediatr. 2013 June ; 25(3): 344–349. doi:10.1097/MOP.0b013e328360bd2e.

RESPIRATORY SYNCYTIAL VIRUS AND ASTHMA: SPEED-DATING OR LONG-TERM RELATIONSHIP?

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Abstract

Purpose of review—Respiratory syncytial virus (RSV) is the most common respiratory pathogen in infants and young children worldwide. Furthermore, epidemiologic evidence has been accumulating that RSV lower respiratory tract infection in infants may be linked to subsequent development of recurrent wheezing and asthma in childhood. This article reviews the epidemiological evidence linking RSV and asthma and some new hypotheses of the cellular and molecular mechanisms of post-viral airway inflammation and hyperreactivity that have been proposed to explain the epidemiologic link.

Recent findings—New epidemiologic studies have suggested that viral pathogens other than RSV, especially human rhinoviruses (HRV), may play an important role in the inception of atopic asthma. Also, recent experimental evidence is challenging the widely accepted axiom that RSV is cleared from immunocompetent hosts within weeks from the onset of the infection. In particular, bone marrow stromal cells may be a frequent target of human RSV infection, develop structural and functional changes when infected, participate actively in the pathogenesis of the acute disease, and harbor the virus chronically allowing persistence of the infection.

Summary—RSV – and possibly other common respiratory pathogens – play an important role not only in the exacerbation, but also in the inception of asthma. The latter effect may involve the persistence of latent virus in extrapulmonary tissues, similar to what has been recently found for some bacterial species. The most immediate consequence of these discoveries is that future prophylactic and therapeutic strategies for common infections caused by viral or bacterial pathogens may have to address the coverage of remote sites of latent persistence or replication, in order to avoid chronic sequelae recurrent wheezing and asthma.

Keywords

Bone marrow; Bronchiolitis; MicroRNA; Nerve Growth Factor; Rhinovirus

INTRODUCTION

RSV epidemiology

The World Health Organization (WHO) estimates that RSV accounts worldwide for more than 60% of acute LRTI in children, and more than 80% in infants < 1 year of age. Therefore, RSV is by far the most frequent cause of pediatric bronchiolitis and pneumonia [1]. Seasonal outbreaks occur each year during the winter months throughout the world, although onset, peak, and duration of the season vary from one year to the next and are

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CONFLICTS OF INTEREST

There are no conflicts of interest.

difficult to predict. In the United States and throughout the northern hemisphere, the annual epidemics usually begin in November, peaking in January or February and ending in May. Regional variability is significant, with the sub-tropical areas like Florida showing an endemic pattern and less predictable epidemic peaks.

Virtually all children have been infected with RSV by the age of 2 years, and nearly half of those will experience two infections [1■]. Of those infants that are infected, 40% will develop a lower respiratory tract infection (LRTI). It is estimated that each year, as many as 126,000 infants (24.3 per 1,000) are hospitalized in the U.S. due to bronchiolitis, with 2 to 5% of these requiring mechanical ventilation. While this represents a large burden, the numbers outside of the U.S. are staggering. Worldwide, as many as 1/200 infants are hospitalized annually for treatment of LRTI, with a mortality rate as high as 5% that results in more than 1 million pediatric deaths per year. For infants under 1 year of age, this is about 10 times higher than the influenza mortality rate.

Previous infection with RSV does not convey persistent immunity even in the presence of significant antibody titers, although higher titers may attenuate the course of the disease [1■]. Reinfection is common, can occur in the same RSV season, and occurs across all age groups. The first and second episodes of infection typically occur in the first two years of life, and these tend to be the most severe. Most subsequent infections occur in the upper respiratory tract and run a milder course, although the illness may still progress to a LRTI with more severe symptoms.

In addition to the significant morbidity and mortality caused by the acute infection, a large proportion of these young patients continue to have recurrent post-bronchiolitis episodes of lower airway obstruction, which may continue for years after the acute infection has resolved [1■]. More recently, RSV has been shown to be a significant cause of respiratory illness among elderly and high-risk adults, and studies in patients with chronic obstructive pulmonary disease (COPD) have raised the possibility of persistent low-grade RSV infection in this population.

POST-RSV ASTHMA

The link between RSV infection and the development of chronic airway dysfunction - usually diagnosed as asthma- has long been debated. There is certainly an increased risk of subsequent wheezing in children who have had RSV infection in early life, but the question remains as to whether RSV is a risk factor, or rather a marker of predisposition to asthma. Two prospective epidemiologic studies support the argument that RSV LRTI is indeed an independent risk factor for recurrent wheeze and asthma.

In an effort to determine the relationship between RSV LRTI in children under 3 years and the development of wheeze and atopy by age 13, Stein et al. conducted a prospective study on a subset of the children enrolled in the Tucson Children's Respiratory Study [2]. Questionnaires were completed by the parents at age 6, 8, 11 and 13 years. Eight hundred and eighty eight children were enrolled, and of these 519 had at least one LRTI; 472 were tested for respiratory viruses, and 207 of those tested were RSV positive. When compared to children who had no LRTI, those with RSV LRTI were 3.2 times more likely to have infrequent wheeze and 4.3 times more likely to have frequent wheeze by 6 years. This risk then decreased, until it became insignificant by 13 years. As in most similar studies, there was no link between RSV LRTI and atopy [2].

Sigurs et al. conducted a prospective study to compare 47 previously healthy children hospitalized with severe RSV bronchiolitis to 93 matched controls [3]. The authors found 30% cumulative presence of asthma in the RSV group, compared to 3% in the control group.

By age 7 years, 23% of the RSV group had physician-diagnosed asthma, compared to 2% of controls. In a multivariate analysis of the data, the combination of RSV LRTI with a family history of asthma was associated with the highest risk. Taken together, these two studies suggest a 30–40% likelihood of recurrent asthma-like episodes after early-life RSV LRTI. Sigurs' studies also show increased risk beyond 13 years of age and propose a link between RSV infection and development of atopy; these discrepancies from other studies may be related to the different severity of the original infection or to differences in the genetic background.

Unfortunately, epidemiologic studies are not suited to resolve whether early-life RSV LRTI are truly causal in subsequent asthma, or more simply precipitate wheezing in children already predisposed by their genetic or epigenetic makeup. Only carefully randomized control trials with specific prophylaxis can conclusively determine if preventing or delaying the first RSV infection lessens the incidence and/or severity of asthma later in life. A recent industry-sponsored, prospective, multicenter trial concluded that RSV prophylaxis decreases by 80% the relative risk of recurrent wheezing during pre-school years in non-atopic children but does not have any effect in children without atopic background [4], suggesting that in the absence of genetic predisposition to atopy RSV plays an important causative role in the pathogenesis of recurrent wheezing. However, it is critical to point out that this study was not randomized, and was also limited to prematurely born children.

RSV vs. HRV

In recent years, a series of publications have proposed that viral pathogens other than RSV, especially human rhinovirus (HRV), may have a stronger influence on the inception of asthma in childhood [5]. In particular, data from the COAST (Childhood Origins of ASThma) birth cohort indicated that HRV-induced wheezing illnesses in the first 3 years of life were associated with 9.8-fold relative odds of asthma at 6 years compared with a 2.6-fold increase among children with RSV. However, it should be noted that all children included in this cohort have a familial predisposition to atopic asthma, and therefore the COAST data may simply reflect a differential susceptibility of atopic airways to HRV infections vs. RSV infections.

This alternative interpretation of the COAST data is supported by a recent study based on the analysis of mother-infant dyads enrolled in the Tennessee Children's Respiratory Initiative (TCRI), which included a total of 383 infants with PCR positivity to either HRV or RSV [6]. This study showed that infants whose mothers had atopic asthma also had increased relative odds of having HRV-induced infections and developed more severe illness from this infection, compared with control infants whose mothers did not have atopic asthma. In contrast, such relationship was not seen in infants with RSV infection, suggesting that a familial atopic predisposition might explain both increased risk of asthma and differential susceptibility to rhinovirus among asthmatic patients.

Another study published by our group almost simultaneously to the Vanderbilt paper proposes a molecular mechanism that can explain the epidemiologic findings outlined above. Our study shows that HRV-16 infection upregulates the prototypical neurotrophin nerve growth factor (NGF) and its cognate receptor tropomyosin-related kinase A (TrkA) in airway epithelial cells [7]. Although we had previously shown a similar effect on the NGF-TrkA axis by RSV (see next section), in the case of HRV neurotrophic upregulation also leads to a strong increase in the efficiency of HRV internalization by increasing expression of the virus ICAM-1 receptor on target cells.

This interaction between NGF and ICAM-1 can explain the different clinical expression of HRV infections in atopic and asthmatic patients [8] because a number of studies have

demonstrated chronically increased expression of NGF and other neurotrophins in the upper and lower airways of subjects with atopy and/or asthma [9]. Thus, it is possible to link this observation with the increase in ICAM-1 expression found in the airway epithelium of atopic subjects [10], which favors both HRV infection and the migration and activation of cellular effectors of allergic inflammation.

CAN RSV PERSIST BEYOND THE ACUTE PHASE?

The epidemiologic evidence of chronic respiratory dysfunction following acute infection by RSV is in strident contrasts with the widely accepted paradigm that this virus is completely cleared from immunocompetent hosts within weeks from the onset of the infection. On the other hand, multiple past attempts to identify the location of the latent reservoir for this virus have been unsuccessful. However, recently published studies have challenged the current paradigm and have impressed a new and unexpected direction to this research. The first of these studies revealed that RSV infection of the human distal airway epithelium, but not of the more proximal sections of the respiratory tract, is responsible for overexpression of both NGF and its high-affinity TrKA receptor, while the low affinity p75^{NTR} is markedly downregulated [11]. This pattern of neurotrophin expression confers protection against virus-induced apoptosis, and its inhibition amplifies programmed cell death in the infected bronchial epithelium.

Thus, NGF functions as an essential autocrine and/or paracrine survival factor deployed early during the infection to prevent or delay apoptotic cell death caused by RSV (Figure 1). The relevance of this finding is closely related to the role of apoptosis as an innate host cell defense mechanism, which in contrast to necrosis limits viral replication, propagation, and inflammation. Similar to human immunodeficiency virus (HIV) infection in macrophages [12], the anti-apoptotic activity of NGF keeps RSV-infected bronchial cells alive to permit completion of the viral lifecycle, is essential to establish a cellular microenvironment favorable to lytic viral replication, and may allow the persistence of latent infection.

Role of miRNAs

The discovery of RNA interference [13] has broadened dramatically our understanding of the epigenetic mechanisms regulating gene expression, and considerable attention has been dedicated to the role of microRNA (miRNA) species [14]. A recent study tested the hypothesis that specific miRNA species modulate the expression of key neurotrophic factor and receptors in human bronchial epithelial cells, and are in particular responsible for the dysregulation of neurotrophic pathways during RSV infection [15].

The results of this study showed that RSV infection modifies miRNA expression patterns in its natural target, i.e., the human bronchial epithelium. In particular, this virus inhibits the endogenous expression of miR-221, which is highly complementary to the mRNAs encoding NGF and its cognate receptor TrKA and can target them for degradation [15]. These observations suggest that, once the physiologic silencing of the anti-apoptotic NGF-TrKA axis is removed, the resulting inhibition of programmed death in the infected cells may favor lytic cycles of viral replication and spreading of infecting virions to neighboring cells (Figure 1). Thus, the study provides the proof of concept that miRNAs are intimately involved in the intracellular mechanisms controlling RSV replication and may be involved in modulating the replicative cycle of the virus in host cells and its ability to persist and maintain a chronic state of airway inflammation and hyperreactivity [15].

Is RSV Infection Local or Systemic?

Although the tropism of RSV for airway epithelial cells is well documented, it is likely that the infection is not always restricted to the respiratory tract. Viremia and extrapulmonary

infections have been shown in experimental RSV infections of rodents [16,17] and may be a frequent occurrence in infants and young children [18,19]. These observations imply that non-respiratory cells and tissues may be exposed to RSV, and that previously unrecognized targets for infection exist which contribute to the acute and/or chronic pathological process. Furthermore, if RSV can spread to remote sites, it could also find sanctuary in immunologically privileged extra-pulmonary cells and tissues that allow a low-grade, sub-clinical infection to persist latently and possibly recur.

The bone marrow microenvironment, and particularly its stromal cells (BMSC), secrete soluble factors that support survival, differentiation, and proliferation of hematopoietic cells, but also provide a protective niche for both normal and abnormal (e.g., malignant) cells, shielding them from intrinsic (immunologic) and extrinsic (pharmacotherapy) host defenses [20,21]. A recent study identified BMSC as a novel target for RSV infection and showed that this infection causes important structural and functional changes in a cell population that is essential to a functional marrow microenvironment and effective hematopoiesis [22■]. But more importantly, the same study was able to detect RSV genome in naïve primary human BMSC from pediatric and adult donors. This confirmed that these cells are frequently infected *in vivo* during the course of natural infections.

RSV infection has important structural consequences on the BMSC cytoskeleton and functional effects on the synthesis of soluble cytokines and chemokines, creating a milieu that favors maturation and mobilization of leukocytes that enter the blood stream directed to the infected lung tissues [22■]. These consequences are primarily a result of the expression and replication of the viral genome, although capsid proteins appear to contribute a direct toxic effect. BMSC infected with live virus also exhibit a reduced ability to support B cell maturation, which may interfere with the humoral immune response against the virus itself and co-infecting pathogens. Collectively, these observations suggest that, albeit the clinical manifestations of RSV are predominantly circumscribed to the respiratory system, the infection may have systemic implications that can affect its severity during the acute phase, as well as its long-term pathologic sequelae [22■].

Thus, RSV may persist both within and outside of the respiratory tract capitalizing on the anti-apoptotic activity of NGF, and particularly BMSC provide the virus with an immunologically privileged extra-pulmonary sanctuary where a low-grade infection can persist latently and possibly recur intermittently, thus providing a plausible pathophysiologic mechanism for chronic post-bronchiolitis airway dysfunction. One of the most intriguing aspects of this discovery is that a subsequent, very recent study found that the bone marrow can also harbor latent *Mycobacterium tuberculosis* [23■]. In particular, viable MTB was found in human bone marrow-derived mesenchymal stem cells from patients who had successfully completed anti-tubercular therapy.

Conclusions

Together, the studies outlined in this review propose the surprising - and perhaps frightening - scenario that the human bone marrow may act as a microbial “clearinghouse” that stores colonies of dormant germs shielded from the host immune system, and potentially capable of reactivation even years after the original infection. Bone marrow stromal cells may be compelled by viral or bacterial pathogens to retain stem cell-like properties or be reprogrammed to a less differentiated status, which has been shown to be more supportive of latent infection compared to more differentiated cells. To this end, we have very recently reported preliminary evidence that RSV avidly infects pluripotent human embryonic stem cells and modulates their differentiation pattern [24■]. The most immediate consequence of these discoveries is that future prophylactic and therapeutic strategies for common infections caused by viral or bacterial pathogens may have to address the coverage of remote sites of

latent persistence or replication, in order to avoid chronic sequelae like recurrent wheezing and asthma.

Acknowledgments

The Author thanks the National Heart, Lung and Blood Institute of the National Institute of Health for the generous support of my research over the past 15 years. I am also very grateful to the many faculty, fellows, technical and administrative staff, without whom my research would not have been possible.

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▪ of special interest

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KEY POINTS

- Solid epidemiologic evidence indicates that both RSV and HRV play an important role in the inception of asthma; however, familial atopic predisposition might explain both increased risk of asthma and differential susceptibility to HRV among asthmatic patients, whereas such relationship has not been seen in infants with RSV infection.
- Epigenetic mechanisms involving specific non-coding RNAs (microRNAs) are intimately involved in the intracellular mechanisms controlling RSV replication in host cells and its ability to persist and maintain a chronic state of airway inflammation and hyperreactivity.
- Albeit the clinical manifestations of RSV are predominantly circumscribed to the respiratory system, recent evidence shows that this virus can spread to the bone marrow, where the resident stromal cells provide an immunologically privileged sanctuary and may harbor a low-grade infection that persists latently.
- The bone marrow mesenchyme may act as a microbial “clearinghouse” that stores colonies of dormant germs shielded from the host immune system, and may be compelled by the same germs to retain stem cell-like properties or be reprogrammed to a less differentiated status, which is more supportive of latent infection.

Figure 1. Model of RSV Persistence

RSV silences miR-221 expression in epithelial cells. The consequent upregulation of the NGF-TrKA axis not only potentiates the local nociceptive innervation and neurogenic inflammation in distal airways, but also functions as a critical virulence mechanism implemented by RSV to coax host cells to resist apoptosis and persist latently in the lungs, and/or in a safe extra-pulmonary niche within the bone marrow mesenchyme where it avoids detection from the immune system. Persistence of RSV virions and chronic upregulation of the NGF-TrKA axis may turn on lytic replication and inflammation in response to viral reinfection or reactivation, contributing to persistent airway hyperreactivity (AHR) and obstructive lung disease.