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REVIEW

Respiratory infections and asthma

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Summary Respiratory tract infections caused by both viruses and/or atypical bacteria are involved in the pathogenesis of asthma. In particular, several viruses such as respiratory syncytial virus, rhinovirus and influenza/parainfluenza viruses may favour the expression of the asthmatic phenotype, being also implicated in the induction of disease exacerbations. Within this pathological context, a significant role can also be played by airway bacterial colonizations and infections due to Chlamydiae and Mycoplasmas. All these microbial agents probably interfere with complex immunological pathways, thus contributing to induce and exacerbate asthma in genetically predisposed individuals.

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Contents

Introduction	776
Asthma and viral infections	776
Respiratory syncytial virus (RSV)	776
Rhinovirus (RV)	777
Influenza and parainfluenza viruses	778
Asthma and bacterial infections	779
Chlamydia pneumoniae	779
Mycoplasma pneumoniae	780
Hygiene hypothesis and asthma	780
Conclusions	782
References	782

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Introduction

Asthma is a chronic inflammatory airway disease characterized by bronchial hyper-responsiveness to a wide range of stimuli, recurrent episodes of wheezing, breathlessness, chest tightness and coughing, which are associated with reversible airflow limitation.¹ The important medical, social and economical implications of asthma depend on its very large epidemiological impact, in that over 150 million people worldwide are affected by this disease, whose prevalence is on the rise especially in the industrialized countries.² Such alarming numbers are prompting huge investments of financial and human resources, aimed at further elucidating the pathogenic mechanisms underlying asthma, as well as at developing new and more effective anti-asthma treatments.

Current clinical and experimental evidence implies that the two essential components of the asthmatic phenotype include bronchial inflammation, mainly involving T lymphocytes, eosinophils and mast cells, and airway remodelling, characterized by structural changes spanning throughout epithelial lining, subepithelial mesenchymal layers, airway smooth muscle, and bronchial vessels.³ These pathophysiologic patterns arise from an intricate network of interactions between genetic and environmental factors, including allergens, pollutants, and infectious agents. With regard to the latter, their role is indeed very intriguing, in that it has been hypothesized that respiratory infections, in addition to promoting both the development and the exacerbations of asthma, might also exert a protective effect against this disease and atopy.^{4,5} Therefore, the aim of this review is to provide a synthetic update about the complex relationships between asthma and respiratory infections caused by either viruses or bacteria.

Asthma and viral infections

In both children and adults, viral infections of the airways may be associated with the development of chronic asthma, as well as with acute disease exacerbations.⁶ These associations can now be reliably confirmed by currently available diagnostic, molecular tools such as enzymatic amplification of viral nucleic acids through polymerase chain reaction (PCR).⁷ Although PCR is not yet widely used in routine medical practice, this technique is very useful for identifying respiratory viruses, especially when performed on samples of induced

sputum, thus being much more efficient than traditional serology and immunofluorescence. With regard to the pathogenesis of asthma and its exacerbations, the most commonly involved viruses include respiratory syncytial virus (RSV), rhinovirus (RV), influenza and parainfluenza viruses, coronavirus, enterovirus, and adenovirus.⁸ Wheezing-associated viral infections are characterized by an age-related distribution. In particular, RSV predominates in children under 3 years of age, whereas RV represents the most frequent cause of infectious respiratory illnesses affecting older children and adults⁹; influenza and parainfluenza viruses affect all age groups.

Respiratory syncytial virus (RSV)

With regard to RSV, it is noteworthy to point out that this virus infects about 70% of infants within the first year of life, and almost 100% by the age of 3 years.¹⁰ RSV often produces only non-complicated infections of the upper airways. Nevertheless, in some cases RSV causes a severe bronchiolitis which is frequently associated with the development of recurrent wheezing and asthma. However, it is not yet clear whether RSV constitutes a direct cause of asthma, or if it rather induces airway obstruction exclusively in individuals with a genetic predisposition to asthma. Therefore, it is very difficult to establish whether the bronchiolitis caused by RSV simply accelerates the onset of asthma, or if this virus instead represents a crucial factor for the subsequent development of the allergic sensitization which characterizes many asthmatic patients. In this regard, conflicting data have been indeed provided by various studies, some of which have allowed to detect, in contrast with others, a close relationship between RSV-induced paediatric bronchiolitis and the clinical manifestations of atopy and asthma. For instance, Stein and colleagues carried out a wide perspective investigation on more than 1000 children, studied since their early infancy until the beginning of adolescence. These authors found that the lower respiratory tract infections caused by RSV within the first 3 years of age, though not so severe to require hospitalization, were associated with an increased risk of wheezing by the age of 6.¹¹ However, such a risk subsequently subsided progressively, thus resulting to be not significant by the age of 13. On the contrary, the Scandinavian group led by Sigurs has more recently shown that children who contracted a severe RSV infection within the first year of life experienced, until the age of about 7 years, a higher frequency of bronchial

obstruction and atopic asthma when compared with children not previously affected by a serious bronchiolitis.¹² Moreover, by continuing to follow-up their study population, the same authors have further confirmed that the risk of developing allergic asthma persisted, until the age of 13, in those subjects who were hospitalized during their first year of life because of bronchiolitis produced by RSV.¹³

Several cellular and molecular mechanisms might contribute to determine an atopic phenotype in children and adolescents with a history of severe forms of bronchiolitis caused by RSV during early childhood. Indeed, RSV may be responsible for an increased risk of allergic sensitization by favouring the creation of a bronchial and bronchiolar micro-environment characterized by high levels of interleukin-4 (IL-4).¹⁴ This cytokine represents the most important factor determining the expansion of Th2 lymphocytes, the cells primarily implicated in the pathogenesis of allergic processes. Furthermore, IL-4 is directly responsible for the antibody class switching which promotes the production of IgE by B lymphocytes. The different biological behaviours induced by RSV might be explained by the tendency of most children with a common, mild RSV infection, to develop a predominantly Th1-mediated antiviral immune response, characterized by an intense production of interferon- γ (IFN- γ). Otherwise, a Th2-dependent immune response associated with high IL-4 levels can occur in very young patients affected by severe bronchiolitis. In this regard, it has been suggested that the individual susceptibility to the development of both allergic sensitization and serious respiratory viral infections, may be due to genetic factors. In particular, a genetically determined delay in the physiological, early post-natal immune deviation from the prevalent Th2 foetal pattern towards the maturation of a Th1 phenotype, could predispose to the amplification and propagation, within the airways, of inflammatory events triggered by both respiratory viruses and inhaled allergens.¹⁵ This hypothesis has been confirmed by the high frequency, recently shown in a population of 105 Korean children hospitalized for severe RSV infections, of a haplotype detected inside the cytokine gene cluster located on the long arm of chromosome 5 (-589T IL-4 gene polymorphism), associated with an overexpression of IL-4.¹⁶ Within such a biological context, a key function is likely exerted by a specific RSV glycoprotein, named protein G, which is able to promote a Th2 immune response.¹⁷ In fact, by comparatively evaluating the consequences of experimentally induced infections caused by either normal or G protein-defective

strains of RSV, it has been observed in mice that this protein plays an essential role in producing airway inflammation and functional respiratory impairment, both evoked by wild-type RSV.¹⁸ Moreover, the RSV-elicited activation of an antiviral protein kinase may favour, in B cells, isotype switching to IgE.¹⁹

Finally, it is also relevant that RSV may contribute to airway neurogenic inflammation by inducing the expression of bronchial NK-1 receptors,²⁰ stimulated by substance P. This effect can thus lead to an amplification of the proinflammatory action of substance P, released by the sensory endings of unmyelinated C-fibres. The importance of such a mechanism is experimentally supported by the demonstrated efficacy of the monoclonal antibody palivizumab, directed against the viral protein F, in reducing the excessive neural inflammation found in the airways of RSV-infected rats.²¹

Rhinovirus (RV)

Differently from children under scholar age, during the following periods of childhood and adolescence, as well as in adults, about 60% of the viral, upper respiratory tract infections involved in asthma exacerbations, are caused by RV.⁸ Therefore, this virus is not only responsible for the common cold, but it also exerts a relevant pathogenic action in acute asthma relapses. From a clinical and functional point of view, airway infection due to some particular RV strains, such as the RV16 serotype, produces in allergic patients a recrudescence of asthmatic and rhinitic symptoms, a deterioration of respiratory function, and an increase in bronchial hyper-responsiveness.²² The latter may persist for several weeks. Indeed, it is now strongly evident that RV, in addition to infecting the upper airways, may also colonize the lower respiratory tract.

The prevalent location of RV-dependent tissue damage is represented by the bronchial epithelium, whose remarkable fragility typical of asthma makes it particularly susceptible to the viral cytotoxic effect, which in turn further facilitates the penetration of allergens and other noxious agents thus triggering a positive feed-forward circuit. The cytopathic action exerted by RV on airway epithelial cells likely constitutes the initial event in asthma exacerbations induced by this virus. The airway epithelial receptor for most RV strains is intercellular adhesion molecule-1 (ICAM-1), which also significantly contributes to the pathogenesis of allergic inflammation.^{23,24} Papi and Johnston²⁵

have shown that RV is able to up-regulate ICAM-1 expression via activation of the transcription factor nuclear factor-kappa B (NF- κ B), thereby increasing its own pathogenic potential. Moreover, Bianco and colleagues have found that also IL-4 and other Th2 cytokines stimulate the airway epithelial expression of ICAM-1, thus increasing the susceptibility of asthmatic patients to RV infections.²⁶ On the other hand, NF- κ B is also involved in transcriptional activation of many genes encoding proinflammatory cytokines and chemokines. In this regard, it is now clear that intracellular oxidant radicals play a key role in RV-induced stimulation of NF- κ B activity occurring in bronchial epithelium.²⁷ These recent advances about the molecular mechanisms underlying the pathogenic action of RV have important therapeutic implications, in that both corticosteroids and reducing agents are able to inhibit the activation of transcription factors responsible for the enhanced expression of proinflammatory genes encoding adhesion molecules and various cytokines.

In particular, airway epithelial cells react to RV infection with an inflammatory response mediated by an increased biosynthesis of interleukins (IL)-6, 8, and 16,²⁸ involved in maturation of T and B lymphocytes as well as in fibroblast proliferation (IL-6), recruitment of neutrophils (IL-8), and chemotaxis of eosinophils and monocytes (IL-16), respectively. Bronchial epithelial cells infected by RV also produce high amounts of the chemokines eotaxin and RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted),²⁸ which play a powerful chemotactic role in eosinophilic airway infiltration. Furthermore, by stimulating the epithelial production of fibroproliferative and angiogenic factors such as the fibroblast growth factor-2 (FGF-2), RV extends its actions to the subepithelial layers, thus contributing to the structural changes responsible for airway remodeling, another essential component of asthma phenotype. Therefore, RV affects the functions of both resident and immune/inflammatory cells, thereby further addressing the immune response of atopic, asthmatic patients towards a predominant type 2 pattern, already characterized by an increased secretion of IL-4. In these subjects, the concomitant reduced production of type 1 cytokines such as IFN- γ , may explain at least in part the defective antiviral defences. Asthma is also characterized by an ineffective presentation of viral antigens, that contributes to impair the immunological mechanisms aimed at neutralizing RV. The resulting persistence of RV infection can thus lead to an exacerbation of asthma symptoms and bronchial hyper-responsiveness.²⁹

Influenza and parainfluenza viruses

The airway immune inflammation occurring in many asthmatic patients can be further amplified by acute viral infections caused by influenza viruses. The latter often exacerbate respiratory symptoms and bronchial responsiveness to allergic stimuli. Utilizing an animal model of asthma, Dahl and colleagues have recently shown that the influenza A virus induces pulmonary changes which last for several weeks after infection resolution.³⁰ In particular, these authors observed that a previous flu infection was able to enhance the cellular response to a subsequent allergen sensitization, as it can be inferred from the increased numbers of eosinophils, lymphocytes and macrophages, detectable in the bronchoalveolar lavage fluid (BALF) obtained from mice infected with influenza A virus when compared with control animals undergoing allergen sensitization, but not previously exposed to viral infection. Influenza viruses also contribute to increase neurally mediated bronchial hypertone, in that viral neuraminidases degrade the sialic acid residues of prejunctional muscarinic M_2 receptors.³¹ By inhibiting acetylcholine release from postganglionic fibres, these cholinergic autoreceptors exert an inhibitory control on vagal neurotransmission. Therefore, the influenza virus-dependent M_2 receptor dysfunction is responsible for an exaggeration of reflex parasympathetic bronchoconstriction.

Endotracheal inoculation of parainfluenza virus induces, in animal models, biological and histological changes reminiscent of asthma. In particular, this viral infection elicits an airway influx of inflammatory cells, bronchial hyper-responsiveness, epithelial damage and bronchiolar fibrosis. Such two latter alterations are likely due to an increased production of transforming growth factor- β (TGF- β) from parainfluenza virus-stimulated alveolar macrophages.³² In fact, it is well known that TGF- β has fibrogenic properties and also induces apoptosis of airway epithelial cells.³³ This suggests that the recurrence of parainfluenza virus infections may contribute, in patients with asthma, to the airway structural changes typical of the disease and at least in part mediated by TGF- β . Apoptosis of bronchial epithelial cells may be viewed as a host attempt to limit viral replication, but at the same time cell death is likely a major determinant of the epithelial damage occurring in asthma. Furthermore, epithelial loss might be the main cause of the defective bronchial synthesis of nitric oxide (NO), detected in association with the parainfluenza virus-dependent increase in airway hyper-responsiveness.

Asthma and bacterial infections

With regard to the role of non-viral infections in asthma inception and exacerbations, the atypical bacteria *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* are currently believed to be the most involved pathogens.³⁴

Chlamydia pneumoniae

C. pneumoniae is a Gram-negative, obligate intracellular bacterium, entirely dependent on energy produced by infected host cells, where it replicates forming characteristic cytoplasmic inclusions. Within the respiratory system, the main cellular targets of this pathogen include epithelial and endothelial cells, as well as monocytes/macrophages. *C. pneumoniae* may cause infections of the upper and lower respiratory tracts.³⁵ Furthermore, recurrence and/or persistence of respiratory infections caused by *C. pneumoniae* are likely implicated in the development and worsening of asthma.³⁶ On the other hand, this microbial agent is characterized by an innate tendency to determine chronic infections, which result to be particularly insidious in that they are frequently quiescent and non-symptomatic. Several different genetic and environmental factors, also including coexistence of other diseases, smoking habit, and the therapeutic use of corticosteroids, contribute to promote the persistence of *C. pneumoniae* infectious capacity.

A number of epidemiological studies have shown that many asthmatic patients have high serum levels of both IgG and IgA anti-*C. pneumoniae* antibodies, which can be considered as markers of persistent infection. In particular, Hahn and colleagues were the first to identify a consistent association between wheezing and anti-*C. pneumoniae* antibody titres in 365 subjects with respiratory symptoms.³⁷ Moreover, these authors also demonstrated that Chlamydial infection was a risk factor for asthma onset in adults. In fact, within the study population, 9 of 19 symptomatic asthmatics exhibited serologic evidence of either a current or a recent infection caused by *C. pneumoniae*. The same authors have further extended their observations thus showing, in adult patients with recently diagnosed asthma, the existence of serologically positive, chronic respiratory Chlamydial infections.³⁸ In addition, a recurrence of dyspnoeic episodes induced by *C. pneumoniae* may precede the development of chronic asthma in previously asymptomatic individuals.³⁹ Cunningham et al.⁴⁰ studied 108 asthmatic

children for 13 months, thus showing that *C. pneumoniae*-specific secretory IgA antibodies in nasal aspirates were much more elevated in subjects with frequent asthma exacerbations. The latter subgroup of children also tended to maintain a longer PCR positivity for *C. pneumoniae*, indicative of a chronic infection. A further confirm of the tight relationships linking the infections caused by *C. pneumoniae* with asthma severity and exacerbations was provided by Cook and colleagues, who demonstrated in a cohort of 169 asthmatic patients that chronic, severe forms of asthma were correlated to high serum concentrations of anti-*C. pneumoniae* IgG and IgA antibodies.⁴¹ More recently, Black et al. observed in 619 asthmatic adults that elevated serum titres of anti-*C. pneumoniae* IgG and IgA were directly associated with the severity of asthma symptoms, as well as with the use of high doses of inhaled corticosteroids.⁴² These same authors also detected an inverse correlation between antibody levels and airway calibre, assessed by measuring forced expiratory volume in one second (FEV₁). Therefore, it can be argued that chronic Chlamydial infections are likely associated with markers of asthma severity. With regard to the relationships between allergen-induced and infection-dependent asthma varieties, von Hertzen and colleagues investigated 332 asthmatic patients thus finding in non-atopic subjects that their disease was significantly associated, differently from atopic individuals, with high concentrations of anti-*C. pneumoniae* IgG.⁴³ However, it is currently not known whether the association between respiratory Chlamydial infections and asthma is causal or coincidental.

In order to settle such a controversy, an useful contribute might be provided by studies performed on asthmatic patients infected by *C. pneumoniae*, with the aim of evaluating the effects of antibiotics, especially macrolides. In this regard, Black et al. showed a significant improvement in evening peak expiratory flow when asthmatic subjects, serologically positive for Chlamydial infection, were treated for 6 weeks with roxithromycin (150 mg b.i.d.)⁴⁴; subsequently to therapy cessation, there was a decrease in the differences detected in comparison with asthmatic individuals treated with placebo. Furthermore, in those patients undergoing treatment with roxithromycin, a tendency was found, though statistically not significant, towards a reduction of asthma symptoms. Kraft and colleagues also demonstrated that clarithromycin, administered for 6 weeks (500 mg b.i.d.), elicited an increase in FEV₁ and a decrease in the bronchial expression of IL-5, but only in subjects who were PCR-positive for *C. pneumoniae*

or *M. pneumoniae*.⁴⁵ Eosinophil levels diminished in the induced sputum obtained from asthmatic adults treated for 8 weeks with clarithromycin (200 mg b.i.d.), as reported by Amayasu et al., who also found a decreased airway hyper-responsiveness in the same patients.⁴⁶ This latter effect was confirmed by Ekici and colleagues after 8 weeks of treatment with azithromycin (250 mg twice a week), which however did not induce any FEV₁ change.⁴⁷ Moreover, Gotfried et al. have recently shown that clarithromycin (250 mg b.i.d.), given for 6 weeks to asthmatic individuals treated with oral prednisone, allowed to reduce steroid dosage without consequences on asthma symptoms and respiratory function.⁴⁸ In order to correctly interpret all these data, it is however noteworthy that macrolides appear to exert, independently from their antimicrobial properties, a relevant anti-inflammatory action.⁴⁹

During the last few years, a considerable progress has been made in our understanding of the cellular and molecular events underlying the potential role of *C. pneumoniae* in asthma pathogenesis. Generally, the development of diseases induced by Chlamydial infections is dependent on immunopathological mechanisms. In this regard, a 57–60-kDa Chlamydial antigen, which is a member of the hsp60 family of heat-shock proteins, seems to possess remarkable immunogenic, proinflammatory, and cytopathic properties.⁵⁰ The immune response to this protein is likely involved in tissue damage caused by recurrent and persistent Chlamydial infections, and infected cells produce proinflammatory cytokines such as tumour necrosis factor- α (TNF- α), IL-1 β , IL-6 and IL-8.^{36,51} In the airways of asthmatic patients, macrophages and bronchial epithelial cells infected by *C. pneumoniae* could continuously or repeatedly release the hsp60 antigen, thus contributing to perpetuate and amplify chronic inflammation, as well as to induce the structural changes typical of asthma. By activating NF- κ B, in fact, hsp60 is able to stimulate the transcription of genes encoding proinflammatory cytokines and adhesion molecules.⁵² An important feature of the amino acid composition of Chlamydial hsp60 is to share a high homology sequence with the analogous human protein. Therefore, the bacterial hsp60 might evoke an autoimmune reaction, eventually persisting even after infection eradication. Recently, an association has been identified between adult-onset asthma and specific antibodies against another Chlamydial antigen, named hsp10.⁵³ In this regard, it is interesting to point out that a surprisingly elevated percentage (65.8%) of asthmatic subjects, who are serologically negative for Chlamydial

infections, exhibit an anti-hsp10 antibody response.

Taken together, the above-mentioned immunoinflammatory pathogenic mechanisms, triggered by chronic infections caused by *C. pneumoniae*, may thus increase airway susceptibility to other environmental stimuli such as allergens and viruses, thereby accelerating asthma progression. Therefore, within this context antibiotics could play a very important therapeutic role, excellently discussed in recent reviews by Blasi et al.⁵⁴ and Cazzola et al.⁴⁹

Mycoplasma pneumoniae

Both serological and epidemiological studies suggest that infections caused by *M. pneumoniae* may be implicated in the pathogenesis of asthma, also regarding its exacerbations. For instance, Martin et al. evidenced, in about half of 55 asthmatic patients, a PCR-documented presence of *M. pneumoniae*, which was also associated with a higher number of tissue mast cell in comparison with PCR-negative subjects.⁵⁵ Furthermore, administration of chlarythromycin (500 mg b.i.d. for 6 weeks) to PCR-positive asthmatics can significantly improve their respiratory function.⁴⁵ *M. pneumoniae* may also trigger Th2-like cytokine responses, associated with elevated serum IgE concentrations. In experimental animal models, mice infected with *M. pneumoniae* may develop airway obstruction and inflammation.⁵⁶

Airway epithelium represents the most important tissue target of *M. pneumoniae*. In vitro, cultured bronchial epithelial cells respond to experimentally induced *M. pneumoniae* infection by enhancing the synthesis of IL-1 β , IL-6, IL-8, TNF- α , RANTES, and TGF- β .^{57,58} The increase in cell culture supernatant levels of RANTES and TGF- β can be inhibited, as shown by Dakhama and colleagues, by a treatment with either erythromycin or dexamethasone.⁵⁸ This implies that in the airways both macrolides and glucocorticoids are potentially very useful in preventing the inflammatory and structural changes caused by *M. pneumoniae*, and especially the TGF- β -induced damage of bronchial epithelium.^{33,58}

Hygiene hypothesis and asthma

The remarkable increase in the prevalence of allergies and asthma, occurred during the last decades especially in the industrialized countries, is frequently attributed to a group of factors which as a whole are referred to the so-called "hygiene

hypothesis".^{5,59,60} This theory presumes that the lifestyle of westernized societies characterized by improved hygienic conditions and lowered risk of infections, due to widespread vaccinations and decreased overcrowding and family size, is responsible for a reduced exposure to microbial agents especially during infancy and childhood. The result of such a "sterilized" environment would be a reduced activation of the Th1-arm of adaptive immunity, which is stimulated by viral and bacterial infections. Indeed, viruses promote the production of cytokines such as IFN- γ , IL-12 and IL-18, that orientate lymphocyte differentiation towards a Th1 pattern.⁶¹ A Th1 response is also favoured by high doses of bacterial components such as lipopolysaccharide (LPS), which activates the innate immune system by interacting with the toll-like receptor 4 expressed by dendritic cells.⁶² Moreover, a delayed hypersensitivity to *Mycobacterium tuberculosis* can be associated with a low risk of atopy.⁶³ Therefore, a reduced Th1 activation due to a lack of infectious stimuli may contribute to polarize the immune response towards a Th2 phenotype, involved in the development of allergic diseases and asthma.

However, recent epidemiological reports have raised reasonable doubts about the supposed protective role of infections on asthma inception and development. For instance, a large study carried out in Europe on more than 18,000 subjects showed a direct, rather than inverse correlation between the number of siblings and the presence of asthma symptoms and functional pulmonary impairment.⁶⁴ Another investigation, performed on a sample of almost 14,000 British children, did not evidence any influence of pertussis vaccination on allergy and asthma.⁶⁵ The relationship between asthma and bacterial endotoxin is also quite intriguing. Studying 226 children under the age of 5 and with a familial history of atopy, Litonjua et al. have recently demonstrated that exposure to high concentrations of endotoxin in house dust was associated with an increased risk of wheezing, which however rapidly declined within a few years.⁶⁶ In this regard, a previous study had detected a direct relationship between inhaled endotoxin levels and the exacerbation of pre-existing asthma symptoms.⁶⁷ Despite the proposed protective effect of tubercular infection against allergy and asthma, no correlation was found between atopy and delayed sensitization to tuberculin in a population of about 500 African children from Gambia.⁶⁸ On the other hand, an intradermal injection of heat-killed *Mycobacterium vaccae*, performed by Camporota et al. in allergic asthmatic patients, did not elicit significant changes with regard to early and late asthmatic reactions to

allergen challenge, bronchial hyper-responsiveness to histamine, serum IgE levels, and IL-5 production from peripheral lymphocytes.⁶⁹

Furthermore, Holtzman and colleagues have recently proposed a new theory, decisively alternative to the hygiene hypothesis, according to which both Th1 and Th2 branches of the adaptive immune response would be implicated in asthma pathogenesis.^{70,71} In particular, within a genetically predisposed individual context, the asthmatic phenotype would develop as a consequence of a concomitant activation of allergic and antiviral mechanisms. Indeed, respiratory viruses might induce bronchial epithelial cells to produce IFN- γ and IL-12, thus favouring the creation within the airways of a local environment characterized by a Th1 immune pattern. At the same time, inhaled allergens would promote a deviation of the immune response towards a Th2 secretory profile, mainly including IL-4, 5, 9, and 13. Therefore, the Th1 and Th2 arms of the adaptive immune system, rather than antagonizing and reciprocally inhibiting each other, may contribute together to unveil and amplify the asthmatic phenotype, featured by chronic inflammation, bronchial hyper-responsiveness, and airway remodelling. This pathogenetic theory appears to be also supported by the above-mentioned data obtained by Dahl and colleagues.³⁰ These authors inoculated intranasally the influenza A virus into some mice which subsequently underwent, several weeks after viral infection, an allergen sensitization. Under such experimental conditions, the influenza A virus dramatically increased the secretion of IFN- γ from pulmonary dendritic cells, thus inducing a strong Th1 response. The latter did potentiate, rather than inhibiting, the expansion of the Th2 lineage triggered by the following allergic challenge. Within genetically mediated host susceptibility, the dual involvement of Th1 and Th2 cells activated by viral agents and inhaled allergens, respectively, may thus determine the immune-inflammatory changes underlying asthma.

Taking together all these considerations, and keeping in mind the complexity of the asthmatic phenotype, it seems unlikely that the increased prevalence of asthma in the industrialized world may be uniquely due to a reduced exposure to infections during childhood. However, the controversial hygiene hypothesis could not be completely inconsistent, but it probably needs to be revised and referred only to a limited group of infectious agents, such as *Toxoplasma gondii*, *Helicobacter pylori* and Hepatitis A virus.⁷²⁻⁷⁴ The latter appears in fact to play a relevant protective role against the development of atopic disorders.⁷⁴ In any case, a

growing body of both in vitro and in vivo evidence does not corroborate the potential utility for asthma prevention and treatment of the immunostimulatory approach, also based on the use of unmethylated cytosine-guanosine (CpG) dinucleotides derived from bacterial genomes.⁷⁵ CpG DNA sequences are potent inducers of the Th1 immune response, which however could result to be not completely effective in preventing or curing atopy and asthma, in addition to representing an eventual risk factor for the development of Th1-mediated disorders like autoimmune diseases and diabetes.

Conclusions

Respiratory infections may significantly affect, via complex cellular and immunological mechanisms, the development and progression of asthma, also including its exacerbations. On the other hand, routine clinical practice clearly indicates that such infections often cause, in asthmatic patients, a worsening of symptoms and a deterioration of pulmonary function. Therefore, anti-bacterial agents such as macrolides and fluoroquinolones may represent a useful therapeutic tool in limiting both the duration and severity of asthma exacerbations induced by atypical bacteria like Chlamydiae and Mycoplasmas. Moreover, further progresses may be achieved in asthma therapy if pharmacological and immunological research efforts will provide new molecules and vaccines, able to be really effective against those viruses most commonly involved in inducing and exacerbating such a widespread disease.

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