Review article

Microorganisms and exacerbation of chronic obstructive pulmonary diseases: pathophysiological mechanisms

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Respiratory tract infections are known to exacerbate bronchial asthma and other chronic obstructive pulmonary diseases (COPD). Viral infections are a major cause of exacerbations of asthma in children, but the association is less clear in adult asthmatics. Bacteria and bacterial products, conversely, are mainly associated with other COPD than asthma, e.g. chronic bronchitis. During the past decade it has been shown that release of mediators and neurotransmitters are of key importance in these inflammatory diseases. It is therefore possible that microorganisms play a role in these events in the airways. The pathophysiological mechanisms, which are only partially known, are reviewed in the following. We will not focus on the clinical investigations, since these have been discussed extensively elsewhere (3, 30, 40, 46, 81, 87).

Experimental upper respiratory infection with rhinovirus in adult patients with allergic rhinitis has been shown to cause increased bronchial reactivity to inhaled histamine and antigen (13, 93). Significant changes in FEV_1 and histamine sensitivity during such an experimental rhinovirus infection were also observed in 4 of 19 adult volunteers with mild to moderate asthma (43). In normal subjects viral infection of the airways causes an asymptomatic decrease in peak flow (38) or an increase in airway reactivity as evaluated by inhalation of histamine (33). This abnormal pulmonary function, presumably of the smaller airways, can persist for up to 8 weeks after the acute infection (41, 75). Thus viral respiratory tract infection results in bronchial hyperreactivity as well as airway obstruction, and these changes may persist for several weeks beyond the clinical infection. In patients with COPD the changes mentioned often exist already, but can be aggravated by the viral infection.

Inhalation of bacteria as well as bacterial endotoxin has been demonstrated to cause airway obstruction. Thus, in patients with chronic non-specific lung disease associated with bronchial hyperreactivity inhalation of *Haemophilus influenzae* (*H. influenzae*) caused a biphasic bronchial obstruction, and the dual response was mimicked by *H. influenzae* endotoxin (101). *Bacillus subtilis* enzymes which are used in washing powders cause respiratory dysfunction among the workers involved in the production. When inhalation tests were performed on the workers a biphasic reaction appeared, where the immediate response subsided within 4 h and the late reaction occurred 5–8 h after the inhalation (31, 32). In normal individuals exposure to endotoxin resulted in an acute decrease in lung function (17). Similar studies have indicated that bacterial endotoxins are responsible for the respiratory symptoms in humidifier disease (76, 77) and for the decrease in respiratory function in the byssinosis syndrome observed in textile workers exposed to cotton dust (57, 78).

Pathophysiological mechanisms

As the inflammatory process in the airways in COPD involves a complex interplay of cell types, mediators and neurotransmitters (2, 56), the theoretical points of action of the microorganisms are numerous. However, a more profound understanding of the pathophysiological mechanisms has been achieved through intensive experimental studies.

Virus

Theoretically, activation of mucosal mast cells to release of mediators via cellbound virus-specific IgE could play a role in the exacerbation of COPD during respiratory tract infections. Welliver et al. (98) found IgE-antibody specific for respiratory syncytial virus (RSV) in nasal secretions from children infected with this virus. Children with clinical evidence of airway obstruction showed higher titers of RSV-specific IgE, and had higher concentrations of histamine in the nasal secretions; the presence of high IgE titers was likewise strongly associated with decreased arterial oxygen saturations. In children with respiratory illness caused by parainfluenza virus infection virus-specific IgE was similarly demonstrated in nasopharyngeal secretions, and the titers were higher in the groups with croup, wheezing or a combination, compared to the patients with upper respiratory illness alone (99). A similar differentiation was also obtained for histamine since it was detected significantly more often and in higher concentrations in secretions from the first mentioned groups. This is interesting since both RSV and parainfluenza virus cause respiratory syndromes peculiar to children, i.e. bronchiolitis and croup. These data point to the possibility of production of virus-specific IgE antibody during respiratory tract infections, and via IgE-mediated mediator release the microorganisms might be responsible for the respiratory symptoms.

IgE-mediated histamine release caused by virus has also been examined in suspensions of peripheral blood leukocytes including basophilocytes. In patients infected with human immunodeficiency virus (HIV) the cells from the AIDS patients responded to both HIV and Herpes simplex virus (HSV) type I, whereas no response was obtained in control cells from normal individuals, which points to the possibility of type I allergic reactions directed against these viruses in AIDS patients (72–74). Basophils from normal individuals do not release histamine when stimulated with live or inactivated viruses as HSV type I, adenovirus type 1, rhinovirus or influenza A virus (12, 18, 47). This was also the case when cells from patients with intrinsic or allergic asthma were challenged with influenza A virus (22). However, some paramyxoviruses, such as RSV, parainfluenza type 1 (Sendai) and type 3 viruses seem capable of releasing histamine from basophils through a non-immunological mechanism (79), although the effect of RSV could not be confirmed by Chonmaitree (18). Further, *in vitro* studies including human epithelial cells and neutrophils, respectively, indicate that RSV via complement activation (90) or via complexes of RSV and antibody (35) should be able to stimulate cells to the release of various mediators, such as histamine, products of arachidonic acid metabolism and oxygen radicals.

Enhancement by viruses of mediator release might be another mechanism in the exacerbation of airway diseases. The first *in vitro* study was performed by Ida et al. (47) who examined the effect of virus on IgE-mediated histamine release from peripheral blood leukocytes from normal individuals. Preincubation of the cells with either adenovirus, influenza A virus, or HSV type I, enhanced the basophil histamine release triggered by anti-IgE. This effect was obtained by live as well as inactivated viruses. It was necessary to preincubate the cells with virus for at least 8 h to obtain the effect, and it seemed that interferon was responsible for the potentiation found in these long-term experiments. Production of interferon was thus observed during the preincubation, and the effect of the virus could be mimicked by interferon. This is interesting since interferon is found in nasal secretions from infants with viral respiratory tract infections (42, 64). The potentiation was confirmed in a study by Busse (12), which also demonstrated that virus potentiates mediator release triggered by IgE-dependent as well as non-immunological (calcium ionophore A23187) mechanisms. However, virus which causes no production of interferon (rhinovirus type 1A), was also able to enhance the anti-IgE-induced histamine release, thus suggesting that other, unknown factors are responsible for the potentiating effect of virus (18).

A more direct effect of virus on the cells was examined in short-term experiments where the leukocytes were exposed to virus and specific antigen (or anti-IgE) for 40 min. In these studies influenza A virus enhanced basophil histamine release triggered by IgE-mediated reactions (22). Potentiation was thus obtained both when cells from normal individuals were stimulated with anti-IgE, and when cells from asthma patients allergic to house dust mite, grass pollen, birch pollen or cat dander were challenged with their specific antigen. It seems doubtful whether a production of interferon could be responsible for the potentiating effect of virus in these short-term experiments, since such a production requires several hours (47). However, influenza A virus appeared to act by means of its surface neuraminidase, since the enhancing effect was mimicked by isolated neuraminidase and was abolished by a neuramini-

dase inhibitor (22) or by monoclonal antibodies directed against the viral neuraminidase (20). One possible explanation for this potentiating effect is an enzymatic effect of the viral neuraminidase; this enzyme is known to cleave off sialic acid from cell membranes (80) thereby causing enhanced cellular responses (37, 44) including basophil histamine release (53, 54). In fact, increased concentrations of sialic acid have been found in sputum and serum from asthma patients during exacerbation due to upper respiratory tract infections (89). On the other hand, the viral neuraminidase is a glycoprotein containing carbohydrates, such as galactose and N-acetylglucosamine (29), and these free carbohydrates were able to abolish the potentiating effect of the virus (26). Therefore, it seems more likely that these carbohydrate residues are responsible for the potentiation by interacting with binding sites on the basophil cell membrane. The change in the cell response induced by influenza A virus comprised both an increased maximum release of histamine, an increased cell sensitivity and a lowering of the threshold to the cell stimulus (27). An increased sensitivity to the allergen is probably more important in asthma than an increased maximum mediator release, since the former was more strongly correlated to the severity of the disease as measured by bronchial provocation with the relevant allergen (65).

The balance between cholinergic and β-adrenergic function might be of importance for airway smooth muscle tone, mediator release and inflammation, and it is likely that this balance is altered by viral infections. Thus, an exaggerated cholinergic reflex response might be the basis for virus-induced bronchoconstriction and increase in airway reactivity to histamine, since pretreatment with atropine of normal subjects with colds prevented these symptoms (33). It was suggested that the reversible airway epithelial damage caused by viral infections exposed afferent nerve endings and accentuated the afferent portion of the reflex arc. However, Buckner et al. (8) demonstrated that the efferent arm of the vagal reflex is increased, since electrical stimulation of the vagus nerve caused greater bronchoconstriction in guinea pigs infected with parainfluenza virus than in uninfected animals. The mechanism might be due to increased acetylcholin release from the parasympathetic nerve terminals caused by a loss of negative feedback control by viral damage of presynaptic M_2 muscarinic receptors (49).

Diminished β-adrenergic function in leukocytes has been demonstrated indirectly by a decreased granulocyte response to isoproterenol in asthma patients, and the response was further impaired during viral upper respiratory infections which provoked asthma (9). The mechanism is obscure, but a similar diminished response was obtained when granulocytes from normal individuals were incubated with rhinovirus or with a mixture of influenza A and B viruses (10, 11). Buckner showed that infection with parainfluenza 3 virus blocked the ability of a β-agonist to inhibit antigen-induced contraction of guinea pig isolated airway smooth muscle (7), demonstrating that the β-adrenergic function was depressed by the virus. It is tempting to speculate that respiratory virus causes similar changes in the function of cells in the airways, especially mast cells and smooth muscle cells.

The epithelial barrier and mucociliary clearance are important for the defense in the respiratory tract. Microorganisms may stimulate mast cells in the epithelium to release histamine, which disrupts the epithelial tight junctions leading to entrance of insulting particles (68). This theory was supported by experiments with human bronchoalveolar cells demonstrating such a mediator release (21), and Hogg and associates have shown that in animals both histamine and antigen challenge can increase the airways' mucosal permeability by disruption of epithelial tight junctions (4, 45). Further, it is well known that infection with RSV, influenza virus or parainfluenza virus results in destruction of the airways epithelium (13, 16, 87). Based on studies in animals it has been found that viral damage of the airways epithelium leads to a loss of neutral endopeptidase (48, 49). Substance P and other tachykinins released from afferent nerves in the lung are normally broken down by this enzyme and the accumulation of these neuropeptides with bronchoconstrictor effects should therefore lead to an enhanced contractile response of the airway smooth muscle (49). An impaired mucociliary clearance appears in subjects with influenza (14), naturally acquired common colds (71), or by infection with *Mycoplasma pneumoniae* (15). The impairment is usually reversible within months. Infections with common respiratory viruses have been shown to be associated with cilia with abnormal microtubular patterns, loss of ciliated cells (16, 71), and depressed mucociliary clearance (14, 100). Further, influenza A virus via its neuraminidase lowers the viscosity of the mucous film in the respiratory tract thus promoting the spread of virus (50). Respiratory viruses such as Sendai virus enhance basophil chemotaxis *in vitro*, and this effect might be due to virus-induced production of interferon (60). It is not known whether a similar effect on migration of inflammatory cells in the airways plays a role during viral infection.

Bacteria and bacterial products

Various bacteria, including the most frequently encountered species in the upper respiratory tract, have been found to release histamine from human mast

cells. These cells comprise pulmonary mast cells obtained by either bronchoalveolar lavage (BAL) (21) or enzymic dispersion of lung tissue (19), tonsillar mast cells (19) and mast cells from nasal mucosa (1). The studies included non-atopic patients without asthma. Whole formalin-killed bacteria were used and it seems that the histamine release response to *Staphylococcus aureus* (*S. aureus*) was higher in epithelial mast cells obtained by BAL than in mast cells from the lung parenchyma. Also bacterial antigens from, for instance, *S. aureus*, *H. influenzae* and *Branhamella catarrhalis* induce histamine release from human BAL-cells (6). Further, *S. aureus* whole bacteria were found to induce synthesis of leukotriene B_4 from BAL-cells (21). Asthmatic subjects are expected to release more histamine than normal subjects since an increase in mast cells and histamine content has been observed in the bronchial mucous membrane (62) and in the lavage fluid (36, 97), and the cells might show an enhanced response to the stimuli, which has been demonstrated in connection with anti-IgE (36, 61). The mediator release triggered by bacteria in epithelial cells might be of importance for the exacerbation of COPD in respiratory tract infections, since histamine is assumed to increase the epithelial permeability with entrance of allergens and other insulting particles (45, 68), and since leukotriene B_4 as a strong chemotactic agent has the potential to facilitate airway inflammation.

IgE-antibodies directed against *H. influenzae*, *S. pneumoniae* and *S. aureus* have been demonstrated in sera from both normal individuals and patients suffering from bronchial asthma and other categories of COPD (70, 95). In patients with chronic bronchitis the IgE RAST scores to whole *H. influenzae* bacteria were significantly higher than in controls (95). Also skin prick tests with bacterial antigens (69, 95) and a positive basophil histamine release response to bacteria in intact leukocytes but not in cells stripped of IgE (67) indicate that allergy to bacteria can be found in some patients. These patients included subjects with intrinsic asthma who were allergic to *H. influenzae* and *S. pneumoniae* (67), patients with Job's syndrome (immune deficiency with hyper-IgE to their own *S. aureus* strains) (51), and different categories of patients infected with *E. coli* and other bacteria (25).

However, bacteria can also trigger histamine release by a non-immunological mechanism verified by release from both intact cells and cells stripped of surface-immunoglobulins (67). The non-immunological mechanism was studied mostly in connection with *S. aureus*, and it was found to operate in the majority of persons tested. These subjects included allergics suffering from hay fever and asthma, children with intrinsic asthma, and normal individuals (25, 34, 51). We suspect that the non-immunological mechanism is a sugar-lectin mediated reaction. This means that sugars on the bacterial cell wall interact with lectins on the basophil cell membrane leading to histamine release (51, 52). The new mechanism may be of importance for our understanding of the role of bacteria in asthma and infectious diseases, since a subject does not need to be sensitized to bacteria in order to induce histamine release. It is also remarkable that this mechanism causes a synergistic enhancement of allergic histamine release. The *in vitro* experiments comprise cells obtained from house dust mite or grass pollen allergic patients. A low histamine release was obtained when the cells were stimulated with small amounts of specific allergen, and this was also the case on challenge with small amounts of bacteria to which the patient was not sensitized. However, the combination of bacteria and specific allergen caused a high mediator release, exceeding the additive effect (67). Also bacterial components such as peptidoglycan, teichoic acid and endotoxins from *H. influenzae*, *E. coli* and *Salmonella* bacteria can aggravate allergic mediator release (23, 67, 91). It is possible that the capability of bacteria and endotoxin to enhance mediator release depends on microbial sugar residues interacting with binding sites on the basophil cell membrane, since the free carbohydrates (galactose and N-acetyl-glucosamine) abolish the potentiating effect (24). Thus it seems that bacteria by their enhancing effect as well as their stimulatory effect may contribute to the exacerbation of airway diseases during infections. This is important since oral intake of galactose has been shown to abolish the potentiating effect in *ex vivo* experiments (unpublished results). The clinical effect of such carbohydrate therapy is, however, not known, but the total lack of adverse reactions makes this an interesting new therapeutic principle.

Release of other mediators has been studied especially in connection with *Escherichia coli* (*E. coli*). Bremm et al. (5) found generation of leukotrienes and lipoxygenase factors from human polymorphonuclear granulocytes during bacterial phagocytosis and interaction with bacterial exotoxins. A similar generation of leukotrienes in human neutrophils was also obtained by bacterial adhesion and toxin production by various strains of *E. coli* (59). The leukotriene B_4 generation was examined after incubation of human cells with 25 different uropathogenic strains of *E. coli*, and the leukotriene production was found to be correlated with the haemolytic activity of the strains in monocytes but not in the neutrophils (92). An infusion of haemolysin-secreting *E. coli* into isolated rabbit lung induced a marked leukotriene generation with predominance of cysteinyl-leukotrienes (39). *E. coli* were also found to produce prostaglandin D_2 in human tonsillar mast cells (19).

Studies with gram-positive bacteria have shown a synthesis of both leukotriene B_4 and C_4 on stimulation with *S. epidermidis* of peritoneal macrophages from patients on peritoneal dialysis (63). Furthermore, delta-toxin from *S. aureus* activates the generation of ^{3}H-platelet-activating-factor (PAF) from exogeneous ^{3}H-lyso-PAF and triggers the production of oxygen radicals in human neutrophils (55). These studies demonstrate that bacteria can induce the release of a variety of inflammatory mediators which all are known to play a role in COPD.

Although bacteria can induce release of mediators and enhance mediator release, some studies also point to an additional effect, i.e. synthesis of histamine by the bacteria. High levels of histamine have been detected in the sputum of patients with asthma and chronic bronchitis, and the level decreases in the remission phases of both diseases. Bacterial synthesis of histamine was suggested since exposure of sputum to 37°C increased the level of histamine, and this increase was abolished by pre-heating at 100°C or by the addition of antibiotics (85). Later, Sheinman et al. (86) demonstrated a synthesis of histamine from histidine by *H. influenzae* using isolates from patients with acute exacerbations of chronic bronchitis and emphysema.

The influence of bacteria on autonomic functions has been studied in guinea pigs sensitized to ovalbumin. In the lung of the *H. influenzae*-treated animals, the threshold for ovalbumin-induced release of prostaglandins and thromboxanes was lowered and inhibition of mediator release by isoprenaline was reduced (82). Further, bronchoconstriction caused by carbachol was potentiated, and isoprenaline caused less relaxation of contracted tracheal spirals in *H. influenzae*-vaccinated guinea pigs compared with non-vaccinated animals (82, 83). It is therefore possible that *H. influenzae* causes an increased sensitivity to antigenic challenge and a decreased activity of β-adrenoreceptors.

A direct effect on the bronchial smooth muscle may also be possible since *E. coli* endotoxin has been shown to enhance the α-adrenergic response in human bronchial smooth muscle; a 1000-fold increase in response was obtained in bronchial smooth muscle preparations taken from patients with chronic bronchitis, whereas an increase of only 2 to 10-fold was observed in preparations from patients with normal lung function (88). A decrease in the number of β-adrenoreceptors was found in the guinea pig lung after vaccination with heat-killed *H. influenzae* and *S. pneumoniae* but not with *S. aureus* (84). This is of interest since the lower respiratory airways of patients with chronic bronchitis are often colonized with *H. influenzae* and *S. pneumoniae* (81).

Impairment of the epithelial barrier caused by bacteria possibly plays a role in COPD, since bacterial IgA-proteases have the ability to destroy the host's secretory IgA (58, 94), which might facilitate colonisation of the airways epithelium (96). Also loss of ciliary function in respiratory tract epithelium can be induced by bacteria, and it is likely that bacterial products, such as lipopolysaccharide are responsible for this effect (28, 96). Some of these bacterial products obtained from *H. influenzae* and *Pseudomonas aeruginosa* also disrupt the ciliated epithelium (28).

Conclusion

Exacerbation of chronic obstructive pulmonary diseases caused by microorganisms and their components is seen in connection with infections and by environmental or occupational exposure. A variety of mechanisms might be involved in the aggravation of COPD: damage of the airways barrier, production of IgE-antibodies directed against the microorganisms, mediator release and reinforcement of mediator release, cellular and neurogenic events, altered cholinergic and β-adrenergic function and constriction of the bronchial smooth muscle. Bacteria can induce mediator release not only by the allergic (IgE-dependent) mechanism but also by a non-immunologic mechanism, which indicates that a person does not need to be sensitized to bacteria to release histamine. An enhancement of mediator release can be obtained by both viruses and bacteria. Mediator release or an enhancement of allergic mediator release by microorganisms or their products may play an important role, since the microorganisms can stimulate superficially lying epithelial mast cells to release histamine, which can disrupt the epithelial tight junctions. This will allow a subsequent stimulation of various submucosal cells and nerve endings by inhaled allergens, virus and bacteria. A vicious circle leading to hyperreactivity and bronchoconstriction can thereby be initiated.

Thus a diversity of mechanisms are involved in the provocation or aggravation of asthma and other obstructive pulmonary diseases by infections. The strategy for further studies should encompass therapeutic manipulation with these targets. This will improve our knowledge of the network of inflammatory reactions, and possibly lead to new therapeutic principles in the treatment of these diseases.

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