**T cells, cytokines and asthma**

This article is based on the Tudor Edwards lecture given at the Royal College of Physicians on 5 March 1993 by A B Kay, Professor of Allergy and Clinical Immunology, National Heart and Lung Institute, London.

**What is asthma?**

Asthma is one of the commonest chronic diseases in developed countries. It affects between three and eight per cent of adults in England and Wales and causes about 2,000 deaths a year [1]. The disorder is characterised by generalised airways obstruction which is reversible either spontaneously or by treatment. A cardinal feature is non-specific bronchial hyperactivity (or hyperresponsiveness) in which the airways are irritable and constrict when exposed to non-specific agents such as sprays, fumes and smoke. It is measured in the clinical laboratory by the methacholine or histamine PC_{20}, the dose which gives a 20% decrease in forced expiratory volume in one second (FEV_{1}). It is widely believed (but not proven) that increased airways responsiveness in asthma is due to inflammation in and around the wall of the bronchial mucosa.

In health, the bronchi are principally lined by pseudostratified squamous epithelial cells and goblet cells, and the diurnal variation in airways calibre is about six per cent or less. In contrast, even in mild asthma there are local inflammatory changes which include oedema of the submucosa. As with inflammation at all mucosal surfaces, there is shedding of epithelial cells, with accompanying mucous hypersecretion [2-4]. Thus, narrowing of the airways in asthma is a combination of bronchial smooth muscle contraction, mucosal oedema and partial or complete occlusion of the lumen with mucous cells and cell debris. In severe asthma lung function is much reduced; there is also considerable diurnal variation in peak flow—30% or more in acute severe asthma. The degree of variability provides an indirect measure of bronchial hyperresponsiveness (Fig 1).

The characteristic features of the histopathology of asthma are:

- hypertrophy of smooth muscle and marked inflammatory infiltrate in which eosinophils and mononuclear cells are prominent;
- local eosinophilia, often intense, with deposition of products from the crystalloid granule of the eosinophil—that is, the basic proteins (major basic protein, eosinophil cationic protein (ECP), eosinophil-derived neurotoxin and eosinophil peroxidase); and
- in addition to shedding of the epithelium, collagen is deposited below the true basement membrane.

**Extrinsic and intrinsic asthma**

The two major variants of asthma are extrinsic (allergic) and intrinsic (non-allergic) asthma (Fig 2). Extrinsic asthma is the commonest variant and usually presents at a young age. It occurs in atopic individuals, ie those with raised levels of allergen-specific immunoglobulin (Ig) E. They frequently give a history of wheezing after direct exposure to allergen and respond well to standard anti-asthma regimes.

In contrast, intrinsic asthma often presents in the third or fourth decade, without evidence of atopic allergy and with normal total and specific IgE serum concentrations. Infection is a common trigger, and the disease can be quite severe and difficult to manage. These patients often require oral as well as inhaled corticosteroids to control their symptoms (‘steroid-dependent asthma’).

**T cells in asthma**

T lymphocytes arise in the bone marrow but differentiate in the thymus where clones directed against self-antigens are eliminated during thymic education. Early thymocytes co-express CD4 and CD8, the phenotypic markers for T helper (TH) and T cytotoxic/suppressor cells, respectively. This is followed by acquisition of the unique T cell receptor which is closely associated with and stabilised by the CD3 family of transmembrane proteins. Antigen is presented to CD4 cells in association with major histocompatibility complex (MHC) Class II molecules and to CD8 cells with MHC Class I molecules. Much of the knowledge both on T cell activation and proliferation and on the cytokines they produce is derived from studies on CD4+ clones. After engagement of the T cell receptor by processed antigen, CD4+ cells begin to synthesise proteins including surface activation markers such as CD69, CD25 (interleukin (IL)-2R), HLA-DR and VLA-1. This is accompanied by transcription, translation and secretion of various cytokines. Cytokines are soluble mediators which can act as growth factors...
and/or inflammatory agents; they are also essential elements in the differentiation and maturation of various cell types.

One of the first observations to focus attention on the T cell in pathogenic mechanisms of asthma came from studies on peripheral blood from patients with acute severe asthma. They had more CD25 (IL-2R) positive cells than control groups (mild asthma, chronic obstructive airways disease and normal individuals) [5]. Furthermore, the proportion of CD25+ cells dropped after successful treatment [6]. Dual immunofluorescence techniques showed that virtually all CD25+ cells are associated with CD4+ rather than the CD8+ cell population. More recently, CD4+, but not CD8+, blood cells from chronic asthmatics were shown to encode messenger RNA (mRNA) for IL-3, IL-5 and granulocyte macrophage colony-stimulating factor (GM-CSF) and that they spontaneously release these cytokines into the supernatant [7].

The direct association of T cells with the pathogenesis of asthma was not firmly established until it became possible to sample tissue directly from the airways using the flexible fibreoptic bronchoscope. Activated T cells were identified in mucosal bronchial biopsies or bronchoalveolar lavage (BAL) cells even in mild asthma and their numbers shown to correlate with disease severity. Transmission electron microscopy revealed more atypical lymphocytes in hyperresponsive mild asthmatics than in control groups [3]. These irregular lymphocytes were demonstrated by immunocytochemistry to be activated T cells (Fig 3). Using an anti-CD25 monoclonal antibody, significantly more CD25+ cells were observed in asthma than in bronchial biopsies obtained from hay fever subjects and normal controls [8,9].

Fig 1. The relationship between airway pathology and lung function. In health (top) the variability in FEV₁ is usually less than 6%. In asthma (centre), even of mild to moderate severity, marked pathological changes (see text) are accompanied by limitation of airflow and an increased diurnal variation in FEV₁. During an 'asthma attack' or acute severe asthma (bottom), FEV₁ is less than 50% of predicted, with great variability (up to 30%) in the diurnal variation. The pathological changes are even more marked. The airway is now considerably narrowed and occluded with mucus, cells and cell debris.

Fig 2. The relationship between atopy and asthma. (TH: T helper)
The other prominent cell type was the eosinophil, as shown by immunostaining with the monoclonal antibody EG2 which recognises a cleaved portion of ECP. The number of EG2+ cells also correlated with clinical features, including FEV₁ methacholine PC₂₀ test. Similar observations were made in biopsies both from patients with intrinsic asthma [10] and in occupational asthma, that is, asthma in association with hypersensitivity to toluene di-isocyanate [11]. Thus, significantly more CD25+ and EG2+ cells were seen in all the major variants of asthma than in allergic rhinitis and controls. In general, the total number of CD45+ cells (total leukocytes), CD3+, CD4+, CD8+ and CD68+ cells were similar in the various types of asthma and in the control subjects [12]. Thus, it was not the total number of T cells and their subsets that was elevated but the number of cells that had been activated, presumably via antigen stimulation. CD25+ cells were shown to be mainly T cells by double staining methods (for bronchial biopsies) and two-colour flow cytometry (for BAL cells) [13]. Dual immunofluorescence with flow cytometry has allowed the study of large numbers of atopic asthmatics in order to correlate the number of CD4/CD25 (activated TH cells) with disease manifestations. For instance, there was an inverse correlation between the percentage of BAL CD4/CD25+ cells and the PC₂₀ methacholine, and a positive association with the severity of disease as shown by a global asthma symptom score.

**Cytokines and asthma**

Early differentiation of eosinophil precursors depends on IL-3 and GM-CSF, and IL-5 produces terminal differentiation of the committed eosinophil precursor [14]. All three cytokines activate mature eosinophils in terms of enhanced phagocytosis and oxidative metabolism and prolong the survival of eosinophils in culture. IL-5, unlike IL-3 and GM-CSF which have many effects on a range of cell types, is relatively selective in its actions for eosinophils, thus raising the possibility that IL-5 may be a critical mediator in the asthma process. IL-5 is a disulphide-linked homodimeric glycoprotein which is highly homologous between species. The monomer has 115 residues (molecular weight: 12,000); the secreted material has a molecular size between 40,000 and 50,000. The molecule exists in an antiparallel (head to tail) configuration [15].

In order to show that specific proteins are involved in physiological or pathophysiological processes, it is first necessary to provide evidence of protein synthesis, storage or secretion in tissue or body fluids in a particular disease state. Transcripts (mRNA) can be identified either by *in situ* hybridisation or Northern blots. *In situ* hybridisation has the advantage of unique sensitivity and with the application of double labelling techniques it is possible to localise transcripts to particular cell types. The translated products stored in cells can be visualised by immunocytochemistry using appropriate specific antibodies. The secreted product can be identified in blood and other body fluids by standard assays such as radioimmunassay, enzyme-linked immunoadsorbent assay (ELISA) or bioassay. *In situ* hybridisation using radiolabelled (³²P or ³⁵S) or non-radiolabelled (digoxigenin) riboprobes has enabled transcripts to be identified for a number of cytokines either from biopsy material or from dispersed BAL cells. Antisense or sense (control) probes were prepared by *in vitro* transcription using the relevant RNA polymerases. Experiments were performed

---

Fig 3. The immunocytochemistry of bronchial biopsies from atopic asthmatics (n = 21) (solid bars), atopic non-asthmatics (n = 10) (hatched bars) and controls (n = 12) (open bars) * indicates significance. (Data from Ref 9)
under high stringency conditions, with the incorporation of various other controls such as pretreatment with RNase. A T cell line from the hyper-IgE syndrome serves as a positive control since these cells are known to synthesise and secrete IL-3, IL-5 and GM-CSF. It was established that IL-5 mRNA positive cells were present in mucosal bronchial biopsies from symptomatic but not asymptomatic mild asthmatics or normal healthy controls (Fig 4) [16]. The biopsies containing IL-5 mRNA positive cells had more eosinophils and CD25+ cells than the IL-5 mRNA negative biopsies. This supports the hypothesis that activated T cells release IL-5 and related cytokines which in turn are involved in eosinophil recruitment, activation and mucosal tissue damage.

Some years ago Mosmann and Coffman suggested that TH cells in mice could be broadly divided into two groups, TH1 and TH2, based on the cytokines they produce in response to specific antigen [17]. TH1 cells produce IL-2 and interferon (IFN)-gamma and are involved in delayed-type hypersensitivity responses, whereas TH2 cells elaborate IL-4 and IL-5 and are associated with increased IgE production. Both cell types elaborate IL-3 and GM-CSF. This TH1/TH2 dichotomy exists also in man; for instance, T cell clones obtained from peripheral blood from patients with allergic rhinitis are TH2-like [18], and individuals sensitive to both purified protein derivatives of tuberculin (PPD) and Toxocara canis produce either antigen-specific TH1- or TH2-type clones [19]. Cells cloned from the conjunctival mucosa from patients with vernal conjunctivitis, or from the skin in atopic dermatitis also produce TH2-type clones [20]. TH1-type clones were obtained from skin biopsies in contact dermatitis [21].

We have provided evidence that specific allergen provokes local accumulation of TH2-type cells in vivo. In these studies, tissue was either obtained from atopic patients and compared with controls, or sampled before and after allergen provocation. Thus, skin biopsies obtained from allergen-induced late-phase reactions were predominantly TH2-type [22] whereas those obtained from tuberculin reactions were TH1 [23]. When BAL cells obtained from atopic asthmatics were compared with normal controls, the pattern was also TH2-type [24], with significantly more IL-5, IL-4, GM-CSF and IL-3 positive cells compared with controls and no appreciable differences in IFN-gamma or IL-2.

Although T cells are a major source of IL-5 and other cytokines, other cell types such as mast cells and eosinophils also synthesise, store and secrete various cytokines. In BAL cells from baseline asthma only the CD2+ cells (obtained by immunomagnetic beads coated with anti-CD2) give positive hybridisation signals for IL-4 and IL-5 [24]. More recently (using a double immunocytochemistry in situ hybridisation technique), BAL eosinophils and mast cells have been shown to be sources of these cytokines.
Nasal biopsies from allergen-challenged late-phase reactions were also TH2-type, raising the possibility that TH2 might be a feature of atopy rather than of asthma [25]. However, more recent work has shown that in both extrinsic and intrinsic asthma eosinophils and IL-5 concentrations are increased, whereas IL-4 and IgE can be detected only in extrinsic disease [26]. It seems unlikely, therefore, that the cytokine profile of intrinsic asthma can be explained on the basis of TH1/TH2 subsets (Fig 2).

**Interleukin-5**

Although the evidence that IL-5 plays a role in the pathogenesis of asthma is persuasive, this has still to be confirmed. In addition to showing that in asthma more cells are mRNA+ for IL-5 in bronchial biopsies and BAL than in controls, IL-5 mRNA+ cell numbers have been found to correlate inversely both with lung function and with the degree of bronchial hyperresponsiveness [27]. Furthermore, provoking asthma by allergen challenge induces BAL cells which contain IL-5 transcripts and the translated product [28]. Conversely, in a placebo-controlled study, successful treatment with corticosteroids reduced the number of IL-5 mRNA+ cells. Late-phase reactions were elicited in extrinsic allergic asthmatics after significant elevations in IL-5 and eosinophils were observed (as well as in IL-4 and GM-CSF) in allergen, as opposed to diluent, inhalation challenge [27,29]. There were also correlations between the numbers of activated TH cells (CD4+/CD25+) and IL-5, CD4, CD25 and eosinophils, IL-5 mRNA+ cells and eosinophils, and eosinophils and the magnitude of the late-phase reaction.

It was also established that a two-week course of oral prednisolone inhibits IL-5 and IL-4 transcripts in BAL cells from patients with moderately severe asthma [30].

A diagrammatic scheme linking mechanisms in episodic and chronic asthma, including the possible interrelationship of activated T cells, TH2-type cytokines and eosinophils, is shown in Fig 5.

**Cytokines in severe asthma**

There is little information on inflammatory changes in the bronchi in chronic severe asthma because of the obvious difficulty in obtaining biopsies or BAL from patients with poor lung function. However, useful information has been obtained from peripheral blood samples. It has been assumed that in many instances bronchial inflammation may be severe enough for there to be a 'spill over' of inflammatory cells and mediators into the circulation. Thus, patients with chronic severe asthma were found to have high serum levels of IL-5, which returned to undetectable levels after seven days of treatment with oral prednisolone [31]. This was not observed in a control group. There were similar decreases in the number of activated T cells (ie CD4+/CD25+ cells).

About 10% of chronic asthmatics have corticosteroid-dependent disease; they are the 'hard core' of the asthma problem. Some of these patients are often relatively corticosteroid-resistant in the sense...
that increasing the dose of steroids does not improve their lung function (whereas withdrawing steroids rapidly leads to acute severe asthma) [32]. Treatment for this group is unsatisfactory since the risk to benefit ratio is not acceptable. These patients are prone to develop complications of long-term steroid therapy such as osteoporosis, peptic ulceration, diabetes and acute psychosis.

T cells from normal individuals, and also from asthmatics who respond clinically to the action of glucocorticosteroids, are sensitive to the action of steroids in vitro: for example, phytohaemagglutinin-induced proliferative responses are inhibited by agents such as dexamethasone, whereas T cells from patients with corticosteroid-resistant asthma are relatively refractory to such inhibition in vitro [33,34]. However, the response of T cells both from steroid-sensitive and from steroid-resistant asthmatics to cyclosporin A in vitro is similar [35].

The immunosuppressants, cyclosporin A and the macrocles FK-506 and rapamycin, effectively prevent graft rejection and other inflammatory processes associated with T cell activation. They are in fact prodrugs that become active only when bound to specific members of the cyclophilin or FK-506 binding protein receptor gene families. The cyclosporin-cyclophilin or FK-506/FK-506 binding protein receptor complexes interact with a key component of the T cell antigen receptor signal transduction pathway, the calcium-calmodulin dependent phosphoprotein phosphatase calcineurin. The drug-receptor complexes inhibit the phosphatase activity of calcineurin and thereby prevent transcriptional activation of the IL-2 gene.

Cyclosporin A is a neutral lipophilic cyclic undeca-peptide extracted from the fungus Tolypocladium inflatum. It has a relatively good safety profile compared with high-dose oral corticosteroids, the major side-effect being dose-dependent renal and hepatotoxicity. Cyclosporin has a wide range of action, and it affects transcription of many cytokines by many cell types, including T cells, eosinophils, mast cells and basophils.

The potential for immunosuppressive agents in chronic asthma was evaluated by performing a double-blind crossover placebo-controlled trial of cyclosporin A in 33 patients with steroid-dependent disease [36]. The average duration of asthma in these patients was 27 years, and they had been taking continuous oral corticosteroids for between three months and 25 years. Their FEV1 was approximately 60% of predicted and they were all taking maximum doses of inhaled corticosteroids in addition to oral prednisolone (5–20 mg). After a four-week run-in period patients received cyclosporin (5 mg/kg/day) or placebo for 12 weeks followed by a two-week washout period, and were then crossed over to the second three-month arm. The trial clinician was blinded, but an unblinded monitor adjusted the cyclosporin A dosage and made random dosage adjustments to the placebo group to avoid bias on the part of the blinded trial clinician. There was only one dropout due to an unwanted effect of the active drug (hypertrichosis). Cyclosporin therapy improved morning peak expiratory flow rate (PEFR) by a mean of 12% ($p < 0.04$) and FEV1, by a mean of 17.6% ($p < 0.001$). The frequency of disease exacerbations requiring an increased prednisolone dose was 48% less ($p < 0.02$) in patients on cyclosporin than in those taking placebo. Diurnal variation in PEFR decreased by a mean of 27.6% ($p = 0.04$).

We believe that cyclosporin may be an effective option in the treatment of chronic severe steroid-dependent asthma. The observations from this study support a role for activated T lymphocytes in asthma pathogenesis.

**Future developments**

There is now considerable interest in newer and more potent immunosuppressants as possible treatments for chronic steroid-dependent asthma. These include the macrocles FK-506 and rapamycin, and mycophenolate mofetil (which inhibits DNA metabolism). FK-506, like cyclosporin, inhibits activation of the resting cell, changing from G0 to G1 phase of the cell cycle, whereas rapamycin interferes with signals required for DNA synthesis (G1 to S phases of the cell cycle). This raises the possibility that combinations of drugs acting at different stages in the cell cycle may represent a novel therapeutic approach.

An alternative strategy, now extensively used in many forms of autoimmune disease and as adjuncts to treatments in transplantation, includes the use of humanised antibodies. These genetically engineered molecules retain only the complementarity repeat regions of mouse origin in the Fab region, and therefore antigenicity is greatly minimised. Antibodies against a number of markers of T cells and their products are under evaluation in several disease states and could be applied to asthma. These involve anti-CD4 and CD25 as well as antibodies to cell adhesion molecules (CAMs) such as the β1-integrins CD11, CD18, and β2-integrins VLA-4 or its ligand V-CAM. Antibodies against cytokines, particularly IL-5, may also represent novel strategies.

Other strategies for inhibiting IL-5 activity involve the development of IL-5 synthesis inhibitors, soluble receptors for IL-5, or monoclonal antibodies to the IL-5 receptor. IL-5 receptor antagonists could also be developed using classical pharmaceutical industry techniques.

Thus, the possibility for treating asthma, particularly chronic asthma, using a variety of new 'immunosuppressive' agents is very real. They could be directed against T cells, T cell activation markers, eosinophil-associated cytokines or molecules involved in T cell or eosinophil adhesion pathways. Delivery could be either by the oral or inhaled route. Such approaches hold exciting prospects for the future.
T cells, cytokines and asthma

References

1. Burney P. Asthma deaths in England and Wales 1951–1985: evidence for a true increase in asthma mortality. J Epidemiol Community Health 1988;42:316–20.

2. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtra T. Damage of airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985;131:599–606.

3. Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural, quantitative study and correlation with hyperreactivity. Am Rev Respir Dis 1989;140:1745–53.

4. Djukanovic R, Roche WR, Wilson JW, Beasley CRW, et al. Mucosal inflammation in asthma. State of the art. Am Rev Respir Dis 1990;142:434–57.

5. Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma: relationship to disease severity and atopic status. Am Rev Respir Dis 1990;141:970–7.

6. Corrigan CJ, Hamid Q, Barkans J, North J, et al. Expression of cytokines (protein and messenger RNA) affecting eosinophil survival by CD4 and CD8 peripheral blood T lymphocytes from asthmatics and normal controls. Am J Resp Crit Care Med 1994;149:495.

7. Azzawi M, Bradley B, Jeffery PK, Frew A, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am Rev Respir Dis 1990;142:1407–18.

8. Bradley BL, Azzawi M, Jacobson M, Assoufi B, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991;88:661–74.

9. Bentley AM, Menz G, Storz C, Robinson DS, et al. Identification of T lymphocytes, macrophages and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial responsiveness. Am Rev Respir Dis 1992;146:500–6.

10. Bentley AM, Maestrelli P, Saetta M, Fabbri LM, et al. Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. J Allergy Clin Immunol 1992;89:821–9.

11. Hamid Q, Barkans J, Robinson DS, Durham SR, Kay AB. Co-expression of CD25 and CD3 in atopic allergy and asthma. Immunology 1992;75:659–63.

12. Robinson DS, Bentley AM, Hartnell A, Kay AB, Durham SR. Activated memory T helper cells in bronchoalveolar lavage from atopic asthmatics. Relation to asthma symptoms, lung function and bronchial responsiveness. Thorax 1993;48:26–32.

13. Clutterbuck EJ, Hirst EMA, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. Blood 1988;73:1504–13.

14. Sanderson CJ. Interleukin-5, eosinophils, and disease. Blood 1992;79:3101–9.

15. Hamid Q, Azzawi M, Sun Ying, Moqbel R, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. J Clin Invest 1991;87:1541–6.

16. Mosmann TR, Coffman RL. Two types of mouse helper T cell clone: implications for immune regulation. Immunol Today 1987;8:223–7.

17. Wierenga EA, Snoek M, De Groot C, Chretien I, et al. Evidence for compartmentalisation of functional subsets of CD4+ T lymphocytes in atopic patients. J Immunol 1990;144:4651–6.

18. Romagnani S. Induction of T1 and T2 responses: a key role for the ‘natural’ immune response? Immunol Today 1992;13:379–81.

19. Maggi E, Biswas P, Del Prete GF, Parronchi P, et al. Accumulation of Th2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. J Immunol 1991;146:1169–74.

20. Kapsenberg ML, Wierenga EA, Stickema FEM, Tiggelman AMBC, Bos JD. TH1 lymphokine production profiles of nickel-specific CD4+ T lymphocyte clones from nickel contact allergic and non-allergic individuals. J Invest Dermatol 1992;89:59–63.

21. Kay AB, Sun Ying, Varney V, Gaga M, et al. Messenger RNA expression of the cytokine gene cluster, IL-3, IL-4, IL-5 and GM-CSF in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 1991;173:775–88.

22. Tasopoulos A, Hamid Q, Varney V, Sun Ying, et al. Preferential mRNA expression of Th1-type cells (IFN-gamma+", IL-2") in classical delayed-type (tuberculin) hypersensitivity reactions in human skin. J Immunol 1992;148:2058–61.

23. Robinson DS, Hamid Q, Sun Ying, Tasopoulos A, et al. Predominant T2-type bronchoalveolar lavage T lymphocyte population in atopic asthma. N Engl J Med 1992;326:298–304.

24. Durham SR, Sun Ying, Varney VA, Jacobson MR, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and GM-CSF in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992;148:2900–4.

25. Walker C, Bode E, Boer L, Hansel TT, et al. Allergic and non-allergic asthmatics have distinct patterns of T cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am Rev Respir Dis 1992;146:109–15.

26. Robinson DS, Hamid Q, Bentley A, Sun Ying, et al. Activation of CD4+ T cells, increased Th2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in atopic asthmatics. J Allergy Clin Immunol 1993;92:313–24.

27. Ohnishi T, Kita H, Weiler D, Sur S, et al. IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late-phase reaction. Am Rev Respir Dis 1993;147:901–7.

28. Bentley AM, Qiu Meng, Robinson DS, Hamid Q, et al. Increases in activated T lymphocytes, eosinophils and cytokine messenger RNA for IL-5 and GM-CSF in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. J All Respir Dis 1993;8:35–42.

29. Robinson DS, Hamid Q, Sun Ying, Bentley AM, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5 and interferon-gamma cytokine gene expression. Am Rev Respir Dis 1993;148:401–6.

30. Corrigan CJ, Haczku A, Gemou-Engesaeth V, Doi S, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5: effect of glucocorticoid therapy. Am Rev Respir Dis 1993;147:540–7.

31. Carmichael J, Paterson IC, Diaz P, Crompton G, et al. Corticosteroid-resistance in chronic asthma. Br Med J 1981;282:1419–22.

32. Poznansky MC, Gordon ACH, Douglas JG, Krajewski AS, et al. Resistance to methylprednisolone in cultures of blood mononuclear cells from glucocorticoid-resistant asthmatic patients. Clin Res 1986;34:901A.

33. Corrigan CJ, Haczku A, Barnes NC, Tsai JJ, et al. Glucocorticoid resistance in chronic asthma. Peripheral blood T lymphocyte activation and comparison of the T lymphocyte inhibitory effects of glucocorticoids and cyclosporin A. Am Rev Respir Dis 1991;144:1026–32.

34. Haczku A, Alexander A, Brown P, Kay AB, Corrigan C. The effect of dexamethasone, cyclosporin A and rapamycin on T lymphocyte proliferation in vitro: comparison of cells from corticosteroid sensitive and corticosteroid resistant chronic asthmatics. J Allergy Clin Immunol 1991;93:510–9.

35. Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin A in corticosteroid-dependent chronic severe asthma. Lancet 1992;339:324–8.

Address for correspondence: Professor A B Kay, Department of Allergy and Clinical Immunology, National Heart & Lung Institute, Dowhouse Street, London SW3 6LY.