THE ROLE OF APOPTOSIS IN THE PATHOGENESIS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Synopsis

COPD is a chronic disease of the airways, usually caused by cigarette smoking and characterised by chronic inflammation and tissue destruction. There are no effective treatments for this debilitating disease and the monetary cost to the Australian community is $800 million per year. In addition, there are devastating social consequences to those with COPD and their families. To date, however, there have been few studies on the cause of defective repair in COPD.

In COPD there is inadequate repair of the chronically damaged and inflamed airway epithelium. There is a school of thought that this inadequate repair may result from an accumulation of apoptotic material in the airways, resulting in secondary necrosis, tissue destruction and chronic inflammation. This thesis is principally an investigation of apoptosis and clearance of apoptotic cells in COPD using samples of peripheral blood, and bronchial brushings and bronchial lavage (BAL) obtained from the airways during fibreoptic bronchoscopy. Importantly, it describes the development of a number of new methods to investigate these biologic specimens.

Increased apoptosis of brushing-derived airway epithelial cells (AEC) and lymphocytes (from BAL) was observed in COPD. Activation of several apoptotic pathways was also increased in COPD, including Fas/Fas ligand, TNF-α/ TNF receptor and TGF-β/ TGF receptor. These results support the hypothesis that excess apoptosis and accumulation of apoptotic material in the airways are key factors in the pathogenesis of COPD.

Whether the increase in apoptotic material in the airways was a result of defective clearance by alveolar macrophages was subsequently investigated. A significantly reduced capacity for alveolar macrophages from COPD subjects to ingest apoptotic AEC was found. This deficiency appeared to be specific for apoptotic cells, as tests carried out in parallel using carboxylate-modified polystyrene microbeads revealed no significant difference between COPD patients and control subjects. The results

demonstrate that the failure to resolve epithelial damage in COPD may result, at least partially, from defects in recognition and/or clearance of apoptotic AEC by AM.

Based on the findings of increased apoptosis of lymphocytes in the airways in COPD as well as reports that lymphocytes from the airway re-enter the peripheral circulation, I investigated the hypothesis that, in COPD, there may be increased T-cell apoptosis in the peripheral blood. As in the airways, significantly increased apoptosis and upregulated TNF-α/TNFR-I, Fas and TGFR was observed in stimulated peripheral blood T-cells in COPD. Whether these findings represent a systemic effect of COPD on peripheral cells or, alternatively, whether these cells have re-entered the circulation after passing through the epithelium, requires further study. This study was then extended to show that the mechanisms by which T-cells in the peripheral blood undergo apoptosis include increased expression of the pro-apoptotic mediator p53, and decreased expression of the receptor for the anti-apoptotic cytokine, IL-7. Given these findings, it would be interesting to apply these techniques to brushing-derived AEC in COPD. Due to time constraints these were not carried out, but one can speculate that similar mechanisms may be involved in the airways.

In airway repair, TGF-β increases apoptosis and inhibits proliferation of AEC. A normal bronchial AEC line (16HBE) was applied as an in vitro model, to further investigate which mechanisms are responsible for control of TGF-β production once the repair process is complete. Both IL-4 and TNF-α, cytokines produced in response to inflammatory stimulus in the airways, were shown to inhibit production of TGF-β by AEC. In COPD, however, where there is increased production of TGF-β and ineffective repair, the inhibitory effects of TNF-α and IL4 on TGF-β production may be overwhelmed by other mechanisms.

An interesting cause for the increase in TGF-β in the airways in COPD was found. Apoptotic AEC were shown to produce cytokines, including TGF-β, *in vitro*. In the

normal lung, increased production of TGF-β by AEC undergoing apoptosis may contribute to inhibition of proliferation, airway repair and reduction of the inflammatory response following acute injury. In COPD, where there is already increased apoptosis and increased TGF-β, the added effect of more TGF-β production by apoptotic AEC may overwhelm the normal regulatory mechanisms.

The findings from this thesis indicate that failure to resolve epithelial damage and chronic inflammation in COPD may result, at least partially, from increased apoptosis, increased activation of apoptotic pathways and defective clearance of apoptotic material by AM. The new insights into the role of tissue defence mechanisms in COPD are relevant in relation to future experimental approaches that may help to design novel new treatment strategies for COPD.