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REVIEW

Virus-induced airway hyperresponsiveness in man

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ABSTRACT: Airway hyperresponsiveness is the most prominent functional abnormality in asthma. Although its aetiology is still unclear, it is well-known that allergen exposure and virus infections can temporarily induce or aggravate airway hyperresponsiveness. Among these environmental factors, virus infections appear to be clinically most relevant, since recent epidemiological studies have shown that most asthma exacerbations in children are associated with positive nasopharyngeal viral identification.

The pathogenesis of virus-induced airway hyperresponsiveness has been investigated by experimental virus infections in animals and in man. Intranasal inoculation and/or inhalation of live attenuated influenza virus, or certain strains of rhinovirus, have been shown to induce airway hyperresponsiveness to various bronchoconstrictor stimuli in man. This indicates that experimental virus-infection, like allergen challenge, is an appropriate investigational model of asthma.

The mechanisms of virus-induced airway hyperresponsiveness are still unclear, but may, in part, be similar to those involved in the pathogenesis of asthma. Currently investigated hypotheses include: epithelial damage or dysfunction, immunological responses, inflammatory mediator release, cholinergic and/or noncholinergic reflexes, and impaired beta-adrenoceptor function. Careful experimental studies, using modern laboratory techniques, are needed to unravel the role of viruses in the development of airway hyperresponsiveness. The results of such studies can potentially lead to an improvement of future asthma management.
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Asthma is a chronic disease, clinically characterized by episodic symptoms of chest tightness and wheezing [1]. This is accompanied by functional abnormalities, such as variable airways obstruction, occurring either spontaneously or following laboratory challenge with inhaled bronchoconstrictor agents [2]. Most patients with asthma show an increase in the ease and degree of airways obstruction in response to inhaled bronchoconstrictors, which is referred to as "airway hyperresponsiveness" [1, 2].

At the bronchial level, asthma is characterized by airway inflammation [3]. Even in mild cases this includes epithelial desquamation, thickening of the subepithelial collagen layer, microvascular congestion, plasma exudate and oedema, smooth muscle hyperplasia and hypertrophy, and (sub)mucosal infiltration with activated lymphocytes, eosinophils and mast cells [3]. This mucosal inflammation in asthma is associated with immunological abnormalities, with distinctive patterns of cytokine expression [4].

In spite of rapidly increasing knowledge on the pathophysiology of asthma, the aetiology and pathogenesis are still poorly understood. This hampers fundamental therapeutic intervention in the disease [5, 6]. One of the causative factors in asthma or its exacerbations might be respiratory virus infections. Epidemiological and experimental studies have indicated that upper respiratory tract infections can indeed lead to the development of airway hyperresponsiveness in man. There is increasing evidence that this is of major importance in clinical asthma, which may have implications in the diagnosis and treatment of the disease.

Asthma and airway hyperresponsiveness

The most relevant abnormality in asthma is acute, potentially life-threatening, airway narrowing [7, 8]. The presence and severity of acute airway narrowing is related to increased airway sensitivity, as well as to an elevated maximal response to inhaled bronchoconstrictor stimuli [7]. These two functional abnormalities are the main components of airway hyperresponsiveness, and can be measured with challenge tests in the laboratory [9].

In general, the degree of airway hyperresponsiveness is only moderately associated with the clinical severity of asthma. This has been observed for both the hypersensitivity (provocative concentration or dose producing a 20% fall in forced expiratory volume in one second (PC_{20} or PD_{20})) [10], and the increased maximal response [11] to histamine or methacholine challenge. Hyperresponsiveness indicates the tendency to develop airways obstruction if an appropriate stimulus is encountered.

Therefore, measurement of airway responsiveness may provide additional information to symptoms and lung function in patients with asthma. It may not only reflect the state of the airways, as a marker of airway dysfunction [12], but may also predict the long-term prognosis of the disease [13].

Measures of airway hyperresponsiveness, such as the PC_{20}, are associated with histopathological abnormalities in bronchial biopsies in "extrinsic" as well as in "intrinsic" asthma [3, 12, 14–19], even though this association has not been confirmed by some investigators [20]. Hyperresponsiveness is associated with epithelial desquamation [14, 15], and with opening of epithelial cell tight-junctions [16]. This fits in with a loss of barrier function, and/or altered metabolic properties of epithelial cells in asthma [9]. In addition, hyperresponsiveness is associated with increased numbers of activated T-lymphocytes and eosinophils [4, 17, 18, 19]. These data have led to the current hypothesis that T-lymphocyte subsets, mast cells and/or basophils activate eosinophils through the release of cytokines, such as interleukin-5 (IL-5) [4, 21, 22], subsequently producing the characteristic pathological abnormalities in asthma [3, 4, 12]. Hyperresponsiveness then develops as a result of increased inflammatory mediator release [23, 24], inflammatory swelling of the airway wall [25], caused by plasma exudation [26] and/or smooth muscle hypertrophy or hyperplasia [27]. Furthermore, hyperresponsiveness might be enhanced by increased thickness of the subepithelial collagen layer [28]. It is likely that neurogenic mechanisms play an important role in these processes, particularly by the local release of pro-inflammatory sensory neuropeptides (tachykinins), the activity of which might be enhanced in asthma [29, 30]. In view of these multiple pathophysiological mechanisms, airway hyperresponsiveness does not seem to have a single cause.

Aetiology of airway hyperresponsiveness

Airway hyperresponsiveness might be determined by genetic factors, as well as by environmental exposures, such as sensitizing agents or virus infections [31, 32]. Hyperresponsiveness is strongly associated with atopy [33], which has been reported to have a (maternal) inheritance on chromosome 11q [34]. However, recently, other investigators have been unable to confirm this type of inheritance, so that, at present, there is doubt whether or not this chromosome is important in atopy [35]. The genetic background of airway hyperresponsiveness itself is also still unclear [36]. The association of its familial component with atopy [31–33] suggests that the inheritance of atopy might enhance the probability of developing airway hyperresponsiveness in predisposed subjects [33, 37].

Environmental exposures seem to play a major role in inducing airway hyperresponsiveness in man [31, 32]. This has been established in epidemiological, clinical, and laboratory studies. Firstly, it is well known that (seasonal) allergen exposure can induce a transient worsening of airway responsiveness to histamine or methacholine in asthma [38]. Since allergen exposure in early childhood is associated with the development of asthma [39, 40], it cannot be excluded that environmental allergens can cause more or less persistent airway hyperresponsiveness. Secondly, environmental toxins, such as occupational sensitizers [41], and cigarette smoke [33, 42], have also been shown to be associated with the induction of airway hyperresponsiveness. All these factors are likely to cause hyperresponsiveness by inducing various inflammatory responses in the airway wall [23].

A history of respiratory illness in childhood is an important risk factor for airway hyperresponsiveness in epidemiological studies [43, 44]. The positive history has not been confirmed by objective criteria in most of these studies, but clinical studies have shown that childhood respiratory illnesses are often associated with viral infections [45, 46]. This suggests that, either viral infections are an early expression of pre-existing abnormalities in the airways associated with hyperresponsiveness [47], or viral infections can lead to the development of hyperresponsiveness [48–50]. There is growing experimental evidence that the latter possibility might play an important role.

Virus infections and asthma

The association between virus infections and asthma can be regarded in different ways. Viruses might be responsible for exacerbations of pre-existing asthma [51]. Then, the infection is a precipitant of a flare-up in asthma symptoms, which has been reported in several clinical and epidemiological studies. Alternatively, based on the association between bronchiolitis in infancy and childhood asthma [46, 52], it can be postulated that virus infections, particularly by respiratory syncytial virus (RSV), contribute to the actual cause of asthma [53]. This would occur, in particular, in a subgroup of children with a genetic predisposition [53]. In both conditions, the viruses are considered to induce airway hyperresponsiveness, either transiently or relatively persistently. This has been examined in epidemiological as well as experimental studies.

Epidemiology

Epidemiological studies have addressed three questions on the association between virus infections and asthma [51]. Firstly, what is the viral identification rate during asthma exacerbations? Secondly, what is the frequency of symptoms during respiratory viral infection in asthmatics? And thirdly, do asthmatics get more respiratory viral infections than non-asthmatics?

During asymptomatic periods, the respiratory viral identification rate in asthmatics seems to be comparable to that in non-asthmatics, at approximately 3% [45, 51]. However, during asthma exacerbations, the identification rate of viruses increases considerably [51], even though the figures vary between different studies.

Initially, cross-sectional studies in children reported that

a virus can be isolated from nose and throat swabs in about 26% of the episodes of wheezy bronchitis [45, 51]. However, by the use of modern virological techniques and polymerase chain reaction, a recent prospective study in children from Southampton has shown a viral identification rate of at least 64% during asthma episodes [54]. In adults, these figures might be less. In a recent cross-sectional study in 17 adult asthmatics presenting to the emergency room, all viral antigen detections and viral cultures were negative [55]. However, in a prospective study in adult asthmatics, BEASLEY *et al.* [56] reported that 10% of all exacerbations, and 28% of the severe exacerbations, of asthma were associated with positive nasopharyngeal viral identification. In these studies, rhinovirus is the predominant individual microorganism, followed by (RSV), parainfluenza, adenovirus, influenza virus, and coronavirus [51]. In asthmatic children, almost 60% of the viral infections during exacerbations appears to be due to a rhinovirus [54], indicating the potential importance of this pathogen in asthma.

What is the proportion of asthmatic subjects who actually develop symptoms of wheezing during an identified respiratory virus infection? Again, cross-sectional studies report variable proportions between 12–56% [51]. In prospective studies by ROLDAAN and MASURAL [57] in children from the Dutch Asthma Centre in Davos, and by BEASLEY *et al.* [56] in adults from an out-patient clinic, the proportion of symptomatic asthmatics during respiratory virus infection was approximately 60%. The predominant viruses in these studies were rhinovirus, coronavirus, RSV, and parainfluenzavirus.

Finally, it appears that at least some asthmatic children are more susceptible to respiratory viral infections than normal controls [51]. In a six month follow-up study in children during the winter, the incidence of confirmed and probable viral infections appeared to be significantly higher in asthmatics than in non-asthmatics, particularly with regard to rhinovirus infections [58]. The reason for this is still unclear. It does not seem to be due to atopy *per se* [59], but may be related to defective production of a protective cytokine, interferon-α [60]. In adults there does not seem to be a tendency for an increased susceptibility of asthmatics to respiratory viral infection, even though clear data on this are lacking [56].

Experimental virus infection in humans in vivo

In 1976, EMPEY *et al.* [61] observed an increase in airway responsiveness to histamine in normal subjects during spontaneous, unidentified upper respiratory tract infection. Airway responsiveness returned to normal within seven weeks after the cold. In addition, these authors observed a lowered cough-threshold for inhaled citric acid during the infection [61]. These findings led other research workers to set up models for experimental virus infection in humans and in animals *in vivo*.

There is some controversy in the literature regarding the development of airway hyperresponsiveness following experimental virus infection in man [62]. This might be due to differences in viral strain, method of inoculation, subject selection, and/or airway responsiveness measurements [62]. In 1965 it had been shown that subcutaneous vaccines with killed influenza induced airway hyperresponsiveness to methacholine in asthmatics but not in normal subjects [63]. These distinguishing results between asthmatics and normals were subsequently confirmed and extended by using nasal application of live attenuated influenza virus and measuring histamine responsiveness in adults [64], as well as in children [65]. In these studies, airway responsiveness was increased at 3 days for up to 2 weeks following the virus inoculation. When the attenuated influenza virus was inhaled by aerosol, in addition to nasal inoculation, airway responsiveness increased even in normal subjects [66]. In general, these findings indicated that experimental virus infection is a clinically relevant *in vivo* model of asthma. This is particularly true for rhinoviruses [67–72].

Rhinovirus inoculation

It appears, based on the epidemiological studies discussed above, that rhinoviruses (RV) are likely to play a predominant role in asthma. Many studies have been performed with rhinovirus inoculation in man *in vivo* [62]. Among these, there have been a number of negative reports on subsequent induction of airway hyperresponsiveness in normal subjects [67–69]. This might have been due to inadequate dosing, producing an insufficient severity of the cold [67, 68], and/or to the choice of the virus strain, *e.g.* RV 2 [69]. Other investigators have been more successful in inducing airway hyperresponsiveness with rhinovirus inoculation [70–72]. The most marked results were obtained in atopic subjects with live rhinovirus 16 [71, 72].

Intranasal inoculation with rhinovirus 16 (RV 16) has been shown to induce airway hyperresponsiveness in subjects with allergic rhinitis [71, 72]. In those studies, RV 16 was given by instilling 320–3,200 tissue culture infective dose 50% ($TCID_{50}$) into each nostril, by pipette and by spraying, on two successive days. The development of common colds was subsequently documented by a questionnaire, by an increase in neutralizing antibodies, and by reisolation of the virus from nasal washes, using cytopathic effects after titration into cell cultures [71, 72]. RV 16 instillation induced significant airway hyperresponsiveness to histamine, methacholine and allergen, with average decreases in PD_{20} of about two to three fold, which persisted for at least 4 weeks [71, 72]. Independently from these effects, RV 16 inoculation increased the likelihood of the development of a late asthmatic response (LAR) to inhaled allergen, although the allergen-induced hyperresponsiveness to histamine remained unchanged [71, 72]. These results suggest that rhinovirus infection enhances inflammatory processes in the airway wall, which may hold important clues to the pathogenesis of airway hyperresponsiveness in asthma.

Recently, the state of the art knowledge on rhinoviruses has been excellently reviewed by DICK and INHORN [73]. Rhinoviruses belong to the picornaviruses, and consist of a single-strand ribonucleic acid (RNA) (7,210 nucleotides,

MW 2.5×10^6) surrounded by a 20-sided protein capsid [73]. The cellular receptor binding sites are located at the base of narrow pores, the major RV receptor group being intercellular adhesion molecule-1 (ICAM-1), the gene of which is mapped to human chromosome 19 [74]. There are at least 100 RV serotypes, causing 30–50% of all acute respiratory illnesses [73].

RV infection occurs predominantly by aerosol transmission, although transmission *via* hand contact cannot be excluded [75]. Less than one $TCID_{50}$ can initiate an infection, the incubation period normally being 2–3 days, whilst nasal secretions may remain infectious for 2–3 weeks after the beginning of the cold [73]. Even though RV replicates well in the upper respiratory tract, it is still unclear whether this also occurs in the lower intrapulmonary airways. RV seems to produce only mild pathological changes, with no discernable cytopathic effects [73]. There is shedding of the nasal ciliated epithelium [76], and an increase in neutrophils in peripheral blood, together with an increase in neutrophils and monocytes in nasal secretions [77, 78]. These inflammatory changes are likely to be accompanied by the generation of cytokines or inflammatory mediators. For instance, it has been established that experimental RV infection induces the generation of kinins (*e.g.* bradykinin) in nasal lavages, associated with increased vascular permeability [78]. In addition, it causes an acute decrease in peripheral blood lymphocyte counts, and primes these cells to release interleukin-2 (IL-2) and interferon-γ (INF-γ) *ex vivo* [79]. With regard to the humoral immune-response, nasal immunoglobulin A (IgA) sharply increases 24 h after infection, followed by an increase in immunoglobulin G (IgG) at 48 h [73]. At 1 week after infection, virus-specific IgA is found in nasal secretions together with serum IgG or immunoglobulin (IgM) antibodies [73]. These antibodies have been detected up to 1 yr after infection.

Taken together, experimental RV infection induces inflammatory changes in the nasal mucosa. It is still unknown whether such changes can also be observed in the intrapulmonary airways after experimental rhinovirus infection. This needs to be examined by bronchoalveolar lavage and bronchial mucosal biopsies taken by fibreoptic bronchoscopy. In addition, it is unclear whether or not the virus is actually present in the intrapulmonary airways after nasal inoculation [62]. Positive rhinovirus cultures from bronchial brush specimens might have been contaminated by the upper respiratory tract during the bronchoscopy procedure [68]. At this stage, the observations in the upper respiratory tract may well serve to generate hypotheses on the mechanisms of virus-induced airway hyperresponsiveness.

Mechanism of virus-induced airway hyperresponsiveness

When looking at the histopathology, the bronchiolitis following spontaneous virus infection in infants (*e.g.* by RSV) closely resembles the inflammatory changes observed in asthma [80]. For instance, there is epithelial shedding, a thickened subepithelial collagen layer, and an enlarged submucosa in both disease entities [3, 80]. Consequently, it has been postulated that virus infections might stimulate inflammatory processes in the airway wall, leading to chronic asthma [80]. This may occur by recurrent infections [62, 80], or by persistent and latent infections [81]. Indeed, by using polymerase chain reaction and *in situ* hybridization, a recent study showed increased integration of the E1A region of the adenovirus into host deoxyribonucleic acid (DNA) of lung tissue (*e.g.* epithelial cells) in patients with chronic obstructive pulmonary disease [82]. This may stimulate tissue growth and cytokine-induced cellular destruction [81, 82]. Therefore, it has been suggested that latent (adeno)virus infections could contribute to the pathogenesis of chronic obstructive pulmonary disease (COPD), which also needs to be examined in asthma.

Several potential mechanisms have been proposed to explain virus-induced airway hyperresponsiveness [48–50, 53, 62, 83, 84]. Some of those arose from animal studies. The various possibilities include: epithelial damage, cholinergic or noncholinergic reflexes, immunological mechanisms, inflammatory mediator release, and/or impaired β-adrenoceptor function.

Epithelial damage or dysfunction

Respiratory virus infections in man not only affect the nasal epithelium [76], but some viruses, *e.g.* influenza type A, also lead to epithelial necrosis in the lower airways within 24 h [85]. After symptomatic recovery, epithelial repair seems to occur rapidly, because at this stage the bronchial epithelial height is significantly increased as compared to healthy controls [86]. The epithelial damage might be the result of a direct cytopathic effect of the virus itself, and can also be caused by the secondary inflammatory response [87]. It is well known that epithelial shedding enhances airway hyperresponsiveness *in vitro* [88]. This might be explained by a loss in barrier function, allowing an increased penetration of irritants or allergens [50], or it could be due to a deficiency in the epithelial release of inhibitory mediators and degrading enzymes, such as prostaglandin E_2 and neutral endopeptidase (NEP) [89].

Cholinergic reflexes

In 1976, Empey *et al.* [61] postulated that virus-induced airway hyperresponsiveness might be due to enhanced activity of rapidly adapting irritant receptors secondary to epithelial damage. Based on the protective effects obtained by inhaled atropine, they reasoned that vagal reflexes are likely to be involved. Further evidence for this came from animal experiments, in which the enhanced *in vivo* response to histamine after parainfluenza-3 (PI-3) infection was prevented by vagotomy or the ganglion blocker hexamethonium [90]. In these studies, electrical field stimulation of the severed vagus nerve led also to enhanced airway narrowing after PI-3 infection as compared to controls [90], which suggested that afferent, as

well as efferent, vagal pathways are involved. Further studies indicated that virus infections can increase presynaptic acetylcholine release [91], either through facilitating mediators or neurotransmitters (substance P?) [29], or by dysfunction of inhibitory (M2) muscarinic receptors [92]. Further studies in man are needed to explore these possibilities.

Sensory neuropeptides

The tachykinins neurokinin A (NKA) and substance P (SP), and calcitonin gene-related peptide (CGRP) are potent pro-inflammatory sensory neuropeptides, that can be released by local axon reflexes [12, 29]. Firstly, virus infection has been shown to enhance the release of bradykinin [78], which is a potent stimulus of C-fibres and, thereby, of axon reflexes [93]. Secondly, viruses may interfere with the breakdown of tachykinins. After Sendai virus infection in guinea-pigs, the *in vivo* airway narrowing response to exogenous SP or to the tachykinin releasing substance, capsaicin, was increased [94]. In contrast to non-infected animals, these responses were not potentiated in the infected animals by an inhibitor of the endogenous neuropeptide degrading enzyme NEP, which is indicative of virus-induced NEP-dysfunction [94]. These findings are in line with those obtained with parainfluenza virus in rats, in which SP-induced microvascular permeability of the tracheal mucosa could not be potentiated by the NEP-inhibitor thiorphan [95]. Therefore, virus infections seem to inhibit the breakdown of neuropeptides in the airways, which might be related to damage of NEP-producing cells, such as the epithelium [89]. These experiments showing virus-induced NEP-dysfunction might be clinically relevant, since endogenous NEP-activity *in vivo* has indirectly been established in normal [96], and mildly asthmatic subjects [97].

Immunological mechanisms

There is recent evidence that viruses can modify specific immune responses by changing the phenotype of helper T-cells towards differentiation into the T_{H1}-profile [98]. This is in remarkable contrast to the predominant T_{H2}-profile in subjects with clinically stable, atopic asthma [22, 99, 100]. The virus-induced T_{H1}-profile is probably due to increased concentrations of INF-γ released by natural killer cells, which are stimulated by the virus or by INF-α and interleukin-12 (IL-12) from antigen presenting cells [98]. Indeed, elevated production of INF-γ (and IL-2) has been measured during virus infection in man in peripheral blood mononuclear cells *ex vivo* [79], as well as in bronchoalveolar lavage fluid *in vivo* [101]. During virus infections, T-cell produced INF-γ might also play a major role in enhancing chemotaxis [102], and/or mediator release [103] from inflammatory cells, such as basophils. These cells and their mediators could subsequently be involved in the development of airway hyperresponsiveness [23, 24]. In addition, it has been observed that viruses, such as RSV [104], and parainfluenza [105], are capable of inducing virus-specific IgE antibody production, particularly in infants who develop wheezing during the infection. This virus-specific IgE may be one of the determinants of virus-induced mediator release and the severity of the clinical response to infection [104, 105]. It cannot be excluded that virus-specific IgE partially explains the elevated total IgE levels in so-called "intrinsic" asthma [106].

Inflammatory mediator release

It has long been recognized that virus infections potentiate inflammatory mediator release *in vitro* and *in vivo* [49, 62]. During experimental infections in man, nasal secretions not only show a rise in kinins [78], but also in histamine levels [104]. Furthermore, the allergen challenge-induced rise in plasma histamine concentration is significantly greater during experimental RV 16 infection, as compared to a non-infected control period [72]. Segmental allergen challenge during experimental virus infection also leads to increased levels of histamine, as well as tryptase, in bronchoalveolar lavage fluid, together with an increased recovery of eosinophils [101]. These findings are indicative of activation of inflammatory cells during virus infection. This has been further examined *in vitro*. Indeed, virus infections enhance basophil histamine release [103, 107, 108], as well as the generation of arachidonic acid metabolites, such as leukotriene C_4, from basophils and macrophages [103, 109].

The involvement of these mechanisms in virus-induced hyperresponsiveness is plausible, as is apparent from elegant animal studies. Parainfluenza type 3 inoculation in guinea-pigs leads to increased maximal airway narrowing *in vitro*, together with an influx of inflammatory cells [110]. In addition, it also leads to airway hyperresponsiveness *in vivo* after 4–8 days, associated with an increase in the number and activity of macrophages, monocytes, lymphocytes, and eosinophils in bronchoalveolar lavage fluid [111]. Incubation of tracheal spirals from non-infected animals with these inflammatory cells, induces hyperresponsiveness *in vitro*, in contrast to the lavage cells obtained from animals inoculated with control solutions [112]. These results strongly indicate that inflammatory mediators and/or cytokines are involved in virus-induced airway hyperresponsiveness. In addition to these effects on inflammatory cells, viral infections are likely to cause an enhanced sensitivity of the airway microvasculature to inflammatory mediators and neuropeptides, potentially leading to plasma extravasation [113]. The inflammatory nature of experimental virus infections in man is further underlined by therapeutic interventions. Successful prophylaxis can be obtained by topical and oral steroids, leading to improvements in nasal obstruction, mucus weight, and kinin concentrations during the first 48 h after nasal inoculation [114]. In addition, combined treatment with INF-α2b, ipratropium bromide and naproxen has been shown to reduce the symptoms and signs after intranasal rhinovirus administration [115]. These observations are in line with those from clinical studies, showing beneficial effects of inhaled steroids

during asthma exacerbations following an upper respiratory tract infection [116].

Beta-adrenoceptor function

Viral infections can interfere with β-adrenoceptor mediated responses [117]. During clinical upper respiratory tract infection, there is a diminished inhibiton obtained by isoproterenol of lysosomal enzyme release of peripheral blood polymorphonuclear leucocytes *in vitro* [67, 118]. This fits in with a loss of β-agonist induced inhibition of bronchial contraction to antigens *in vitro* in guinea-pigs infected with para-influenza virus *in vivo* [119]. These findings can be explained by virus-induced alterations of β-adrenoceptors on leucocytes, such as mast cells. This is potentially caused by various T-lymphocyte derived cytokines, which inhibit the β-adrenoceptor function of leucocytes, but not of airway smooth muscle *in vitro* [120]. The effect of virus-infection on β-adrenergic function of human airways *in vivo* has not been investigated.

Conclusions

It has long been recognized that virus infections are associated with worsening of asthma symptoms. More importantly, bronchiolitis in infancy appears to be a risk factor for subsequent development of clinical asthma and airway hyperresponsiveness. Therefore, it can be postulated that either virus infections occur more easily in children with pre-existing airway abnormalities, or that viruses are actually one of the causes of asthma. Careful prospective studies in infancy and childhood are needed to answer this question.

In spite of a divergent immunological profile, the pathology of respiratory virus infections shows similarities with that of asthma. Therefore, common inflammatory mechanisms might be involved in the pathophysiology of both entities. This is supported by recent studies in animals and in man, in which airway hyperresponsiveness has been induced by experimental virus infection. These findings indicate that virus-induced airway hyperresponsiveness might by an important investigational model of asthma in humans *in vivo*, beside the widely used model of allergen-induced hyperresponsiveness.

The role of virus infections in the pathogenesis of airway hyperresponsiveness can now be studied adequately according to Koch's postulates, by using the modern techniques that are available in molecular biology, immunology, pathology and physiology [121]. Such studies are likely to have implications for the future diagnosis and treatment of asthma.

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