Pathophysiology of viral-induced exacerbations of COPD

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Abstract: Inflammation of the lower airways is a central feature of chronic obstructive pulmonary disease (COPD). Inflammatory responses are associated with an increased expression of a cascade of proteins including cytokines, chemokines, growth factors, enzymes, adhesion molecules and receptors. In most cases the increased expression of these proteins is the result of enhanced gene transcription: many of these genes are not expressed in normal cells under resting conditions but they are induced in the inflammatory process in a cell-specific manner. Transcription factors regulate the expression of many pro-inflammatory genes and play a key role in the pathogenesis of airway inflammation.

Many studies have suggested a role for viral infections as a causative agent of COPD exacerbations. In this review we will focus our attention on the relationship between common respiratory viral infections and the molecular and inflammatory mechanisms that lead to COPD exacerbation.

Introduction

Many epidemiological and clinical studies have suggested a role for respiratory viral infections in the natural history of chronic obstructive pulmonary disease (COPD), particularly during their exacerbations highlighting the need for development of effective vaccines and/or treatment for these viruses. The precise cellular and molecular mechanisms underlying these events are still largely unknown (Caramori et al 2006; Mallia et al 2007).

Lower airways inflammation is a central feature of many lung diseases, including COPD. Although the specific characteristics of the inflammatory responses and the site of inflammation differ between one disease to another, they always involve recruitment and activation of inflammatory cells and changes in structural cells of the lung. Inflammatory responses are associated with an increased expression of a cascade of proteins that includes cytokines, chemokines, growth factors, enzymes, adhesion molecules and receptors. In most cases the increased expression of these proteins is the result of enhanced gene transcription: many of the genes are not expressed in normal cells under resting conditions but they are induced in the inflammatory process in a cell-specific manner. Transcription factors regulate the expression of many genes, including inflammatory genes and play a key role in the pathogenesis of respiratory inflammatory diseases, including COPD (Caramori et al 2004).

In this review we will provide an overview of the relationship between respiratory virus infection and the molecular mechanisms involved in the activation of airway inflammation in COPD exacerbations.

Clinical impact of viral infections in COPD

COPD is a disease state characterized by airflow obstruction that is not fully reversible. The airflow obstruction is usually both progressive and associated with an abnormal

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inflammatory response of the lung to noxious particles or gases. The main cause of COPD is cigarette smoking (GOLD 2006).

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of mortality worldwide (GOLD 2006). Costs of COPD are mainly related to exacerbations requiring hospitalization (Sullivan et al 2000). In addition to their enormous acute morbidity, mortality and cost, exacerbations are also associated with major reductions in long term quality of life and lung function (Seemungal et al 1998; Donaldson et al 2002).

Despite the clinical and economic importance of severe COPD exacerbations, their etiology and mechanisms are poorly understood.

Both bacterial (Sethi and Murphy 2001; White et al 2003) and viral infections (Seemungal et al 2000, 2001; Aaron et al 2001; Rohde et al 2003; Wedzicha 2004; Beckham et al 2005; Papi et al 2006) have been detected at increased frequencies during COPD exacerbations. The most frequently respiratory viruses involved in the etiology of COPD exacerbations are represented by rhinoviruses, influenza viruses, coronaviruses, and respiratory syncytial virus (RSV). More rarely parainfluenza viruses and human metapneumoviruses (HMPV) have also been identified (Rohde et al 2003; Hamelin et al 2005; Falsey et al 2006; Papi et al 2006).

A recent study has recently addressed the relative importance of viral versus bacterial infections in the etiology of severe (hospitalized) COPD exacerbations (Papi et al 2006). Viral and/or bacterial infection was detected in 78% of COPD exacerbations (29.7% bacterial, 23.3% viral, 25% viral/bacterial co-infection). Infectious exacerbations had longer hospitalizations and greater impairment of several measures of lung function than non-infectious exacerbations. Importantly, exacerbations with co-infection had more marked lung function impairment and longer hospitalizations. Similarly, when COPD exacerbations not requiring hospitalization were evaluated, a greater lung function fall was documented in those patients where both bacterial infection and cold symptoms were present simultaneously (Wilkinson et al 2006). This data suggests that bacterial and viral infections could synergistically cooperate to increase severity of the exacerbations. Whether viral infections can lead to bacteriological exacerbation (of previously colonizing bacteria) is still an open question that deserves properly designed longitudinal studies.

Determining the etiology of exacerbations will inform appropriate antibiotic and antiviral therapy. Antibiotic therapy is already widely used, but not always appropriately, in the treatment of COPD exacerbations (GOLD 2006). Indeed, a recent meta-analysis supports antibiotics only for those patients with COPD exacerbations with increased cough and sputum purulence who are moderately or severely ill. Analysis restricted to community-based studies did not find any differences between antibiotic and placebo (Ram et al 2006). In asthma, recent study showed a signification reduction of symptoms at exacerbations in those patients receiving telithromycin as compared to placebo. Given that no relationship between bacteriologic status and the response to study treatment was found, the mechanisms of such a benefit remain unclear (Johnston et al 2006). Therefore, (i) there is an urgent need to develop simple clinical or biological markers to identify those patients at highest risk of bacterial infection and who will benefit most from antibiotic therapy, (ii) further studies are needed to evaluate whether novel class of antibiotics can be more effective in the treatment of COPD exacerbations.

Antiviral therapy, and in particular anti-rhinovirus therapy, is a "still theoretically" relevant therapeutic option since two thirds of exacerbations are associated with viral infections (Seemungal 2001). Indeed, so far no study has investigated whether currently available antiviral treatment can reduce viral induced COPD exacerbations. The use of anti-influenza neuraminidase inhibitors in COPD exacerbations during influenza epidemic appears appropriate in non vaccinated patients and may reduce the number of hospitalizations (Lalezari et al 2001; Cooper et al 2003; Kaiser et al 2003), though further controlled clinical trials are needed to confirm therapeutic benefit.

Susceptibility to virus infections in COPD patients

Viral infections are the most frequent cause of COPD exacerbation. Whether COPD patients are more susceptible to virus infection as compared to normal subjects is still debated. A recent study documented that patients with frequent COPD exacerbation have more frequent episodes of naturally occurring colds as compared to patients with infrequent exacerbations (Hurst et al 2005). These results suggest that COPD subjects with frequent exacerbations may represent a subgroup particularly susceptible to viral infections. Thus, while there is solid evidence of impaired innate (Wark et al 2005; Contoli et al 2006) and possibly acquired (Gern et al 2000; Papadopoulos et al 2002) immune responses to viral infection in asthmatic patients, it is not yet clear whether COPD patients have increased susceptibility to viral infections. Intriguingly patients experiencing frequent colds had a significantly higher exposure to cigarette smoke (Hurst 2005). Recently, using a mouse model of cigarette smoke exposure, it has been

demonstrated that cigarette smoke increases susceptibility to viral infections possibly via alteration/inhibition of immune response (Robbins et al 2004).

Another possible mechanism leading to increased susceptibility is related to up regulation of ICAM-1, the receptor for the major group of human rhinoviruses. Latent expression of adenoviral E1A protein in alveolar epithelial cells of patients with emphysema increases ICAM-1 expression and this could be a potential mechanism for greater susceptibility to rhinovirus infection in COPD (Retamales et al 2001).

Patients with COPD are chronically colonized with airway bacteria, and the bacterial load is related to airway inflammation and disease progression (Patel et al 2002). It has been postulated that bacterial colonization could contribute to increased susceptibility to viral infection in COPD patients for example by increasing ICAM-1 expression in bronchial epithelial cells either directly or through induced inflammation (Sajjan et al 2006).

Further studies are required to investigate the interaction between chronic bacterial colonization and respiratory viral infection and in particular whether chronic bacterial colonization can increase susceptibility to viral infection or vice versa.

Mechanisms of rhinovirus and respiratory syncytial virus-induced COPD exacerbations

As the contribution of viruses to COPD exacerbations has only recently been appreciated little research has been carried out into the mechanisms of virus-induced inflammation. Performing airway sampling at exacerbation in COPD patients is even more difficult than in asthmatics due to their older age and high prevalence of co-morbidities. One way to overcome these obstacles is the development of a human experimental model that would allow studies to take place under controlled conditions. The first step towards development of such a model has been recently realized with the reporting of the first study evaluating the effects of an experimental viral infection in COPD patients (Mallia et al 2006).

COPD exacerbations are associated with an increased numbers of inflammatory cells in the lower airways and increased/decreased release of pro-inflammatory/anti-inflammatory mediators (Wedzicha and Donaldson 2003). Through these mechanisms respiratory viral infections may enhance the pathological processes associated with cigarette smoking and contribute to the lung pathology and loss of lung function associated with COPD. The type and the degree of activation of the different inflammatory cells recruited to the lung during COPD exacerbations has not been well studied (Zhu et al 2001; Qiu et al 2003).

Cells and molecules involved in respiratory virus-driven airway inflammation in COPD exacerbations

Despite growing clinical evidence for a role of respiratory viral infections in the pathogenesis of COPD exacerbations, the precise mechanisms of respiratory virus-induced airway inflammation and of host defenses against respiratory viruses are poorly understood (Johnston 2005).

Most of the data available relate to rhinovirus (RV) and respiratory syncytial virus (RSV), ie, the respiratory viruses that appear to be more frequently involved in COPD exacerbations.

Rhinovirus

Human rhinoviruses (RV) are the largest genus in the Picornaviridae family. They are single-stranded RNA viruses. Rhinovirus is the main cause of the common cold and several studies supported the role of this pathogen in both asthma and COPD exacerbations. Recent data documents that rhinovirus can reach and replicate in the lower airways.

There is striking genetic diversity of the RV strains circulating in a given community during a short time (Oliveira et al 1993). Rhinovirus serotypes are divided into two groups on the basis of receptor specificity. The "major" receptor group (including RV14 and RV16) utilizes intercellular adhesion molecule-1 [(ICAM-1); CD54] as a receptor for infecting the target cells (Greve et al 1989; Terajima et al 1997). Using ICAM-1, RV can infect airway epithelial cells, but there is little evidence of productive replication in other cells such as monocytes, macrophages and eosinophils granulocytes. The "minor" receptor group, do not bind ICAM-1 and instead bind the LDL receptor and related proteins (Hofer et al 1994).

Rhinovirus is transmitted by aerosol through infectious droplet nuclei emanating from infected subjects (Myatt et al 2003; Samet 2004).

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is a member of the family Paramyxoviridae, subfamily Pneumovirus; it is an enveloped RNA virus with a negative-sense, nonsegmented, single-stranded RNA genome.

RSV is a major respiratory pathogen, it is most common in infants where it causes a range of illnesses from asymptomatic infection through upper respiratory tract infection, bronchiolitis,

and pneumonia (Falsey and Walsh 2000; Hall 2001; Falsey et al 2005). In addition, during the past two decades, a growing number of studies have clearly established RSV as a severe pathogen in certain adult populations. The elderly, those with underlying cardiopulmonary disease (chronic heart failure, chronic obstructive pulmonary disease, bronchial asthma) and the immunocompromised patients appear to be at greatest risk of developing severe, even life-threatening lung disease following RSV infection (Hall 2000; Walsh and Falsey 2004; Falsey 2005; Sethi and Murphy 2005).

The primary site of RSV replication are the airway epithelial cells, whereas other cell types (monocytes/macrophages, airway smooth muscle cells eosinophils, neutrophils, mast cells, dendritic cells and endothelial cells) are targets of abortive replication or viral attachment only (Arnold and Konig 2005; de Graaff et al 2005; Guerrero-Plata et al 2006).

In human bronchial epithelial cells RSV colocalize with ICAM-1, and this binding can be inhibited by an antibody to the fusion F protein. These data suggests that RSV interaction with ICAM-1 involves the F protein and facilitates RSV entry and infection of human bronchial epithelial cells (Behera et al 2001). Interestingly in vitro RSV infection of human lung endothelial cells increase their expression of ICAM-1 (Arnold and Konig 2005).

Since RSV replication is largely restricted to airway epithelial cells, an hypothesis is that inflammatory cell recruitment by the infected cells will start the later immunopathology (Becker et al 1992; Becker and Soukup 1999). The effects of RSV on airway inflammation may be therefore partly mediated by sequential production of pro-inflammatory cytokines/chemokines in the infected airway epithelium.

Respiratory viruses and transcription factors

The activation of several nuclear factors has recently been studied in COPD (Caramori 2004). Several studies have shown that both the promoter genomic regions and the related transcription factors that regulate inflammatory mediator production are deeply affected by respiratory virus infection. Most of the data published until now on this issue are mainly related to rhinovirus and respiratory syncytial virus infections. More research is required to clarify the molecular events that determine the changes in gene transcription determined by virus infection in target cells.

Molecular mechanisms of rhinovirus induced inflammation

To date the specific molecular mechanisms involved in the production of pro-inflammatory mediators by bronchial/lung cells after rhinovirus infection are still not fully characterized. However, some studies have examined at the molecular level the transcription factors involved in RV-induced production of pro-inflammatory mediators from structural cells of the lungs.

Rhinovirus infection of primary bronchial epithelial cells induces a rapid increase of intracellular oxidants (superoxide anion) production that is maximal at the time of nuclear factor κB (NF-κB) activation. Intriguingly, it has been shown, in the same model, that reducing agents inhibit RV-induced ICAM-1 up-regulation, ICAM-1 promoter activation and NF-κB activation (Papi et al 2002). This data suggests that modulation of RV induced intracellular oxidant burst can be a novel pharmacological approach to treat/prevent RV induced COPD exacerbations.

Several transcription factors appear to be activated after rhinovirus infection on a variety of respiratory cells (Caramori 2006). These proteins include NF-κB, AP-1 and GATA families. Activation of intracellular signaling pathways is induced by rhinovirus in epithelial cells, which may be dependent on surface receptor binding (ICAM-1), or by intracellular products during viral replication such as double-stranded RNA (dsRNA). It has been shown that dsRNA can activate components of several signaling pathways including protein kinase R, nuclear factor-κB (NF-κB) and p38 mitogen-activated protein kinase (MAPK) (Alexopoulou et al 2001). However, to date, no studies have investigated the role of these molecules in rhinovirus infection.

Rhinovirus-induced activation of NF-κB leads to an increased expression of proinflammatory cytokines (IL-1, IL-6, granulocyte colony-stimulating factor (G-CSF) and GM-CSF), CXC chemokines (IL-8) and ICAM-1 (Papi and Johnston 1999b), whereas expression of VCAM-1 seems to be mediated by the activation of both NF-κB and GATA proteins (Papi and Johnston 1999a). It has been recently suggested that early activation of p38 MAPK pathway by rhinovirus infection, which induces the activation of many transcription factors, could be a key event in the regulation of rhinovirus-induced cytokine transcription, and may provide a new target for inhibition of rhinovirusinduced asthma exacerbations.

Molecular mechanisms of respiratory syncytial virus induced inflammation

RSV-induced cytokine production in airway epithelial cells seems to play an important role in the pathogenesis of RSV respiratory tract infections.

Recent studies on RSV-epithelial cell interactions have demonstrated that RSV can act at the molecular level by altering

cytokine gene transcription (Jamaluddin et al 1998; Mastronarde et al 1998) or mRNA stability (Koga et al 1999).

As for RV it has been shown that early activation of mitogen-activated protein kinases (MAPKs) by RSV infection is a key event in the regulation of RSV-induced pro-inflammatory transcription factors activation and cytokine/chemokines transcription in bronchial epithelial cells, however both p38 MAP kinase, mitogen-activated protein kinase kinase kinase 14/ NF-κB-inducing kinase (MAP3K14/NIK) and extracellular signal-regulated kinases (ERKs) pathways seem to be important (Chen et al 2000; Kong et al 2004; Chouldhary et al 2005; Rixon et al 2005).

Several transcription factors appear to be activated after RSV infection on a variety of respiratory cells. These proteins include mainly the NF-κB and AP-1 families. NF-κB is activated by the M2-1 protein of RSV (Fiedler et al 1996; Mastronarde 1998; Casola et al 2000; Chouldhary 2005; Reimers et al 2005; Spann et al 2005).

Link between virus infection, type of inflammation, dyspnea and sputum purulence

The mechanisms by which virus-enhanced inflammation may pave the way to COPD exacerbations are so far unknown. The recent availability of a human model of RV-induced COPD exacerbations (Mallia et al 2007) will foster the research and the progress in this important area. In COPD this is so far the only model where a specific etiology has been experimentally proven to induce exacerbation. In this pilot study mild to moderate COPD patients were safely experimentally infected with rhinovirus providing for the fist time that, in in vivo experimental condition, rhinovirus infection in COPD patients is per se sufficient to trigger exacerbations. Lower respiratory tract symptom scores were significantly increased compared to baseline on days 7 to 14, with peak lower respiratory tract symptoms on days 10 and 11. When the individual symptoms were analyzed separately, all five lower respiratory symptom domains (wheeze, cough, sputum production and dyspnea) increased from baseline however the increases were only statistically significant for wheeze, cough and sputum production, but not for dyspnea. In terms of recovery, all symptom domains other than sputum production had recovered to baseline by day 20, however full recovery of sputum production took almost 4 weeks (Mallia et al 2006). This important data suggests that the link between virus-induced COPD exacerbation and increased dyspnea and sputum production is probably very complex and needs dedicated studies for a better comprehension.

Intriguingly, it has been recently shown that viral induced severe COPD exacerbations with or without concomitant bacterial infection are characteristically associated with sputum eosinophilia whereas sputum neutrophilia is present during exacerbations in all subgroups independently from etiology (Papi et al 2006). This finding is in line with previous studies showing that in vivo experimental rhinovirus infection in normal subjects leads to lower airway eosinophilia (Fraenkel et al 1995). Thus, enhanced airway eosinophilic inflammation of the airway may be a hallmark of viral infection. Further studies are required to confirm and extend this pivotal observation and to evaluate whether modulation of rhinovirus-induced activation of pro-inflammatory mediators (ie, NF-κB) might be of any relevance in the treatment/prevention of virus-induced COPD exacerbations.

Conclusions

Many studies conducted in the last decade have produced convincing data on the molecular mechanisms involved in the pathogenesis of natural and experimental respiratory virus infections in humans, particularly rhinovirus (RV) and respiratory syncytial virus (RSV). Most of the studies have been performed on human respiratory epithelial cells, which can be directly infected by RV and RSV. Their infection by RV and RSV determines the production of several pro-inflammatory molecules (such as cytokines, chemokines and adhesion molecules). Conversely, very few studies have been conducted in vivo on the molecular mechanisms underlying the pro-inflammatory derangement induced by respiratory viruses in the airways during COPD exacerbations. Collectively these studies suggest a critical role for several transcription factor families, including the NF-κB, AP-1 and GATA families, in the production of pro-inflammatory mediators after RV and RSV infection. The relative importance of each cell, mediator, signalling pathway and transcription factor will hopefully be clarified in the next few years. The recent development of the first human model of virus induced COPD exacerbation, that has been demonstrated to be feasible and so far, safe, will facilitate identification of novel pharmacological targets that will provide opportunities to develop new treatments for exacerbations of COPD.

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