



# Allergens and bacteria interaction in the induction of basophil activation: is this the lost ring between allergy and infections in pediatric patients?

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## Purpose of review

The aim of this review is to describe the relationships between allergy and infectious diseases, with specific attention on bacterial infection. According to the most recent literature, bacteria could be considered also as one of the major causes of asthma exacerbations that we need to further explore.

## Recent findings

The availability of novel methods to detect large panels of pathogens, including viruses and bacteria specific for the upper respiratory tract, together with the capacity of evaluating how basophils are activated, has changed the point of view of the mechanisms related to asthma exacerbations. The recent finding that basophils are activated in the presence of suboptimal doses of allergens and bacteria has been recently described and may explain the clinical behavior of allergy exacerbation. Indeed the activation of basophils induces the secretion of mediators, which, at bronchial level, may cause bronchospasm that leads to an asthma exacerbation in patients with infection. The contrary is also true.

## Summary

Although the explanations for these facts are numerous, at present, bacteria seem to play a relevant role in the worsening of asthma in infected patients.

## Keywords

allergens, bacteria, basophils, FcE receptors, Toll-like receptor

## INTRODUCTION

Respiratory infections are associated with wheezing illnesses at all ages and have a significant impact on the natural history and severity of asthma, affecting, in childhood, about 10–15% of patients. The first evidence that asthma exacerbations in children were associated with infections was reported in 1995 [1]. Viruses were detected in 226 of 292 reported episodes (77%); in 14 (4.8%), two viruses and, in one, three viruses were detected. Enterovirus and, in particular, rhinovirus (RV) infections of the respiratory tract were detected using suitable virological methods. Other viruses such as coronavirus (CoV), influenza viruses (IV), parainfluenza viruses (PIV), respiratory syncytial virus (RSV) and a few others were also identified. The high frequency of virus isolation in these patients documents the high quality of the study. Viral infections were also repeatedly indicated as a cause or concause of the well known 'September asthma epidemics' [2]. All those results, although of primary interest, depicted

a very complex problem. Indeed, it has been demonstrated that asthma exacerbation requiring hospitalization accounts for a major fraction of the total cost of asthma care in society [3–5]. It has also been hypothesized that chronic viral and bacterial infections or colonization with pathogenic bacteria could lead to chronic lower airway inflammation, impaired mucociliary clearance, increased mucus production and eventually asthma [6]. Other studies

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**Curr Opin Allergy Clin Immunol** 2012, 12:000–000

DOI:10.1097/ACI.0b013e328350fd91

## KEY POINTS

- Asthma exacerbations are strictly related to infections. In the past, viruses were considered the main infectious agent, whereas at present, microbes also seem to be involved.
- From an experimental point of view, the presence of suboptimal doses of bacteria in the culture medium is able to induce the activation of basophils, only if stimulated with suboptimal doses of allergens.
- Activated basophils have the capacity of secreting all the mediators of allergy in the environment, thus mimicking the situation of an allergic patients infected by a pathogen.
- These laboratory evidences should be the link between infections and allergy.

reported that infants colonized with bacteria were at risk of asthma at 5 years of age [5]. In this context, strategies for defining the cause of the exacerbations and the optimal therapeutic regimens, required not only to treat the illness but also to prevent any progression of the disease, are needed.

## PATHOGENS INVOLVED IN ASTHMA EXACERBATION

As stated before, respiratory tract infections caused by viruses [7,8] have been shown to be epidemiologically associated with asthma in the following ways: first, viruses associated with infantile wheezing have been theorized to lead to the inception of the asthmatic phenotype [7,8]; second, infants who experienced severe viral respiratory infections are more likely to develop asthma later in childhood [9,10].

### Virus infections

Several studies, generally based on the recently described multiplex molecular biology-based techniques, have observed a relationship between various viral infections and asthma exacerbations.

### Human rhinoviruses

Among respiratory viruses, human rhinovirus (HRV) infections preceded as many as 50% of asthma exacerbations in children [11,12,13<sup>■</sup>,14]. However, other authors were unable to detect any difference in microbial pattern between patients with asthma exacerbations and 'nonasthmatic' patients [14]. In this context, three updated reviews [15,16<sup>■</sup>,17<sup>■</sup>]

describing the present features of the infection of asthmatic patients are now available. Of note, HRV infections seem to aggravate allergic asthma [18]. In addition, the intercellular adhesion molecule-1, which is variably expressed on respiratory epithelia during allergic inflammation [19], is the major receptor for human rhinoviruses [20]. Very recently, it has been shown that allergic sensitization precedes HRV wheezing but the opposite is not true [21].

### Influenza viruses

INV also accounted for substantial morbidity (up to 200 annual outpatient visits per 1000 children) in children with asthma and other chronic conditions [22–24]. Nevertheless, a meta-analysis failed to support the protective effect of influenza vaccination in asthma exacerbations [25].

### Parainfluenza viruses

PIV have been identified as pathogens potentially involved in asthma exacerbation since the original observations in 1995 [21]. From that moment, a number of recent works [13<sup>■</sup>,26] have confirmed their role.

### Adenoviruses

It has been demonstrated that 94% of children with steroid-resistant asthma had detectable adenovirus antigens compared with 0% of controls [27]. In adults, both with and without asthma, evidence of adenoviral infection has been observed to be as high as 50% of the individuals tested [5]. Nevertheless, the virtual absence of any new report on adenoviruses infections in asthma exacerbation in the recent bibliography may indicate either a well known role of this pathogen in at-risk patients or the absence of any significant association detectable using molecular biology tests.

### Metapneumoviruses

Newly discovered respiratory viruses such as human metapneumoviruses may also play a role [28–30]. Further studies are necessary to define the actual role of these viruses that, in clinical experience, are slowly but continuously spreading in pediatric population.

### Bocaviruses

A more recently described parvovirus called human bocaviruses was identified in children with lower

RTIs, but its relation to asthma exacerbation is uncertain [31–33].

## Coronaviruses

Respiratory viruses, such as human coronaviruses (CoV) [34], have been described in asthma exacerbation. A similar finding was described in the first work on this topic [1]. Even for CoV, the use of large cohorts of patients and highly sensitive and specific assays is needed to better define the actual role.

## Respiratory syncytial virus

RSV is another major pathogen causing asthma exacerbation [11,13<sup>\*</sup>]. RSV is virtually the most frequent virus isolated in small patients with bronchiolitis, wherein bronchospasm is frequent. Of note, RSV was used in a recent set of experiments that showed that, in mice, an early exposure to an infectious agent might be responsible for asthma in the following months [35<sup>\*</sup>].

## BACTERIAL INFECTIONS

There is a body of literature regarding the association between chronic *Mycoplasma* or *Chlamydo-*  
*phila* infection and asthma in children and adults.

### *Mycoplasma pneumoniae*

*Mycoplasma pneumoniae* (MYP) is detected by PCR in more than 50% of patients with asthma, but it was also found in 11% of upper airway secretions from patients with stable asthma [36–39]. Martin *et al.* [5] evaluated 55 adult patients with chronic asthma and 11 controls for infection with *Mycoplasma*. Of the asthmatic patients, 56% had a positive PCR for MYP. Only one of 11 controls was MYP positive. A significantly greater number of tissue mast cells were observed in the group of positive patients. In another study by Biscardi *et al.* [38], 119 children hospitalized for a severe asthma exacerbation were tested by serology for acute infection due to MYP. Acute MYP ‘serological’ infection was found in 20% of the patients during the current exacerbation. Out of 51 patients experiencing their first asthma flare, MYP was demonstrated in 50%. Out of the children infected with MYP and experiencing their first asthma exacerbation, 62% had recurrent asthma but only 27% without these infections had recurrent asthma [40]. Although Cunningham *et al.* [41] have reported infection in up to 25% of children with wheezing, others have not been able to confirm these observations [42]. Moreover, when the same group of

children was evaluated for the relative contributions of *Mycoplasma* and *Chlamydia* infections to acute exacerbations, viruses were a far more common cause [8,42]. Ongoing research will further determine the role of macrolide antibiotics in asthma management [43]. Their effects in asthma can be partially explained by the antimicrobial activity, as asthmatic patients are more prone to infections and infections are a well known cause of exacerbation. Beyond this, it has been shown that macrolide antibiotics also have immunomodulatory and antiinflammatory activities. In particular, both in-vitro and in-vivo data have shown that these drugs reduce airway mucus secretion, inhibit the bacterial adhesion, reduce the production of reactive oxygen species and inhibit neutrophil activation [44]. Despite this, the results from meta-analyses are still inconclusive because some symptomatic improvement was demonstrated, but no change in functional parameters or exacerbation rates was observed [45].

### *Chlamydo-* *phila pneumoniae*

The first relationship between asthma and CHP, an obligate intracellular respiratory pathogen, was described in 1991 in 19 wheezing adult asthmatic patients, of whom nine were found to have serologic evidence of CHP [46]. However, results of diagnostic tests in the upper and lower airways do not always agree. More recently, CHP was linked to asthma exacerbation in children [47]. In school-age children with wheezing, a surprisingly high prevalence of low-grade CHP infection in nasal aspirates has been observed [41]. The detection of CHP infection by PCR and serology (secretory IgA) was similar during symptomatic and asymptomatic episodes (23 vs. 28%, respectively). Children who experienced multiple episodes also tended to remain PCR positive for CHP. Moreover, CHP-specific secretory IgA antibody levels were more than seven times higher in patients who reported four or more exacerbations. In another study [48] of 70 pediatric patients, 40% of PCR CHP-positive samples were obtained from patients with asthma: a significantly higher proportion of asthmatic patients (34.3%) resulted positive for CHP, when compared with controls (11%). Asthma-related symptoms resolved or significantly improved in half of adult patients with asthma seropositive for CHP who were treated with macrolides or doxycycline [36,41,44,47–49]. In more recent years, the role of bacterial infections in the development of asthma exacerbation has been further evaluated [17<sup>\*</sup>,27], but the original role of MYC and CHP remained just as described in original works.

*Streptococcus pneumoniae* (SCP), *Haemophilus influenzae* (HeI) and *Moraxella catarrhalis* (MXC) were suspected to have a role in asthma exacerbations a few years ago [6]. Only recently, but in adults with COPD, novel evidence of their role was described [50].

## FACTORS INVOLVED IN THE ASSOCIATION BETWEEN INFECTIONS AND ASTHMA EXACERBATIONS

Several different triggers can start asthma exacerbation during an infection. Indeed, the actual relationship can be considered as asthma causing infections as well as infections causing asthma. Basically, the two factors are strictly connected to each other and the first cause can hardly be distinguished from the second one.

### Mechanical factors

Viral infections hurt airway epithelial cells and cause both edema and leakage of serum proteins. This results in obstruction and wheezing. In addition, an inflammatory response is started to control the infection, causing secretion of cytokines and chemokines that enhance airway inflammation. In addition, a sentinel role of the airway epithelium in asthma pathogenesis has been suggested [51].

### Environmental factors

These factors may act synergistically with viral infections. Allergy increases the risk in infancy [52] as well as exposure to air pollutants [53].

### Vitamin D defect

A number of observational studies [54–57] in adults and children suggested that those with low vitamin D serum levels have a higher incidence of virus-induced asthma exacerbation. Genetic polymorphisms in vitamin D receptor-dependent pathways were also associated with the development of asthma [58,59] and vitamin D deficiency related to the seasonal variation in symptomatic respiratory infections [60]. Of note, vitamin D treatment of airway epithelial cells promotes the healing of epithelial barrier [61].

### Secreted cytokines

Interleukin 13 (IL-13) is Th2 cytokine that slows down the restoration of function [62] and promotes hypersecretion of mucin [63]. From a phenotyping

perspective, a genome-wide airway epithelial cell expression array identified three IL-13-induced genes forming a signature that distinguished asthmatic patients from healthy ones [64]. For activation pathways, two different signatures were identified: the first showed an activation of genes of innate immunity and of an antigen-independent T-cell activation mediated by IL-15; the second documented specific lymphocyte activation through antigen receptors and subsequent recruitment of cells of adaptive immunity [65].

### Chemokines

Nasal chemokines have been associated with neutrophilic influx and cold symptoms [66].

### Interferons

Interferon  $\beta$  (IFN- $\beta$ ) is responsible for the stimulation of many of the genes subsequently induced by HRVs [67]. Production of IFN- $\beta$  and IFN- $\gamma$  in response to HRVs discriminates healthy from asthmatic patients [68,69], a finding that was not confirmed using a genome-wide expression array [70].

### Toll-like receptors

It has been shown that HRV-stimulated signaling by Toll-like receptor 3 (TLR3) induces IFNs [71]. In addition, ATP, present in the airway at higher concentrations during allergic or viral challenges, is involved in the amplification of immune response initiated by TLR [72]. Heat shock protein 60 (HSP60), the major CHP antigen, is an inducer of macrophage inflammatory responses mediated by TLR4 [73].

## BASOPHILS, ALLERGY AND MICROBES: THE LABORATORY EVIDENCE

In the recent South American Congress on Asthma in Cordoba (Argentina) we have presented novel findings: the activation of basophils, mediated by cross-linking of the allergen to the specific IgE captured by surface Fc epsilon receptors, can be significantly potentiated in the presence of bacteria (Fig. 1). Bacteria alone may induce the activation of basophils (measured as expression of CD63 on cell surface), by using high concentrations of microbes. At suboptimal concentrations, neither allergen nor bacteria activate basophils, but, given together, activation occurs. Thus, the link between allergy and infections is the capacity of bacteria to induce the activation of basophils (which, in turn, secrete inflammation and allergic mediators) in the

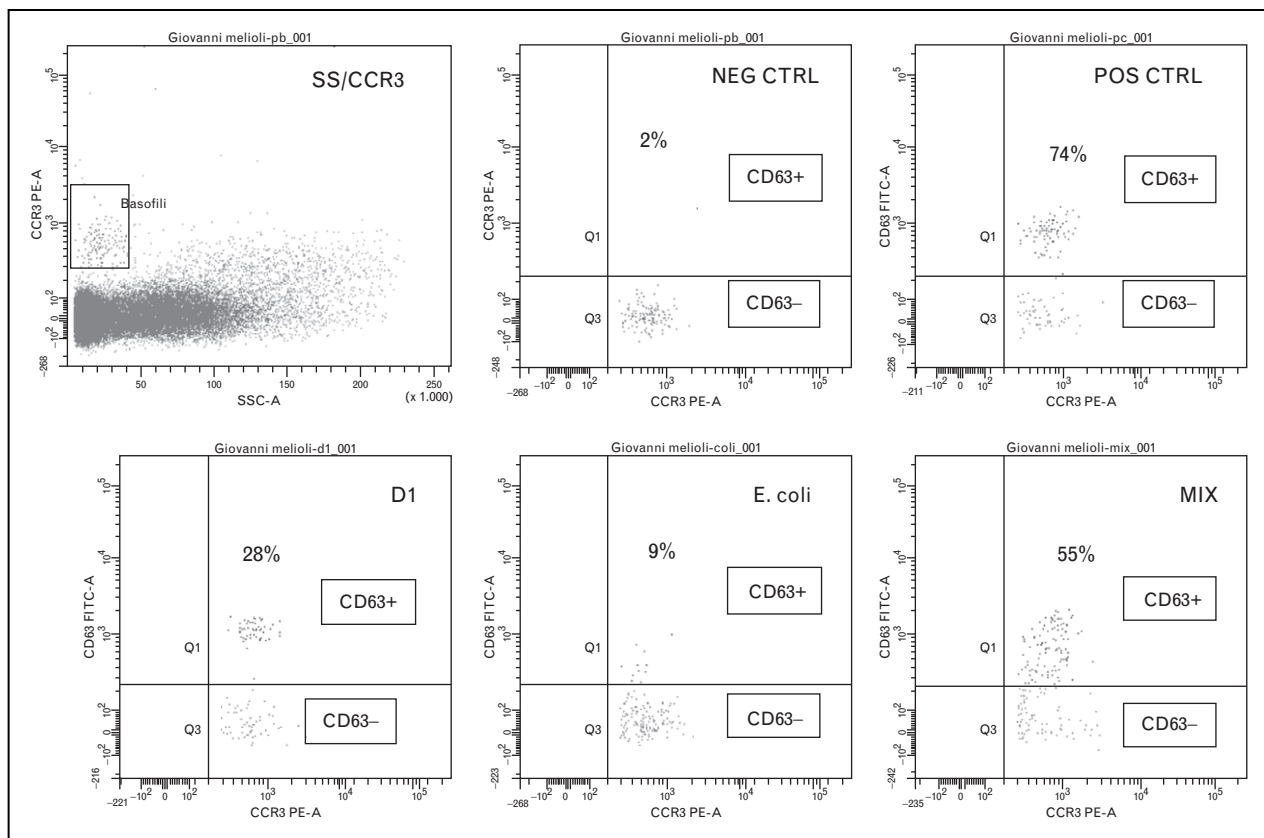


**FIGURE 1.** Basophil activation test. Synergic effect of suboptimal doses of allergen and microbes.

presence of ineffective doses of allergen and vice versa, thus offering an intriguing explanation for the asthma exacerbation, mediated by factors released by basophils, during infections.

## CONCLUSION

Despite the large number of studies performed in this field, the reason why asthma exacerbations occur during infections of the respiratory tract is still uncertain. Several pathogens as well as other mechanical, immunological and biochemical causes have been taken into consideration. The recent demonstration that basophils, in the presence of nonactivating doses of allergens, acquire the capacity of degranulating in the presence of bacteria seems to have shed some light on this argument. In addition, the evidence that antibiotic therapy may be active and that the mechanisms of recovery are strengthened in the presence of drugs reducing the microbial contamination indicates that, at least in certain conditions, the microbe–allergy association can be considered as the start of asthma exacerbation and any possible control of this possibility should allow more efficient control in the patients.

## Acknowledgements

The authors thank Associazione Ricerca Malattie Immunologiche e Allergiche (ARMIA) for supporting the literature review and Lisa Foppiano for linguistic revision and English editing.

## Conflicts of interest

The authors have no conflict of interest to declare.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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