

Case report

Cyclosporin A treatment and airways inflammation in corticosteroid-dependent asthma

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Cyclosporin A is a potent immunosuppressive agent which inhibits activation of T cells and other inflammatory cells. It has been shown to be of clinical benefit in patients with corticosteroid-dependent asthma, but there are no data on its *in vivo* effects on airways inflammation. In this report, we describe the case of a 47-year-old man with chronic severe corticosteroid-dependent asthma who made a dramatic clinical response to therapy with cyclosporin A. Fibreoptic bronchoscopy with bronchoalveolar lavage and endobronchial biopsy were performed before and after a 12-month period of treatment with cyclosporin A and demonstrated a concomitant reduction in airway inflammatory indices.

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The view of asthma as a chronic inflammatory condition has become widely accepted in recent years, largely on the basis of studies using fibreoptic bronchoscopy to obtain bronchoalveolar lavage (BAL) fluid and biopsies from the airways (reviewed by Djukanovic et al. [1]). Most asthmatics respond well to treatment with inhaled corticosteroids, which decrease symptoms, improve physiologic indices, and reduce the expression of inflammatory markers in the airways (2, 3). However, a small proportion of patients require long-term treatment with systemic corticosteroids. In these patients, alternative immunosuppressive agents are sometimes considered in the hope of both improving control of their asthma and lessening the incidence of adverse events resulting from long-term corticosteroid usage.

Cyclosporin A is a potent immunosuppressive drug that has been used in the management of a number of chronic inflammatory conditions, such as psoriasis, Crohn's disease, and rheumatoid arthritis (4). The role of cyclosporin A in asthma is less certain. However, recent placebo-controlled studies of its use in corticosteroid-dependent asthma

have suggested an ability to improve pulmonary function and reduce the number of disease exacerbations (5), and to exert a steroid-sparing effect (6, 7). To date, however, no published studies have described the *in vivo* effects of this agent on airways inflammation in asthma.

We here report the findings in a patient with chronic severe corticosteroid-dependent asthma who underwent fibreoptic bronchoscopy with BAL and endobronchial biopsy before and after a 12-month period of treatment with cyclosporin A.

Case report

A 47-year-old man was referred to us for assessment of chronic severe corticosteroid-dependent asthma. He worked as a computer operator for a telecommunications company, and there was no history suggestive of exposure to any occupational sensitizer. He was atopic, with positive skin prick tests to *Dermatophagoides pteronyssinus*, *D. farinae*, and cat allergen, although his total serum IgE was not elevated at 40 IU/ml (normal <81 IU/ml). He also had perennial rhinitis and had suffered from

eczema as a child. There was no family history of atopic disease and he had no other significant past medical history. He smoked 25 g of tobacco weekly and had previously been a heavier smoker.

He first developed asthma at the age of 2 years, and throughout his childhood and adult life he had often been admitted to hospital for treatment of acute exacerbation. This usually responded to treatment with oral corticosteroids, and he had never required mechanical ventilation. Corticosteroids were initially prescribed on an intermittent basis, but at the time of assessment he had taken continuous oral prednisolone for the previous 18 years at a dose never below 20 mg daily. As a result of prolonged corticosteroid usage, he had developed a number of adverse effects. Radiology of his thoracic spine revealed osteopenia with end-plate compression at a number of vertebrae, and bone densitometry measurements indicated that the bone mineral density of his lumbar spine was only $58 \pm$ (95% confidence intervals) 6% of the predicted value. Upper gastrointestinal tract endoscopy, performed as part of the investigation of iron deficiency anaemia, showed patchy gastritis and moderate duodenitis. Other corticosteroid-related adverse effects included a markedly cushingoid appearance, proximal myopathy, easy bruising, and depression.

At the time of referral, he was receiving treatment with prednisolone 30 mg daily. His other medication at this time consisted of theophylline SR 400 mg at night and inhaled therapy with beclomethasone dipropionate 2000 μ g daily, and salbutamol and ipratropium bromide as required. Spirometry revealed an FEV₁ of 1.57 l (43.3% pred.) and FVC 3.69 l (98.9% pred.). Peak expiratory flow (PEF) monitoring at home showed values of 160–200 l/min (pred. 530 l/min). Plain radiography of his chest was unremarkable.

Fibreoptic bronchoscopy was performed with BAL and endobronchial biopsy to determine the reason for his poor control. In view of the findings, which, as shown below, indicated persistent airways inflammation despite long-term corticosteroid treatment, the immunosuppressive agent azathioprine was commenced at a dose of 50 mg twice daily. At first, this appeared to bring about some improvement with a decrease in symptoms, a modest rise in FEV₁ to 2.15 l, and some reduction in corticosteroid requirement. However, these effects were not maintained, and 10 weeks after commencing azathioprine, there had been no sustained increase in FEV₁ and he had needed to increase his dose of prednisolone back to 30 mg daily.

Azathioprine was therefore withdrawn and cyclosporin A was introduced as an alternative

immunosuppressive agent. Baseline renal and hepatic function were normal, and a starting dose of cyclosporin A of 450 mg (7.5 mg/kg) daily was prescribed. However, whole-blood cyclosporin A levels were supratherapeutic on that dose, and his renal function deteriorated with a progressive rise in serum creatinine from 91 to 223 μ M over 6 months. The dose of cyclosporin A was reduced to 300 mg (5 mg/kg) daily, and his renal function thereafter improved with a fall in serum creatinine to around 140 μ M. Subsequent cyclosporin A levels were acceptable in the range 100–400 ng/ml.

His clinical response to cyclosporin A was impressive. After 6 weeks of treatment, he felt symptomatically better, his PEF measurements had risen to 360 l/min, and it was possible to start reducing his prednisolone from its previous level of 30 mg daily. After 6 months, he had been able to decrease the dose of oral prednisolone to 10 mg daily, and at this stage, he felt very greatly improved symptomatically and only rarely needed to use inhaled bronchodilators. His PEF had risen further to 400 l/min with a diurnal variation of around 50 l/min, and his FEV₁ was 2.35 l (64.7% pred.). Further reduction of prednisolone dosage was achieved in steps of 1 mg per month, and 12 months after commencing cyclosporin A he remained on only 3 mg prednisolone daily with an FEV₁ of 2.65 l (73.0% pred.) and an FVC of 4.75 l (106.7% pred.). Theophylline SR was continued over this time, and although serum levels were consistently subtherapeutic, he was unable to tolerate higher doses because of nausea. His inhaled medication remained as before, comprising beclomethasone dipropionate 2000 μ g daily and inhaled bronchodilators as required. At this point, he underwent repeat fibreoptic bronchoscopy to reassess the state of his airways inflammation.

Over the following 6 months, he remained on the same dose of cyclosporin A. A short Synacthen test showed evidence of adrenocortical suppression; therefore, the dose of prednisolone was not reduced below 3 mg daily. His other medication remained as above. His PEF measurements at home were stable at around 400 l/min, and he did not experience any further exacerbation requiring an increase in prednisolone dosage or hospital admission.

Material and methods

Fibreoptic bronchoscopy, BAL, and endobronchial biopsy were performed according to our previously published protocol (8). BAL fluid was centrifuged at 200 g for 10 min at 4°C, and the cell pellet was resuspended in phosphate-buffered saline. Total cell numbers were counted with a Neubauer improved haemocytometer (BDH, Dagenham,

Essex, UK), and cytocentrifuge slides were prepared and stained using the Hema "Gurr" rapid staining kit (BDH, Lutterworth, Leicestershire, UK). Differential cell counts were performed, counting 500 cells per slide.

For flow cytometry, 50- μ l aliquots of the suspensions of BAL fluid cells were incubated with pairs of directly conjugated mouse monoclonal antibodies (mAbs) (all Becton Dickinson, Oxford, UK) at previously determined dilutions. The mAbs used were fluorescein-isothiocyanate (FITC)-conjugated anti-CD3 in combination with phycoerythrin (PE)-conjugated anti-CD4, anti-CD8, anti-CD25, or anti-HLA-DR. After incubation for 30 min at 4°C, the cells were washed, and 5000 events per sample were acquired and analysed by two-colour flow cytometry with a FACScan analyser (Becton Dickinson) and appropriate software (FACScan Research and Lysis, Becton Dickinson). Lymphocytes were gated with a forward and side scatter dot-plot, and a second gate was set on CD3+ cells. Cells which were CD4+, CD8+, CD25+, and HLA-DR+ were identified and expressed as a percentage of the CD3+ lymphocyte population.

Bronchial biopsies were fixed in acetone, and processed into glycolmethacrylate (GMA) resin, and immunohistochemical staining was performed as previously described (9). The following mouse mAbs were used: anti-CD3 (UCHT1), anti-CD4 (Becton Dickinson), anti-CD8 (Dako, High Wycombe, UK), and anti-CD25 (Dako). Positively staining cells were counted in the entire area of subepithelial tissue, and data were expressed as the average number of cells per mm² in three nonserial sections.

Results

Inflammatory markers in BAL fluid and bronchial biopsies are shown in Table 1. After cyclosporin A treatment, there was a reduction in the proportion of lymphocytes from 27.6% to 12.8%. As the total number of leukocytes recovered per ml of BAL fluid was greatly reduced, there was also a marked decrease in the absolute number of lymphocytes from 4.22 to only 0.13 $\times 10^4$ /ml. Few eosinophils were evident either before or after treatment. FACS analysis showed that at the time of the first bronchoscopy, despite long-term therapy with oral prednisolone, there was persistent activation of BAL fluid T cells, as indicated by the cell-surface expression of CD25 (7.4%) and HLA-DR (9.6%). On the second occasion, however, the number of identifiable CD3+ cells was too low for FACS analysis to be performed. In bronchial biopsies, there was a reduction in the numbers of total

Table 1. Inflammatory markers in bronchoalveolar lavage fluid and bronchial biopsies before and after cyclosporin A treatment

	Before cyclosporin A	After cyclosporin A
BAL fluid		
Total leukocytes ($\times 10^4$ /ml)	15.3	1.0
Differential cell counts (%)		
Macrophages	65.4	76.8
Lymphocytes	27.6	12.8
Neutrophils	6.4	7.4
Eosinophils	0	0.2
Epithelial cells	0.6	2.8
FACS analysis (% CD3)		
CD4+	33.9	Insufficient
CD8+	47.4	identifiable
CD25+	7.4	CD3+ cells
HLA-DR+	9.6	for analysis
Bronchial biopsies		
Cells/mm ²		
CD3+	48.7	16.9
CD4+	44.7	1.7
CD8+	22.8	11.7
CD25+	2.6	0

T cells and individual T-cell subsets, most marked in relation to the CD4+ population. Whereas a small number of cells expressing the activation marker CD25 were evident in the first biopsy specimen, none could be identified on the second occasion.

Discussion

A number of published clinical studies have examined the effectiveness of low-dose cyclosporin A therapy in asthma, with conflicting results. In an initial open-label study, Szczeklik et al. described beneficial results in 6/12 patients with severe corticosteroid-dependent asthma but no discernible effect in the remainder (10). After cessation of therapy, these improvements were not maintained (11). In a subsequent randomized, placebo-controlled, crossover study, Alexander et al. reported that, over a 12-week treatment period, cyclosporin A treatment resulted in significant improvements in pulmonary function in subjects with corticosteroid-dependent asthma and a reduction in the number of disease exacerbations requiring an increase in the dose of prednisolone, but again the response was variable with some subjects exhibiting very large improvements while others showed no response (5). In addition to improving lung function, Lock et al. reported that, as compared with placebo, low-dose cyclosporin A therapy allowed a significant reduction in oral corticosteroid dosage in

patients with severe asthma over a 9-month period (7). On the other hand, Nizankowska et al., in a study of parallel-group design, found no evidence of improved lung function in response to cyclosporin A after 12 weeks of treatment, although there were some minor changes in symptom scores (6). In the same study, however, these investigators were able to confirm that cyclosporin A was more efficient than placebo in reducing the requirement for systemic corticosteroids (6).

In the present report, we describe the case of a patient with previously poorly controlled corticosteroid-dependent asthma who showed a dramatic clinical response after the introduction of cyclosporin A treatment. In association with this clinical improvement, the bronchoscopic findings revealed a marked reduction in the expression of airway inflammatory markers. This was evident both in BAL fluid, where there was a reduction in the percentage and absolute number of lymphocytes and a striking reduction in the expression of T-cell activation markers, and in bronchial biopsies where there were also decreases in the numbers of T cells, most marked in relation to the CD4+ population.

T cells are believed to play an important role in the pathogenesis of asthma via their ability to release cytokines which induce the recruitment and activation of other inflammatory cells, especially eosinophils (reviewed by Corrigan & Kay [12]). Activation of T cells has been described in peripheral blood (13), BAL fluid (14), and bronchial biopsies (15) in asthma. Treatment with corticosteroids normally leads to a reduction in the degree of T-cell activation in the airways in parallel with a decrease in symptoms and an improvement in physiologic indices (2, 3). However, there is evidence of persistent T-cell activation in the airways of asthmatics who remain clinically unresponsive to corticosteroids (A. E. Redington, unpublished observations). Previous investigators have reported that in patients with corticosteroid-resistant asthma, peripheral blood T-cell activation *in vitro* is not suppressed by dexamethasone but can be inhibited by cyclosporin A (16). Furthermore, clinical response to cyclosporin A in chronic severe asthma is associated with a reduction in concentrations of CD25 in serum (17). Clearly, it is necessary to exercise caution when attempting to interpret data from a single patient in view of the possibility of random variation in inflammatory indices. However, the marked reduction in all T-cell indices observed after cyclosporin A treatment in the present report, in association with the impressive clinical response, suggests that these two events may be causally related. In addition to its suppressive effects on T cells, cyclosporin A can inhibit the

release of mediators from other cell populations involved in allergic inflammation, including mast cells (18) and eosinophils (19), and these effects may also be relevant to its therapeutic benefit.

Cyclosporin A has a number of well-recognized and in some cases potentially serious adverse effects, including nephrotoxicity, hypertension, hepatotoxicity, peripheral neuropathy, and hypertrichosis (20). The drug must therefore be used with caution, and blood pressure, renal function, and cyclosporin A levels should be monitored regularly throughout the period of treatment (20). However, in previous clinical studies of low-dose cyclosporin A in the treatment of asthma, it appears to have been generally well tolerated (5–7, 10). In the present case, the only problem encountered was a decline in renal function. This was probably related to the high dose of cyclosporin A used initially (7.5 mg/kg), which was associated with supratherapeutic blood cyclosporin A levels. The risk of cyclosporin A-induced nephropathy is related to the dose of cyclosporin A, serum creatinine, and age, and can be reduced by avoiding doses above 5 mg/kg daily (21). In this case, the renal impairment responded partially to a reduction in cyclosporin A dose, although the serum creatinine remained persistently 50–65% above the baseline value.

In summary, the present report provides further evidence that cyclosporin A can be an effective option in the treatment of selected patients with severe chronic corticosteroid-dependent asthma, although its widespread use is likely to be precluded by the high incidence of adverse effects and the costs associated with treatment and monitoring. In addition, our findings represent the first *in vivo* evidence of the ability of cyclosporin A to exert an anti-inflammatory effect in the airways in asthma. Larger studies will clearly be required to examine more systematically the effects of this agent on airway inflammatory indices. We believe that fiberoptic bronchoscopy with BAL and endobronchial biopsy may prove useful in evaluating patients with difficult-to-control asthma when treatment with cyclosporin A or other immunosuppressive agents is being considered. This approach might allow the identification of patients who genuinely have corticosteroid-unresponsive airways inflammation, and thus avoid the administration of a potentially toxic drug to patients who are unlikely to benefit.

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