Proteases and Chemotactic Factors in BAL Fluid From Subjects With Subclinical Emphysema*

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Abbreviations: IL = interleukin; NE-α1 PI = neutrophil elastase-α1 protease inhibitor complex

It is generally accepted that proteases released from neutrophils and/or macrophages are involved in the development of emphysema associated with cigarette smoking. However, it has not been resolved which of these inflammatory cells is the main source of proteases responsible for lung destruction. To address this issue, we compared the concentrations of neutrophil elastase-α1 protease inhibitor complex (NE-α1 PI) and cathepsin L in BAL fluid between asymptomatic volunteers aged > 40 years who had emphysema detected by CT scans and those who had a similar smoking history but did not have emphysema. NE-α1 PI originates mainly from neutrophils, while cathepsin L is from macrophages. The immunologic levels of both proteases were significantly higher in the subjects with subclinical emphysema; but, when subjects aged < 60 years were chosen for comparison, there was no difference between the two groups for cathepsin L, although the difference remained for NE-α1 PI.1,2 These results suggested that neutrophils are more important than macrophages in the early development of emphysema.

We then used enzyme-linked immunosorbent assays to determine the levels of chemotactic factors for neutrophils and monocytes in BAL fluid. The factors studied were interleukin (IL)-8 and leukotriene B4 as key chemotactic factors for neutrophils and monocytes in BAL fluid. The factors measured were proteins and/or cytokines measured by enzyme-linked immunosorbent assay (ELISA) in BAL fluid among these chemotactic factors had a strong positive correlation with NE-α1 PI (r = 0.74, p < 0.01), and was significantly elevated in the subjects with subclinical emphysema (p < 0.01). To examine the possibility that alveolar macrophages are responsible for the elevation of IL-8 in BAL fluid, we measured IL-8 in alveolar macrophage-conditioned media and alveolar macrophage IL-8 messenger RNA by competitive reverse transcriptase-polymerase chain reaction. We did not, however, find any significant differences between the two groups in these measurements. These studies indicate that neutrophils recruited and/or activated by IL-8 appear to be crucial in the pathogenesis of subclinical emphysema. The source of IL-8 remains to be determined.

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Latent Adenoviral Infection in the Pathogenesis of Emphysema*

The Parker B. Francis Lectureship

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Abbreviations: ALRI = acute lower respiratory tract infection; ICAM = intercellular adhesion molecule; IL = interleukin; RSV = respiratory syncytial virus

Cigarette smoking causes airway and alveolar inflammation in everyone who smokes and is the major risk factor for developing COPD. However, as only 10 to 15% of heavy smokers develop emphysema and airways obstruction,1 other risk factors must influence cigarette smoke-induced lung inflammation to cause the lesions that produce COPD. Several factors that add risk to cigarette smoking have been identified,2 but this brief review is designed to concentrate on lower respiratory tract infections with particular reference to the hypothesis that latent adenoviral infection is capable of amplifying cigarette smoke-induced lung inflammation to produce emphysema.

Acute lower respiratory tract infection (ALRI) in childhood is a major worldwide health problem ranked first among conditions contributing to the global burden of disease.3 Viral infections contribute about 20 to 30% of all cases of ALRI,4,5 and in a community setting, approximately 53.5% of cases of viral etiology are attributable to respiratory syncytial virus (RSV), 13.9% to adenovirus, 7.0% to influenza, 4.7% to parainfluenza, 2.3% to more than one virus.4 Studies of pediatric hospital admissions for ALRI, on the other hand, show that the proportion of viral infections fall to 36.3% of all cases with RSV, and adenovirus accounting for the bulk of these admissions.5

An important feature of the adenovirus compared to the other common respiratory viruses is that it has a DNA rather than RNA genome. Portions of this viral DNA persist in host cells after viral replication has stopped as either a circular extra chromosome (plasmid) or by inte-

References

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migration into the host DNA. This persistence may be important in the pathogenesis of the known sequelae of adenoviral infection that include the Swyer-James syndrome (or unilateral hyperlucent lung), permanent airways obstruction, bronchiectasis, bronchiolitis obliterans, and steroid-resistant asthma.\textsuperscript{7–12} Our working hypothesis that this latent adenoviral infection also plays a role in the pathogenesis of COPD, because a protein expressed by the latent viral DNA amplifies the expression of genes that are activated in cigarette smoke-induced airway inflammation.

The adenoviruses are icosahedral particles that are 70 to 100 nm in diameter that consist of a protein shell surrounding a double-stranded DNA core.\textsuperscript{13} The genomes of >100 types isolated so far have the same general features, in that they consist of approximately \(35 \times 10^6\) base pairs that contain two origins for DNA replication, five early transcription units, two delayed early units, and one major late unit that is processed to generate five different families of messenger RNA.\textsuperscript{14} The replication of the adenovirus is dependent on transcriptional activation that is initiated by activation proteins encoded by one of the early transcription units referred to as the \textit{E1A} gene.\textsuperscript{15,16} During an acute infection, interactions between the adenoviral \textit{E1A} protein and host transcriptional elements create conditions that are favorable to viral replication. For example, they induce quiescent cells to enter the S phase of the cell cycle and inhibit host cell apoptosis.\textsuperscript{14} They also increase the transcription of viral and host genes by interacting with the DNA binding sites of host transcription factors.\textsuperscript{14} Early studies using classical hybridization techniques showed that the viral DNA remained in tonsils\textsuperscript{16,17} and peripheral blood lymphocytes\textsuperscript{18} long after viral replication stopped. Polymerase chain reaction based studies have shown that the adenoviral DNA from the \textit{E1A} gene is present in human lungs,\textsuperscript{19} and immunohistochemistry has shown that \textit{E1A} protein can be demonstrated in epithelial cells on the surface of conducting airways, epithelial glands, and in type II alveolar cells.\textsuperscript{20} The essence of our working hypothesis concerning latent adenoviral infection in emphysema is that the \textit{E1A} protein in alveolar epithelium amplifies the transcription of host genes expressed during cigarette smoke-induced lung inflammation and increases the migration of inflammatory cells into the alveolar surface.

The opportunity to study lung tissue from a group of children who died of ALRI of proven adenovirus etiology using \textit{in situ} hybridization showed that the virus targets epithelial cells in both the conducting airways and the gas exchanging surface of the lung where it primarily infected the type II cells.\textsuperscript{21} In the normal lung, the type II cells cover approximately 7% of the alveolar surface, produce surfactant, and are the progenitors of the type I cell that cover the remaining 93%.\textsuperscript{22} The recognition that inflammatory cells migrate out of vessels and into the alveolar airspace by passing between the type I and type II cells\textsuperscript{23} puts the type II cell in an ideal position to influence the inflammatory response in alveolar tissue (Fig 1). Over the past 11 years, we have conducted experiments to determine if the persistence of latent adenoviral infection in lung epithelial cells might amplify cigarette smoke-induced alveolar inflammation.

The demonstration of more \textit{E1A} DNA in lung tissue from patients with COPD than in tissue from age- and gender-matched control subjects with similar smoking histories\textsuperscript{19} and the immunohistochemical studies showing that there was \textit{E1A} protein in airway surface epithelial cells in epithelial glands and in type II alveolar cells on the gas exchanging surface of the lung,\textsuperscript{22} encouraged us to initiate an \textit{in vitro} investigation of a type II-like A549 cell.\textsuperscript{24,25} The A549 cell line is derived from a peripheral lung carcinoma that has many type II cell characteristics. When these cells are transfected with \textit{E1A} DNA, they demonstrate lamellar bodies and tight junctions consistent with a type II alveolar cell phenotype;\textsuperscript{26} and when challenged with inflammatory stimuli, they produce excess interleukin (IL)-8 and intercellular adhesion molecule (ICAM)-1.\textsuperscript{1,29,30} Subsequent studies demonstrated that the presence of \textit{E1A} protein leads to the activation of nuclear factor \(\kappa B\)\textsuperscript{26} to initiate these changes;\textsuperscript{27} and very recent studies have shown that the excess ICAM-1 and IL-8 production induced by tumor necrosis factor-\(\alpha\) becomes steroid resistant in \textit{E1A} transfected cells.\textsuperscript{28} The observations suggest that latent adenoviral infection of type II cells in \textit{in situ} might provide a mechanism that could amplify cigarette smoke-induced lung inflammation and make it steroid resistant.

When guinea pigs are exposed to cigarette smoke, they develop lung inflammation involving both the conducting airways and gas exchanging surface,\textsuperscript{29,30} and chronic exposure produces lesions consistent with human emphysema.\textsuperscript{31} When guinea pigs with latent adenoviral infection\textsuperscript{32}...
are exposed to a single dose of cigarette smoke, they develop an excess inflammatory response39 that could lead to excess emphysema. In a recent collaboration with the University of Pittsburgh, we compared tissue from patients with mild disease obtained by lung resection for tumor to advanced disease obtained from patients undergoing lung volume reduction surgery. Our preliminary findings suggest that there is a greater inflammatory response in the lungs in advanced emphysema that is associated with a greater prevalence of E1A protein in the type II alveolar cell.34 Collectively, these animal and human studies suggest that advanced emphysema is associated with amplification of the cigarette smoke-induced inflammation, and that this excessive response to cigarettes is related to the presence of latent adenoviral infection.

In summary, the cigarette smoking habit is the number-one risk factor for the development of emphysema and chronic airways obstruction, but only 15 to 20% of heavy smokers develop this complication.1 Lower respiratory tract infection is one of the factors that contribute to the risk of developing COPD, and our studies of latent adenoviral infection suggest mechanisms by which latent adenoviral infection results in amplification of cigarette smoke-induced lung inflammation. Although the studies presented here have focused on adenovirus, the field is open to the possibility that other infectious agents might produce similar results.

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HIV Infection Increases Susceptibility to Smoking-Induced Emphysema*

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Background: A number of uncontrolled reports have suggested that prior to the development of AIDS-related pulmonary complications, individuals infected with HIV may develop an accelerated form of lung damage consistent with an emphysema-like process. Confirming this observation may have important implications for our understanding of emphysema pathogenesis.

Methods: We prospectively evaluated 114 clinically stable HIV-positive subjects with high-resolution CT (HRCT) of the chest. Forty-four HIV-negative subjects matched for age and smoking history served as control subjects. Scans were interpreted for the presence and severity of emphysema. BAL was performed in agreeable subjects to exclude occult opportunistic infection and to examine cellular characteristics that might be correlated with emphysema development.

Results: The percentage of subjects meeting emphysema criteria was significantly higher in the HIV-positive group. Among clinical variables, low body mass index (BMI) and cigarette smoking were independently correlated with emphysema. There appeared to be a marked susceptibility to smoking-induced lung damage among HIV-positive subjects: nearly 40% with a >12-pack-year history of cigarette smoking met criteria for emphysema, vs 0 of 14 control subjects with a similar smoking history (p < 0.01). BAL revealed no occult pathogens to explain the HRCT changes; however, the percent of lymphocytes bearing the cytotoxic phenotype was highest among HIV-positive smokers with emphysema.

Conclusions: HIV infection is associated with an increased susceptibility to smoking-associated lung damage. The presence of emphysema is associated with decreased BMI and increased BAL cytotoxic lymphocytes.

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Airway Inflammation and Hyperresponsiveness to Adenosine 5′-Monophosphate in COPD*

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Abbreviations: AMP = adenosine 5′-monophosphate; BHR = bronchial hyperresponsiveness

COPD is often accompanied by bronchial hyperresponsiveness (BHR). Measurement of BHR may yield information about airway inflammation, and it has been suggested that indirect stimuli might have a closer relation with inflammation than direct stimuli such as histamine or methacholine. In order to get a better understanding of the role of BHR to adenosine 5′-monophosphate (AMP) in COPD, we investigated inflammatory indices in induced sputum, BAL fluid, and bronchial biopsies. We studied 18 nonatopic, nonreversible subjects with COPD, 12 with BHR to AMP (mean ± SD age, 63 ± 8 years; FEV1 percent predicted, 56 ± 13%), and 6 without BHR to AMP (mean ± SD age, 60 ± 6 years; FEV1 percent predicted, 65 ± 11%), and compared these with 11 healthy nonatopic control subjects (mean ± SD age, 58 ± 8 years; FEV1 percent predicted, 104 ± 11%).

Subjects with COPD with BHR in comparison to those without BHR had significantly higher numbers of mucosal CDS+ cells (median, 550 cells/µL; range, 30 to 1,340 cells/µL² vs median, 280 cells/µL; range, 110 to 450 cells/µL², p = 0.045) and higher percentages of sputum eosinophils (median, 2.7%; range, 0.5 to 8.5% vs median, 0.6%; range, 0 to 0.8%; p = 0.004). Otherwise, no differences between the two groups with COPD were observed.

We submit that hyperresponsiveness to AMP in COPD is associated with airway inflammation that is characterized by increased numbers of mucosal CDS+ cells and sputum eosinophils. A logical next step is to investigate whether AMP is a marker of inflammation in COPD that has prognostic relevance for the course of the disease and for the effects of treatments, including steroids.

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