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## Neutrophil elastase and obliterative bronchiolitis

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**Abstract** Bronchoalveolar lavage levels of elastase were assayed to determine the timing and magnitude of elevations in elastase relative to both fibrosis, as indicated by hyaluronate (HA) levels, and decline in FEV<sub>1</sub> characteristic of the clinical syndrome of obliterative bronchiolitis (OB). Samples were collected from 48 heart-lung or single lung transplant recipients. Regression analysis was performed and demonstrated that high levels of elastase occurred with active

decline in lung function and in association with high levels of HA. This study suggested that intense neutrophil elastase release occurs concurrent with the development of OB and may contribute to the destruction of bronchiolar architecture.

**Key words** Lung transplantation · Obliterative bronchiolitis · Elastase · Hyaluronic acid · Bronchoalveolar lavage · Neutrophils

### Introduction

Although the destruction of bronchioles in obliterative bronchiolitis (OB) may result from persistent and severe acute rejection [1–3], the destructive potential of neutrophils in this process remains undefined. Neutrophils are generally found in the more severe grades of pulmonary rejection [4]. The proteolytic enzyme elastase (EL), contained in the azurophilic granules of neutrophils, is a mediator of tissue injury, and can degrade basement membrane constituents and damage the epithelium in a number of pulmonary diseases.

Neutrophils are a regular feature of acute rejection of the lung, both in transbronchial biopsies [4] and in bronchoalveolar lavage (BAL) [5, 6]. In order to demonstrate neutrophil involvement in the pathogenesis of OB, it is necessary first to quantify neutrophil activity, which has previously been described in BAL using neutrophil EL assays. Second, it is necessary to demonstrate that a rise in neutrophil activity is either contiguous

with, or precedes, evidence of fibroblast activity, such as is putatively provided by BAL HA levels. Third, such changes in neutrophil EL must be closely linked to the decline in FEV<sub>1</sub> that characterises OB. EL levels should be higher in patients who develop OB than in non-OB well patients. Finally, EL levels should rise following persistent acute lung rejection.

### Methods

BAL levels of EL were assayed to determine the timing and magnitude of elevations in EL relative to both fibrosis, as indicated by hyaluronate (HA) levels, and decline in FEV<sub>1</sub> characteristic of the clinical syndrome of OB. A total of 46 heart-lung transplant (HLT) and 2 single lung transplant (SLT) patients, with a mean age of 31.2 years (range 17–57 years), on average 21.3 months post-transplantation (range 1–65 months) were lavaged on a total of 60 occasions with 50-ml aliquots of 0.9% saline into each lobe of both lungs and samples were analysed for both HA and EL. Lung function was recorded immediately prior to lavage. Comparison with peripheral blood white blood cell count (WBC) and peripheral blood neutrophils was also made.

## Results

The mean lavage return volume varied between 50 and 60% for each lobe and lingula with a range of SE of 1–2.4, showing no significant difference in return from the different areas of the lungs. Regression analysis was performed and the results are described below using the logarithm of the geometric means of HA and EL data.

LOG<sub>10</sub> HA vs. LOG<sub>10</sub> EL:  $r = 0.70$ ,  $t = 9.27$ ,  $df = 93$ ,  $P = 4.2 \times 10^{-15}$

LOG<sub>10</sub> EL vs. FEV<sub>1</sub>:  $r = -0.53$ ,  $t = -5.89$ ,  $df = 90$ ,  $P = 1.1 \times 10^{-7}$

LOG<sub>10</sub> EL vs. FEV<sub>1</sub>/FVC:  $r = -0.50$ ,  $t = -5.42$ ,  $df = 88$ ,  $P = 6.1 \times 10^{-7}$

LOG<sub>10</sub> EL vs. WBC:  $r = 0.40$ ,  $t = 3.48$ ,  $df = 66$ ,  $P = 9.1 \times 10^{-4}$

LOG<sub>10</sub> EL vs. neutrophils:  $r = 0.28$ ,  $t = 2$ ,  $df = 49$ ,  $P = 0.049$

Comparison of those patients with transbronchial evidence of early OB compared with those without such changes using Student's *t*-test indicated LOG<sub>10</sub> EL levels were higher in those patients with bronchiolar obliterative changes ( $t = -3.1$ ,  $df = 87$ ,  $P = 0.003$ ).

## Discussion

High levels of EL occurred with the active decline in lung function and in association with high levels of HA. We have previously demonstrated the powerful association between decline in FEV<sub>1</sub> and persistent rejection and OB (1). The only relationship with immunosuppression was positive, with higher immunosuppression associated with increasing EL levels, suggesting clinical concern in these patients. This study suggested that intense neutrophil EL release occurs concurrent with the development of OB; high levels of EL may mediate bronchiolar injury. We concluded that for patients with declining lung function characteristic of OB in whom conventional therapy has failed, anti-neutrophil chemotherapy may be of benefit.

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