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Asthma and viruses: is there a relationship?

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1. ABSTRACT

Asthma is a multifactorial disease in which many factors play a role in its development and exacerbations. Viral infections are known to be the main cause of asthmatic exacerbations and are often the first manifestation of asthma in preschool age. However, there is much evidence suggesting a role of viral infections even in asthma development. Respiratory Syncytial Virus (RSV). has been first associated with an increased risk to develop asthma, but recently new viruses have been proposed to be involved in asthma pathogenesis. Further studies will be needed to demonstrate a causative role of viral infections in asthma development, in order to implement preventive strategies in high-risk children.

2. INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways characterized by recurrent episodes of wheezing, breathlessness, chest tightness and coughing. The most important element in the pathogenesis of asthma is expiratory airflow obstruction, caused by airway hyperresponsiveness, persistent airway inflammation, mucus hypersecretion and airway remodeling. (1). Asthma is a complex disease with an intricate interplay of genetic and environmental factors.(2).

Viral infections of the upper respiratory tract are known to be the main cause of asthmatic exacerbations, (3, 4). but in recent years there is increasing interest regarding

their potential involvement in asthma development. (5). Indeed, there are remarkable clinical findings that respiratory viral infections are also related to the early development of asthma as well as its exacerbations. (6). Asthma begins in preschool age and its first manifestation is often represented by wheezing during viral infections. Respiratory viruses infections are thought to be the cause of at least 90% of wheezing episodes during early childhood. (5). However not all the children who wheeze during viral respiratory infections will develop asthma, as shown in the "Tucson Children's Respiratory Study" (TCRS).. Only a minority of them, known to have the "Atopic Wheezing phenotype" of TCRS, will develop atopic asthma during their life. (7). The relationship between respiratory viruses and asthma is therefore controversial. The classes of viruses which first have been associated with an increased risk of asthma were Respiratory Syncytial Virus (RSV). and Human Rhinovirus (HRV).. (5). Due to innovative techniques in molecular biology, new viruses have been recently involved in the pathogenesis of asthma. (8).

3. RHINOVIRUS: NOT ONLY THE MAIN CAUSE OF VIRAL ASTHMA EXACERBATIONS, BUT ALSO A RISK FACTOR FOR ASTHMA DEVELOPMENT

Human Rhinoviruses (HRVs)., single-stranded RNA viruses belonging to the family of Picornaviridae, were considered the most frequent cause of the common cold since their isolation in the 1950s. (9). HRVs are a large family of genetically diverse RNA viruses, previously subdivided into two species (HRV-A and HRV-B)., but recently a novel group of HRVs, HRV-C, was identified by means of RT-PCR. (9, 10). The 100 classical serotypes belong to species A and B and approximately 50 newly identified types are HRV-Cs. (11). In recent years it was discovered also that HRVs are important in lower respiratory tract infections and asthma exacerbations, and not only in common cold and upper respiratory tract infections. (12). Since Rhinovirus replication is optimal at 33-35°C, infections were once thought to be restricted to upper airway tissues. However, temperatures observed in the tracheobronchial tree allow Rhinovirus replication since they are lower than body core temperatures because of external air temperature and ventilation rate. (13, 14). Furthermore many HRV serotypes can replicate in lower airway cells even at core temperature, although greater viral replication occurs at cooler temperatures. (15). Indeed temperature preferences may vary between different species of Rhinoviruses, which therefore have different probability to cause lower or upper respiratory tract infections.

HRVs are the most common viral cause of asthma exacerbations. (16). Khetsuriani *et al* have found that these viruses might account for approximately 30% of asthma exacerbations in children. However this incidence may be underestimated since only symptomatic children with respiratory viral infections were considered. (17). Indeed another study investigating outpatient asthmatic exacerbations demonstrated HRVs positivity in almost 53% of specimens. (18). In particular HRV-Cs appear to be the class of Rhinovirus more involved in lower respiratory tract infections and asthma exacerbations, compared with other HRV species. (19, 20). HRV-C is by far the most important virus group in acute asthma. (21). This novel group of Rhinoviruses may be more virulent than HRV-A or HRV-B, frequently causing severe illnesses in infants. (5). Children with HRV-C infections were more likely to be hospitalized for respiratory illnesses such as severe bronchiolitis and asthma exacerbations and to require supplemental oxygen. (10, 22). HRV-C infected children had also lower FEV_1 than other HRVs, confirming the greater severity of these genogroup C HRVs infections. (23). Nevertheless HRVs may result in a wide range of illness severity, ranging from asymptomatic infection to hospitalization, depending on many host, viral and environmental factors.

Asthmatic children are more susceptible to symptomatic Rhinovirus infections. (24). It's likely that atopic asthmatic children produce less IFN-g and more IL-10 than healthy individuals during HRV infections. (25). This deficient immune response to HRVs might thus lead to more frequent or persistent Rhinovirus infections. (17). Other host risk factors for severe HRV-associated illness in addition to asthma are "atopic traits" such as eczema, allergic sensitization and parental atopy or some host phenotypic characteristics such as neonatal age, chronic respiratory disease, male gender and reduced lung function. (11). Both viral infections and allergic sensitization appear to contribute to loss of asthma control, resulting in a very high risk of severe asthma exacerbation in children with both these conditions. (26).

Regarding HRV infections seasonality, many studies have reported that peak periods are in fall and to a lesser extent in spring. (27). In particular, HRV infections have been shown to rise in September, about a week after the beginning of schools. In the same period, also usually increase asthma exacerbations. (28). In recent years, it has been suggested that this "September epidemic" of asthma exacerbations can be caused by HRV infections that spread rapidly after the beginning of a new school year. (29). In a more recent study, Lee *et al* have described a less marked seasonality in Rhinovirus infections, with a high infection rate even in winter. Two are the potential explanations for this result: the first is the inclusion of not only symptomatic children with HRV infections but also the asymptomatic ones; the second is the enrollment of younger children which are more likely to be chronically infected. (11).

Asthmatic children are more likely to wheeze after viral infections than healthy children. In the last years it has been observed that asthma is often preceded by viral wheezing episodes in infancy. On the other hand, the first clinical manifestations of asthma often occur during viral respiratory illnesses, so it is difficult to attribute wheezing episodes to asthma during infections. (30). Whether or not these viral infections of the lower respiratory tract are causal in asthma development is controversial. Maybe viruses are more likely to infect intrinsically predisposed children to early childhood asthma compared to healthy ones. (5). Carroll *et al* first showed a correlation between

the severity of bronchiolitis in infancy and the increased risk for early childhood asthma and for higher asthma-specific morbidity. (31). As regards the role of HRV infections in asthma development, significant considerations are emerging. (32, 33). So far studies have focused mainly on the relationship between Respiratory Syncytial Virus (RSV). infections and increased risk of asthma. (34). However in the latest works it seems that HRV infections are a better indicator of asthma risk than RSV infections. (35). An important birth cohort study investigating the relationship between HRV wheezing illness and subsequent asthma development was The Childhood Origins of ASThma (COAST). study. (36). In the 289 high-risk newborns enrolled in COAST study, symptomatic Rhinovirus illnesses during infancy were the most significant risk factor for preschool wheezing development. (32). Also according to Jackson *et al,* early Rhinovirus wheezing illnesses are the most important predictor of subsequent asthma development in high-risk children. (30). Another high-risk cohort of 198 children was followed from birth to 5 years in Australia by Kusel *et al.* They reported that acute severe lower respiratory tract infection (LRI). caused by HRV in the first year of life were associated with asthma and persistent wheeze at the age of 5.(37). The recent study of Midulla *et al* has confirmed that HRV infection is a major independent risk factor for recurrent wheezing. In this study HRV was the virus most likely to be associated with recurrent wheezing during a 12-month follow-up after the first episode of bronchiolitis in this study.(38). Also Carroll *et al* have reported an increase of approximately 25% in the risk of asthma development in children who had bronchiolitis during non-winter period, when Rhinovirus are predominant, compared to them who had bronchiolitis during the RSV-predominant winter period. (39). However children with HRV infections were found to be older and to have more frequently atopic dermatitis and eosinophilia compared to RSV infected children. (40). For this reason, further studies are needed to understand whether HRVs are more involved in asthma development than RSV or whether HRV infect children predisposed to develop asthma more frequently than RSV.

Thanks to recent findings about the role of HRV in asthma development, future strategies may be implemented to prevent asthma development in high risk children. Treatment protocols with anti-inflammatory drugs and immunomodulatory therapies during Rhinovirus infections in high-risk children should be studied to assess if early intervention can lead to disease modification.(37).

4. INFLUENZA AND THE COMPLEX RELATIONSHIP WITH ASTHMA EXACERBATIONS

Asthma is a high-risk condition for flu diseases, however the role of Influenza virus as causal agent of asthma exacerbations is still unknown. (41). Studies on hospitalized children have found that Influenza virus causes only 3-4% of asthma attacks, but this percentage might be underestimated. Indeed Influenza virus is much more prevalent in ambulatory patients with asthma exacerbations than in hospitalized children, since flu rarely requires hospitalization.(42).

Miller *et al* observed that asthmatic children were respectively fourfold and twofold more likely to have influenza-related hospitalizations and to have outpatient visits than healthy ones. (43). Despite influenza vaccination is recommended for asthmatic children, there is conflicting evidence about its effectiveness in reducing asthma exacerbations. Moreover it has emerged in the past that influenza vaccination might trigger asthma attacks. (44). That's probably why vaccination rate among asthmatic children remains low (<30%). (43)., although vaccine safety has been widely proven. (45, 46). In their randomized placebo-controlled study, Bueving *et al* demonstrated that influenza vaccination doesn't reduce the number and severity of influenza-related asthma exacerbations. (47). In his review, Bueving has also suggested to reconsider influenza vaccination recommendations in children with mild to moderate asthma. (48). On the other hand, Kramarz *et al* have noted that influenza vaccination was associated with a decreased incidence of asthma exacerbations, if asthma severity was taken into account as potential confounder. They observed that asthmatic children with more severe disease and higher incidence of asthma attacks were more likely to be vaccinated and thus vaccinated and control group could not be comparable. Therefore they used a self-control method to compare the frequency of asthma exacerbations before and after vaccination in the same children. (49). Since studies on this topic are few and conflicting, question remains open and it is still unclear what's the degree of protection against asthma exacerbations provided by flu vaccination. (50).

The diffusion of the novel H_1N_1 influenza A virus has stimulated research on the relationship between this pandemic infection and asthma. H_1N_1 flu, first reported in USA in 2009, showed from its outset some unusual features like high morbidity, rapid dissemination and involvement of young adults and children. (51). Asthma was the most common underlying condition in children hospitalized with H_1N_1 infection and was a risk factor for severe H_1N_1 flu much more than it normally was for other influenza viruses causing seasonal flu. The proportion of asthmatic children requiring hospitalization was two to five times higher during the pandemic H_1N_1 than seasonal influenza. (52). However the probability to have a serious illness seems to have no clear relationship with asthma severity. (53). Given the increased severity of novel H_1N_1 influenza infections, preventive strategies for annual influenza vaccination should be considered in asthmatic children.(53).

According to what we said so far further studies will be needed to investigate the role of influenza viruses in asthma exacerbations. The association between asthma and increased influenza morbidity doesn't mean a direct involvement of this virus in asthma attack indeed. (8).

5. RESPIRATORY SYNCYTIAL VIRUS: A LONG STORY AS A POTENTIAL CAUSATIVE FACTOR IN ASTHMA DEVELOPMENT

RSV is a single-strand RNA virus that belongs to the family of Paramyxoviridae and causes lower respiratory tract disease in infants and young children. RSV infections typically occur in winter season, with an average peak in

December. (54). Almost every child is infected with RSV within 3 years of life. RSV is the most common cause of severe respiratory tract infections such as bronchiolitis in infants and young children and is responsible for the majority of hospital admissions worldwide. (55). Unlike other viruses, RSV has been initially studied for its involvement in the development of asthma and it was only subsequently investigated as a potential cause of asthma exacerbations.

Since "Tucson Children's Respiratory Study" has been published it is well known that a subset of children with very early RSV wheezing episodes is 3 to 5 times more likely to wheeze at the age of 6 years. This increased risk decreased significantly at the age of 13 years. This subgroup has also lower levels of lung function reversed after bronchodilator. (7). Thereafter many studies have investigated the role of RSV infections in asthma development regardless of other risk factors and genetic predisposition, with conflicting results. In a Swedish study, Sigurs *et al* have shown that children hospitalized in infancy for severe RSV bronchiolitis had higher rate of allergic sensitization and asthma development up to age 7½. (34). In later studies the same authors have observed that the increased risk of having allergic asthma was still present in early adolescence (56). and even in early adulthood. (57). They have suggested that reduced performance in spirometric tests after RSV may reflect airway remodeling. (57).

The prospective controlled long-term follow-up study of Ruotsalainen *et al* has recently shown that patients hospitalized for RSV and HRV bronchiolitis in infancy had an increased asthma risk at 15-18 years of age compared to healthy controls. However, the risk was higher after HRV than after RSV bronchiolitis. (58). Wu *et al* have also speculated that the risk to develop early childhood asthma could be linked to the relationship between the period of birth and the winter virus peak, with the highest estimated risk if the birth was about 4 months before the winter virus peak. (59). Before dealing studies which had opposite results, we will now describe a recently published hypothesis by Krishnamoorthy *et al*, describing a potential mechanism whereby RSV infections increase susceptibility to asthma and allergic disease. (60). According to the authors, RSV would affect early mucosal tolerance and lung microenvironment, inducing a TH_2-type inflammatory response in T-reg cells and thus impairing tolerance to inhaled allergens. Indeed RSV infection in mice induced GATA-3 expression and TH_2 cytokine production and upregulated IL-4Rα expression in $FOXP_3^+$ T-reg cells. More inflammed airways, hyper-reactivity and higher allergen-specific IgE were observed in infant mice infected with RSV compared to uninfected control mice. (60). However it's still unclear whether RSV plays an active and causative role in asthma onset or whether RSV infections identify children predisposed to develop asthma. (61).

In the prospective 20 years follow-up study of Korppi *et al*, RSV infections in infancy were not a risk factor for asthma or bronchial reactivity, but only for lung function abnormalities in young adults, regardless of atopy. According to the authors, bronchiolitis doesn't predispose directly to atopy and asthma onset, but it's only an indicator of immunologic abnormalities, which are common to both conditions. (62). In a review on the long-term effects of RSV bronchiolitis it also seems unlikely that this infection can be a cause of atopic asthma. The authors are also critical about several other studies investigating the association between early respiratory infections and asthma. (63). On the other hand in a more recent systematic review of Perez-Yarza *et al,* a significant association between RSV infection in childhood and the development of subsequent episodes of recurrent wheezing or asthma was observed.(64). Poorisrisak *et al* studied cohabiting monozygotic twin pairs discordant for hospitalization for RSV bronchiolitis in infancy to evaluate whether RSV has a causal role in asthma onset or whether it identifies children genetically predisposed to develop asthma. The authors found no differential effect from severity of RSV infections on the development of asthma and allergy in monozygotic twins. (65). So whether RSV causes asthma in previously healthy infants or whether these infants are predisposed to asthma and RSV infection is their first virus-induced asthma exacerbation remains an open question. (61). According to Wu and Hartert, investigating several retrospective studies, these two explanations don't exclude each other and both are likely to be relevant in asthma development.(66).

Anyway RSV has not been only investigated as a potential causative factor, but also as a triggering factor for asthma exacerbations. Aeffner and Davis argued in their publication that RSV is the main cause of asthma exacerbations in children under the age of 2, but liable pathogenic mechanism is still unknown. (67). Whatever the precise relationship between RSV and asthma, RSV continues to be the leading cause of severe respiratory tract disease in infants and young children, so the development of an RSV vaccine would be a useful means of primary prevention.(68).

In conclusion, the relationship between RSV and asthma is not completely explained and a causal link cannot be demonstrated with descriptive studies, so prospective randomized trials will be needed in the future. (34). If a causative role of RSV in asthma development will be confirmed, immunoprophylaxis against RSV or prevention of winter RSV infections during early infancy could prevent asthma. (66, 69).

6. DOES HUMAN METAPNEUMOVIRUS HAVE A ROLE IN ASTHMA DEVELOPMENT?

Human Metapneumovirus (hMPV). is a Paramyxovirus discovered in 2001 and identified worldwide as a common cause of acute respiratory tract disease, particularly in infants and young children. (70, 71). HMPV infections are clinically similar to those due to RSV, ranging from mild upper respiratory tract disease to severe bronchiolitis and pneumonia requiring hospitalization. (72, 73). Moreover co-infection of these viruses isn't uncommon and appears to be more severe than a single infection. (74). Also the seasonality of hMPV is

comparable to that of RSV, with a peak incidence in winter. (75, 76).

Some studies have investigated the possible association between hMPV and asthma exacerbations in children (77, 78). and in adults (79)., suggesting that hMPV might be responsible for acute asthmatic attacks. This is confirmed by the paucity of asymptomatic hMPV infections. In the study on adults of Williams *et al* it was noted that asthmatic subjects infected with hMPV had more severe and less controlled asthmatic disease than uninfected asthmatics. (79). Given the strong similarity between hMPV and RSV, it was assumed that even hMPV could play a role in asthma development. To date only one study in literature has investigated this relationship, noting that hMPV bronchiolitis in infancy was more likely to be associated with asthma in preschool age compared to RSV bronchiolitis. (80). The hypothesis that hMPV may predispose to asthma is supported by studies carried in guinea pigs, demonstrating a long-term pulmonary inflammation associated with airway obstruction and hyperresponsiveness induced by hMPV infection. (81). In the study of Alvarez *et al* the immune response to hMPV in a mouse model was associated with aberrant immunity, induction of a TH_2-type response and virus persistence, quite different from the immune response to RSV. (82). This association between hMPV infections and asthma development should be confirmed by prospective future studies.

7. A NEW VIRUS RECENTLY PROPOSED TO BE LINKED WITH ASTHMA: HUMAN BOCAVIRUS

The Human Bocavirus (HBoV). is a newly discovered Parvovirus, first identified in 2005 by Allander *et al* in nasopharyngeal aspirates of children with lower respiratory tract infections. (83). Since then many studies have investigated its prevalence and its role in human respiratory diseases.

Parvoviruses are nonenveloped, single-stranded DNA viruses, so called because they're the smallest known viruses. (84). Peak incidence of HBoV in infants and young children has been observed during winter. (85). Human Bocavirus has shown to be a widespread respiratory virus, causing wheezing and bronchiolitis in infants at a higher age than RSV but younger than Rhinovirus. HBoV also causes illnesses that are longer compared to HRV ones and that have a similar clinical severity to those of RSV. (86). Since the first studies were published, it appeared that HBoV had a high frequency of coinfections. Almost half of the HBoV-positive samples were positive even for other respiratory viruses. The most likely explanation for this high coinfection rate is that Bocavirus may be an exacerbating factor which increases the severity of other virus infections, especially of RSV. (85).

Studies on Bocavirus are limited by the fact that identification can only be done through molecular techniques such as polymerase chain reaction (PCR)., because this virus has been only recently isolated in culture. (87). However HBoV PCR-based diagnosis is problematic because many patients have low viral load and HBoV can persist in the respiratory tract for a longer time compared to other viruses. Therefore a serological diagnosis seems to be more useful and to correlate better with clinical symptoms. (88). Human Bocavirus has been recently detected even in blood and fecal samples, suggesting that respiratory HBoV infections are systemic and induce a B cell immune response that is at the base of serological diagnosis. (89). It was recently discovered that there are several members of the genus Bocavirus, but only HBoV1 appears to cause wheezing and respiratory tract infections. Instead HBoV2-4 occur mainly in the gastrointestinal tract and elicit weaker B-cell responses than HBoV1. However it has to be taken into account that members of Rhinovirus family may serologically cross-react, resulting in overestimation of HBoV1 prevalence. (90).

The role of HBoV in asthmatic disease is still unclear. Indeed studies published to date are inconclusive, although they appear to identify this virus as a cause of asthma exacerbations. For example, the prospective study of Vallet *et al* suggests that HBoV is a common cause of asthma exacerbations requiring hospitalization. (91). HBoV has been recently cultured in differentiated human airway epithelial cells (92). and therefore promising studies on the role of Bocavirus in asthma exacerbations are expected in the future.

8. CONCLUSIONS

Much evidence is reported in the literature suggesting a role of viral infections in acute exacerbations and in the development of asthma. However we still cannot draw any conclusions about the existence of a causal relationship. Indeed asthma is a multifactorial disease in which several factors contribute to its development. Viral infections, as well as other environmental factors such as exposure to allergens, interact in a complex way with the genetic ground.

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Abbreviations: RSV: respiratory syncytial virus; TCRS: Tucson children's respiratory study; HRV: human rhinovirus; FEV_1: forced expiratory volume in the 1st second; IFN-g: interferon-gamma; RNA: ribonucleic acid; COAST: Childhood Origins of ASThma study TH_2: T-helper 2; T-reg cells: regulatory T cells; FOXP3: forkhead box P3; IL-4Rα: interleukin-4 receptor α; hMPV: human metapneumovirus; HBoV: human bocavirus.

Key Words: Asthma, Children, Viral infections, Multifactorial disease, Review

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