Dossier : Allergy

Antimicrobial strategies: An option to treat allergy?

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Abstract

Respiratory infections by bacteria and viruses often trigger symptoms of asthma in both adults and children. This observation and subsequent mechanistic studies have demonstrated important interactions among allergens, microbes and the atopic host. The mechanisms responsible for microbe-induced asthma exacerbations are only incompletely understood. A focal point of current research is the inflammatory response of the host following an encounter with a pathogenic microbe, including variations in chemokine and cytokine production and resulting in changes in bronchial hyper-responsiveness and lung function. Direct bronchial infection, exposure of nerves with resulting neurogenic inflammation and a deviated host immune response are among the mechanisms underlying these functional disorders. Lately, suboptimal innate immune responses, expressed as defective interferon production, have gained attention as they might be amenable to intervention. This review describes the suggested mechanisms involved in the complex interactions between 'asthmagenic' microbes, the immune system and atopy, based on in-vitro and in-vivo experimental models and epidemiological evidence. In addition, it provides a synopsis of potential therapeutic strategies either directly against the microorganisms or in respect to the associated inflammation.
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1. Introduction

The prevalence of allergies and asthma has been increasing, especially in children, for the last several decades. Modern lifestyle and various environmental factors significantly influence the onset of these complex, chronic disorders. Considerable research effort has focused on the potential effects of exposure to pollutants, aeroallergens and infectious agents that could adversely affect lung development or function but also precipitate asthma exacerbations [1].

It is widely recognized that respiratory viral infections are among the most important triggers of asthma exacerbations, both in children and adults [2−4]. The association between upper respiratory viral infections and asthma exacerbations in children was demonstrated almost three decades ago using virus cultures and serological techniques [5]. These findings were subsequently confirmed and expanded using more sensitive techniques for virus detection, such as reverse transcription polymerase chain reaction (RT−PCR) assays in well-designed longitudinal studies [6−9]. After implementation of these techniques more than 80% of reported episodes of wheeze or drop in lung function could be attributed to respiratory viral infections [7], rhinovirus (RV) being the most prevalent virus. Similar studies in adults implicate respiratory pathogens in almost half of the exacerbations, rhinovirus being once again the prevailing virus [8,10,11].

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In addition, there is the evidence for an association between 'atypical' bacterial respiratory pathogens and the pathogenesis of asthma, with *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* most commonly implicated. However, many studies investigating such a link have been uncontrolled and have provided controversial evidence, mainly reflecting the difficulty in accurately diagnosing infection with these pathogens [12,13].

Taking into account these strong associations, it is conceivable that antimicrobial agents and/or strategies may have the potential to reduce the burden of asthma-associated morbidity.

2. 'Asthmagenic' viruses

The majority of viruses implicated in the pathogenesis of asthma exacerbations are single-stranded RNA viruses, including RV, influenza and parainfluenza (PIV) viruses, respiratory syncytial virus (RSV) [3,7,14], coronaviruses [15] and the newly described human metapneumovirus (MPV) [16] and bocavirus [17—19]. Among the double-stranded DNA viruses adenoviruses have also been involved [20].

In the human respiratory tract, all the above agents are able to produce a spectrum of clinical acute infection phenotypes, ranging from the common cold, croup and acute bronchiolitis, to pneumonia, although each virus has increased propensity for a particular clinical disease (e.g. parainfluenza for croup, RSV for severe bronchiolitis, influenza for pneumonia) [21,22]. There is some evidence that adenovirus can also cause a latent infection in the human lung [23].

The spectrum of viral-triggered asthma exacerbations and reported viral prevalence may vary according to various factors. The age of the subjects is important [24], since, overall, the frequency of viral respiratory illness is highest in children up to 4 years of age, gradually declines in teenagers, rises again in young parents exposed to children, re-declines in older adults, while the elderly are again more susceptible [25]. In addition respiratory viral infections have strong seasonal patterns although sporadic cases or nosocomial outbreaks can occur [6,26—30]. The presence or absence of a lipid-containing envelope affects viral survival in the environment [31,32]. In temperate areas, the enveloped viruses, (e.g. influenza virus, RSV and coronavirus), are, characteristically, prevalent during middle-winter periods, whereas non-enveloped ones, such as RVs, are found most often in spring and fall. RV characteristically produces epidemics soon after children return to school. September epidemics of asthma exacerbations coincide with such increase in the rate of respiratory track infections [28,29]. There is evidence that different infectious agents may induced asthma exacerbations of varying severity, however, stronger evidence is needed in this respect [6,33—35]. Finally, the type of diagnostic test used to identify infection may considerably affect epidemiological results [7,36].

Although RSV remains the agent associated with the majority of cases of bronchiolitis requiring hospitalization, recent evidence suggests that in the community RV is the most prevalent virus at all ages [35]. Furthermore, with a few exceptions, studies assessing virus-induced exacerbations of asthma have shown that RVs are the prevalent agents, attributing for 50—80% of virus-confirmed cases or around half of all exacerbations studied [7,8]. There is also evidence that RV may be more 'asthmagenic' than other viruses (Papadopoulos et al, unpublished).

RVs belong to Picornaviridae family and probably represent the most abundant pathogenic microorganisms universally. These viruses have small RNA genomes are non-enveloped and are capable of surviving on surfaces for several hours under ambient conditions [37]. More than 100 serotypes of RVs are identified and numbered. They are divided into major (90%) and minor (10%) groups depending on their receptor specificity. Major RVs attach to the intracellular adhesion molecule-1 (ICAM-1) while minor group RV binds the low-density lipoprotein receptor. In vitro and in vivo, RV infects the bronchial epithelium and upregulates a range of pro-inflammatory cytokines, chemokines, adhesion molecules, mucins and growth factors, all of which are thought to contribute to lower airway inflammation and consequent effects on lung function [38,39]. A large number of these mediators are upregulated partly or solely through the transcription factor NF-κB [40—42].

3. Viral infections and acute exacerbations of asthma

Several mechanisms have been suggested as part of the complicated pathways leading from a viral infection to an acute asthma exacerbation. These include direct infection of the lower respiratory tract [43,44], induction of local inflammation [38,41,45], increase in bronchial reactivity [43,44, 46,47] and induction of bronchial obstruction [48]. Local inflammation produced after bronchial epithelial cell infection, neurogenic inflammation induced directly or indirectly through the epithelium, and the immune response of the host are probably the most important.

There is increasing evidence that the epithelium of the lower airway does not simply act as a physical barrier. Not only it has important regulatory role on the immune response inasmuch it may act as an antigen presenting structure [49] but also contributes to the inflammatory response following a viral infection through the production of cytokines and chemokines (e.g. IL-6, IL-8, IL-11, TNF-α, RANTES, GM-SCF, eotaxin 1 and eotaxin 2) that attract inflammatory cells involved in asthma exacerbations [50,51]. The epithelial cells' structure and function is altered after the infiltration with inflammatory cell and the oedema of the airway wall.

It has been recently suggested that epithelial cells from asthmatic subjects may have an abnormal innate response to infection by RVs, resulting in increased virus replication and cell lysis compared with cells from healthy normal controls. Cells from asthmatic individuals did not produce enough interferon-beta (IFN-β) in response to infection, leading to a reduced apoptosis rate, a consequent increase in viral replication within the cells and finally increased cytotoxicity because of the increased viral load [52].

Another proposed mechanism by which acute infections might enhance airway narrowing and hyper-responsiveness is the stimulation of the airway neural network which may lead to neurogenic inflammation [39,44,53,54]. Virus-mediated damage to the epithelial layer can expose the dense sub-epithelial nerve endings, increasing stimulation of sensory nerves by inhaled particles or pro-inflammatory mediators. Sensory nerves can directly release neuropeptides which may trigger reflex bronchoconstriction. Among the mediators of neurogenic inflammation, nerve growth factor (NGF) may have an important role in the pathogenesis of hyper-responsiveness induced by respiratory viruses. It has been documented that NGF induces a selective up-regulation of the high-affinity neurokinin 1 receptor (NK1) for the tachykinin substance P (SP) [55]. SP is a neuropeptide released from sensory nerves with both bronchoconstrictive effects and immunomodulatory properties which regulates the functions of all white blood cells by affecting their migration and response to various mitogens and allergens [56]. One recent study [57] focused on neural development in the lungs during early life and has proposed that this process is under the control of NGF and its corresponding receptor TrkA. These factors control the branching of nerves into the developing lungs and are downregulated with age. NGF is strongly upregulated during RSV infections, especially in infants and such overexpression may result in prolonged viral clearance from the infected epithelial cells.

Finally, another interesting pathway attributes to protracted inflammation, associated with an imbalance in T_H1/T_H2 immune responses. In the lower airways of atopic asthmatic individuals a T_H2 environment predominates. Although IFN-γ, and IL-12 (T_H1 cytokines) are produced both in normal and atopic asthmatic subjects, the ratio between IFN/IL-4 is considerably reduced in asthmatics compared with normal subjects [58]. Furthermore, atopic individuals may have impaired antiviral responses concerning IFN-α [59,60] or/and IFN-β [52] or/and IFN-γ [50,61,62] reduced secretion, something that may result in prolonged bronchial inflammation and increased asthma severity. It has been also demonstrated that this impairment is extended to cell recruitment, since in asthmatic patients there seems to be an increased number of eosinophils, compared with normal individuals which also indicates a difference in the immune response to viral infections [54,63].

4. Interaction between viral infections and other environmental stimuli

In the natural history of asthma exacerbations, interactions between viral infections and other environmental stimuli are often noted. In several occasions synergistic effects have been shown. This is important as it implies that therapeutic results may occur with treatment of only one of such factors. An association between upper respiratory tract infection and air pollution, especially NO_2, and tobacco smoke exposure, has been observed in children [64−66]. There are several potential mechanisms by which pollutants can exacerbate

asthma interacting with respiratory viral infections. Direct effects of the pollutant on the airways include epithelial damage and an acquired ciliary dyskinesia; release of pro-inflammatory mediators and increases in IgE concentration may follow. Indirectly NO_2 can also impair local antiviral immunity in the airways [67−69].

Recent studies have suggested that viruses and allergens may have a synergistic effect on individuals with asthma [43,70]. This has been shown for both clinical outcomes [71] (symptoms severity) and in experimental models, where there is evidence that viral infections enhance allergen induced inflammatory responses, eosinophil recruitment, histamine release and late phase airway response [72].

5. Atypical bacterial infections and asthma

Although there is increasing evidence from controlled studies to support an association between atypical bacterial infection and both chronic stable asthma and acute exacerbations of asthma, it is still unclear whether such association is causal or patients with asthma are just more susceptible to colonization and/or infection with atypical bacteria.

Nevertheless, case reports, but also controlled trials during several decades have suggested that postinfectious wheezing may respond to antibiotic therapy, in particular to macrolides [73,74]. Problems with diagnostic techniques for acute and chronic infections with *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* have made difficult to conduct and interpret epidemiologic studies of the potential relation between these microorganisms and asthma. To add to this complexity, macrolides and ketolides have been shown to have anti-inflammatory properties [75,76], making it difficult to assess the true role of infection.

In asthmatic patients, exacerbations can be associated with an increase in antibody titres to *Chlamydophila pneumoniae* or *Mycoplasma pneumoniae* [13,74,77,78]. It has recently been proposed that *C. pneumoniae* might modulate epithelial cell apoptosis by upregulating both pro-apoptosis and anti-apoptosis genes. It has been suggested that *C. pneumoniae*-induced inhibition of apoptosis increases the longevity of the host cell, enhancing the survival of *C. pneumoniae* in patients with chronic asthma [79]. Consequently, bronchial infection with atypical bacteria is likely to be associated with increased airway inflammation and thus possibly increasing asthma severity and airway remodelling. Although, these organisms are common causes of infection, not all infected patients develop or exacerbate their asthma. This suggests that certain individuals may be genetically predisposed to the chronic effects of atypical bacteria, or be genetically susceptible to infection [52], rendering them more likely to be persistently infected. Only a few studies have investigated the possibility of such susceptibility. In one of them [80], isotype-specific serologic tests have been performed for *C. pneumoniae*, and the results have been compared with variations in mannose-binding lectin (MBL), a complement component that is important in clearance of respiratory pathogens. The presence of variant alleles in MBL was associated with increased susceptibility to other

types of respiratory infections, significantly increasing the risk of asthma development among children infected with *C. pneumoniae*. On the other hand, a recent study, detailed below, showed improved outcomes when a ketolide was used in patients with asthma exacerbations [81].

6. Therapies

Although there has been much progress in understanding the mechanisms of microbe-induced asthma exacerbations, there is a need for the development of new therapeutic agents as well as preventive strategies. Both antimicrobial and immune modulators could have therapeutic benefits in this respect.

Rhinovirus is the key virus accounting for the majority of exacerbations both in children and adults and thus the effective treatment or prevention of that infection would be a major asset in asthma therapy. Unfortunately, there is currently no available antiviral therapy of clinical value and vaccination seems to be far away because of the large number of RVs' serotypes (more than 100). Although this genetic diversity has hampered vaccine development, modern vaccination strategies (such as recombinant proteins, reverse genetics, replication defective particles and other techniques) may make it feasible to induce cross-reactive neutralizing antibodies to the majority of serotypes and produce an effective vaccine [82].

In contrast to RV, RSV has gained more attention because of its association with severe bronchiolitis in infancy. Ribavirin, although initially promising, did not find a place in majority of cases, although still included among possible choices for severe bronchiolitis. Passive immunoprophylaxis by monthly administration of anti-F monoclonal antibody (palivizumab) reduces the risk of lower respiratory tract RSV disease and hospitalization in high-risk infants and children [83]. However, it cannot be used widely or in an outpatient basis. No vaccine against RSV is available yet, but studies of intranasal live-attenuated vaccine in children and injected subunit vaccine in elderly persons are ongoing [84].

Influenza viruses A and B cause annual outbreaks of illness worldwide. A variety of antiviral agents are available for treatment of influenza. The previous generation of agents, amantadine and rimantadine, have demonstrated clinical efficacy, however, potential side effects and most importantly resistance considerably reduced its usefulness [85–87]. The more recent neuraminidase inhibitors, zanamivir and oseltamivir, are active against both A and B viruses, including the avian influenza A/H5N1 strain [87,88], and are promising as important tools against a pandemic. More possibilities for anti-influenza agents are being explored [89]. Influenza vaccination is available in two forms: an intramuscular preparation containing formalin-inactivated virus and purified surface antigen and an intranasal spray containing live attenuated viruses [90]. The efficacy of these vaccines is approximately 70–90% in young adults, especially when the vaccine antigen and the circulating strain are closely matched. Immunization in healthy working adults is

associated with fewer upper respiratory illnesses and fewer visits to physicians' offices [90–93]. However, the use of influenza vaccines in reducing virus-induced exacerbations remain controversial [94,95]. Concerning the other asthmagenic viruses (coronaviruses, adenoviruses, human metapneumovirus, bocavirus), clinically available therapeutic or prophylactic agents are still awaited.

As mentioned above, a causal link between deficient interferon-impaired apoptosis and increased virus replication has been demonstrated, suggesting that type I interferons might be useful in the treatment or prevention of virus-induced asthma exacerbations. Type I IFNs include the numerous IFN-αs, IFN-β and the newly identified IFN-λs [96]. In the past, IFN-α2 was shown to be effective when given prior to experimental RV infection [97–99], or as a prophylactic therapy [100,101], in a context of natural RV infections, however cost and side-effects have prevented its exploitation in the common cold and/or asthma exacerbation fields. IFN-β has not been very effective in preventing experimental or natural RV colds [52,102–104], but its effects on asthma exacerbations have not been investigated. Promising data have been recently published about IFN-λs [105]. It should be noted that in addition to the antiviral approach, combinations of IFN-α with intranasal ipratropium, or oral naproxen, or chlorpheniramine, or ibuprofen have been tested with promising results, but these were also not commercialized [106,107].

Although quite active in vitro [108], glucocorticosteroids (GCs) so far have been disappointing in their ability to control symptoms in models of experimental RV challenge of asthmatics [109,110] and high-dose steroids remain only partially effective at controlling virus-induced exacerbations of asthma [111,112]. A synergistic effect of GCs with long-acting β_2 agonists (LABA) has been shown both in in vitro studies and clinically. LABAs act via a G protein coupled receptor, activate adenylate cyclase and through the second messenger cAMP, induce intracellular signalling events affecting a broad range of physiological processes, providing by this way an extra potentiality to enhance the anti-inflammatory properties of GCs when acting together in a combination therapy [113]. Studies have confirmed clinical benefit in exacerbations, although the viral origin of these events has not been confirmed [114,115].

Evidence suggests that leukotrienes play a key role in viral-induced respiratory illness [116,117]. The leukotriene receptor antagonist, montelukast, has proven efficacy in the control of asthma exacerbations in adults [118], but also in preschool and school children with persistent [119,120] and intermittent asthma [121]. In addition, montelukast significantly reduced symptoms and exacerbations from respiratory syncytial virus postbronchiolitis in infants without asthma [122].

Other agents, including antihistamines [123] and antioxidants [124] can block pro-inflammatory mechanisms induced by virus infections in airway epithelial cells, although in vitro evidence has not been paralleled by convincing clinical data.

Although viral infections are of major interest, the potential role of antibacterial therapy should also be discussed. A

number of different antibacterial agents, namely tetracyclines, macrolides, quinolones, azalides and the ketolide telithromycin have in vitro and in vivo activity against the common atypical bacteria *C. pneumoniae* and *M. pneumoniae* [125−128]. Clarithromycin, roxithromycin, azithromycin [73,74,129,130] and recently telithromycin [81], have shown some clinical benefit in patients with chronic stable asthma or acute exacerbations. In the most recently published double-blind, randomized, placebo-controlled study [81] telithromycin (at a daily dose of 800 mg for 10 days) provided improvement in symptoms and lung function among adult patients with acute exacerbations of asthma. The study design did not incorporate an analysis of the mechanism by which telithromycin was associated with improvement, but the data imply a benefit not solely attributable to an antimicrobial effect. It should be noted that resistance need also to be considered before initiation of long term therapy in order to control or to prevent probable bacteria-induced asthma attacks.

Although promising, many questions should be answered before antibacterial therapies can be proposed for the treatment or prevention of asthma exacerbations. Certainly, a combination of the antimicrobial with anti-inflammatory approaches seems reasonable. There is evidence suggesting that a window of opportunity exists between appearance of a viral infection and the initiation of an exacerbation [131]. On the other hand, more controlled studies with macrolides, especially in children, are urgently needed as management of exacerbations is an important unmet need in this age group.

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