Local endobronchial allergen challenge allows a high dose of allergen to be delivered to a single airways segment to study the release of spasmogenic mediators [1] and recruitment of T cells and other leucocytes in small blood vessels in the subjacent bronchial mucosa [2], in comparison to matched control sites. Six hours after challenge, we found an increased number of eosinophils and neutrophils with up-regulation of the endothelial adhesion molecules ICAM-1 and E-selection [3]. We have extended these studies to address the cellular changes 24 hours after local allergen exposure.

caine. Endobronchial challenge was performed in the medial segment, right middle lobe with 20 ml of prewarmed allergen solution, the concentration used being calculated by skin test titration. Control challenge was performed with 20 ml saline diluent in the anterior segment, right upper lobe. The airway was observed for 5 min to confirm bronchoconstriction. A second bronchoscopy was performed with identical premedication 24 h later. Bronchoalveolar lavage (BAL) was performed in each of the challenged segments with 120 ml isotonic warm saline and four mucosal biopsies were obtained from each segment. Mucosal biopsies were processed into glycol methacrylate and stained with monoclonal antibodies and an indirect peroxidase technique. BAL cells were processed for flow cytometry and in a small number of cases, samples were studied by PCR for cytokine transcription.

Methods
Ten subjects with mild atopic asthma (mean age 33 years) were recruited by advertising for volunteers. All 10 had atopic asthma as defined by history, increased bronchial responsiveness to
histamine and strong (> 5 mm) reactions to mixed grass pollen our house dust mite. All were using inhaled bronchodilators as required, but none were on inhaled corticosteroids. All were non-smokers. Bronchoscopy was performed by a standard technique using premedication with nebulised albuterol followed by atropine 0.6 mg and midazolam 5-8 mg intravenously, while local anesthesia was achieved with 2-4% lidocaine.

Results

Compared with the saline control sites, BAL samples from the allergen sites showed increased numbers of macrophages (95.2 vs. 70.6, p < 0.05), neutrophils (33.8 vs. 20.6, p < 0.05), lymphocytes (21.65 vs. 11.92, p < 0.05), and eosinophils (16.3 vs.0.9, p < 0.01). Flow cytometry showed similar proportions of CD3+, CD4+, CD8+, CD25+, and HLA-DR+ T cells at both sites. In contrast, the mucosal biopsies showed no significant differences in eosinophil or neutrophil numbers but there was a trend for increased numbers of CD3+, CD4+ and CD8+ T cells at the allergen challenge site. There was considerable variation between individuals in the pattern of cellular recruitment and these differences were not statistically significant. Expression of the T cell activation marker CD25 was increased in biopsies from the allergen site in 2 subjects only. At both sites, there were similar proportions of vessels staining for the adhesion molecules ICAM-1, E-selectin and VCAM-1 with a slight excess of VCAM-1 staining at allergen sites. Staining for LFA-1, VLA-1 or VLA-6 showed no difference between the allergen and saline sites but there was a slight excess of VLA-4+ cells and a parallel reduction in CD44+ staining at the allergen sites.

Discussion

In comparison with our previous studies (6 h after challenge), this series showed a greater degree of BAL eosinophilia but less tissue eosinophilia. It is conceivable that acute allergen exposure leads to recruitment of eosinophils which then migrate through into the BAL compartment but recruitment of new eosinophils does not continue after the first 6 h. These subjects were relatively mild asthmatics and it remains possible that the mechanisms of allergic inflammation in very mild asthmatics may differ from the processes that occur in patients with more severe disease. This may explain the relative lack of T cell activation found in the present study and may account for differences from other models such as allergen inhalation [4, 5].

References


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