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Clinical response to cyclosporin in chronic severe asthma is associated with reduction in serum soluble interleukin-2 receptor concentrations

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Clinical response to cyclosporin in chronic severe asthma is associated with reduction in serum soluble interleukin-2 receptor concentrations. A.G. Alexander, N.C. Barnes, A.B. Kay, C.J. Corrigan. ©ERS Journals Ltd 1995.

ABSTRACT: Activated T-lymphocytes play an important role in asthma pathogenesis and release soluble interleukin-2 receptor (sIL-2R), which can be detected in the serum. In a recent randomized, cross-over trial we showed that cyclosporin, an inhibitor of T-lymphocyte activation, improved lung function in patients with chronic severe asthma. To investigate whether changes in serum sIL-2R concentration could be related to clinical response we prospectively compared serum sIL-2R concentrations in patients during cyclosporin and placebo treatment.

Peripheral venous blood was obtained from 22 patients during the last 4 weeks of both the cyclosporin and placebo treatment periods and serum stored at -80°C pending measurement of sIL-2R concentration by enzyme immunoassay.

Soluble IL-2R was detected in all samples at a concentration range of 191–2,297 U·ml^{-1}. Mean serum concentrations of sIL-2R were significantly lower on cyclosporin therapy (560 U·ml^{-1}) as compared with placebo (676 U·ml^{-1}). The decreases in serum sIL-2R concentrations associated with cyclosporin therapy in these patients correlated with the percentage increases in their morning peak expiratory flow rate (PEFR) measurements on cyclosporin as compared with placebo.

These data demonstrate that in patients with chronic severe asthma, cyclosporin therapy which results in clinical improvement is associated with a decrease in serum concentrations of sIL-2R. This is compatible with the hypothesis that cyclosporin ameliorates asthma, at least partly, by inhibition of T-lymphocyte activation.
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Activated (CD25+) CD4+ T-lymphocytes and their products are features of acute and chronic asthma, and the degree of T-lymphocyte activation can be correlated with disease severity [1–3]. Increased numbers of activated CD4+ T-lymphocytes and elevated serum concentrations of soluble interleukin-2 receptor (sIL-2R) were found in the peripheral blood of patients with acute severe asthma [1]. The numbers of activated CD4+ T-lymphocytes and the concentrations of serum sIL-2R were decreased following 3 days of hospital therapy, and there were significant correlations between both of these decreases and the corresponding increases in peak expiratory flow rate (PEFR). Elevated numbers of activated CD4+ T-lymphocytes have been demonstrated in the bronchial mucosa [2, 4] and bronchoalveolar lavage [3] of patients with mild atopic asthma compared to normal controls.

We postulated that cyclosporin, which inhibits T-lymphocyte activation [5], would be an effective therapeutic agent in asthma. In our recent randomized, placebo-controlled, double-blind, cross-over trial of the efficacy of oral cyclosporin in patients with oral glucocorticoid-dependent chronic severe asthma, cyclosporin therapy for 12 weeks resulted in a mean increase above placebo of 12.0% in PEFR ($p<0.004$) [6].

We also sought to obtain evidence, prospectively, that any clinical improvement with cyclosporin therapy might be related to T-lymphocyte inhibition. We hypothesized that the concentration of sIL-2R, which is a specific marker of T-lymphocyte activation readily measurable in serum [7–10], would be reduced in the serum of these asthmatic patients to a degree correlating with the degree of clinical improvement. Our objective in the present study was, therefore, to measure serum concentrations of sIL-2R in the trial patients during both cyclosporin and placebo therapy, and to relate any changes in these concentrations to clinical improvement as measured by changes in PEFR.

Methods

Patients

The clinical trial has been described in detail previously [6]. Briefly, documented chronic asthmatics aged

Table 1. – Baseline (pretrial) patient characteristics, treatment and respiratory function data

Sex M/F		8/14
Age yrs*		49 (34–62)
Duration of asthma yrs*		26 (5–51)
Skin-prick test +ve# n		14
IgE IU·ml^{-1}*		127 (6–540)
Continuous oral prednisolone yrs*		9.9 (0.3–26)
Prednisolone dosage mg·day^{-1}*		8.9 (5–20)
Inhaled glucocorticoid usage µg·day^{-1+}		1660 (93)
Oral theophylline usage n		15
PEFR	l·min^{-1+}	242 (16.1)
	% predicted	55
FEV_1	l^+	1.80 (0.17)
	% predicted	64
VC	l^+	3.16 (0.25)
	% predicted	85

*: data presented as mean and range in parenthesis; +: data presented as mean and SEM in parenthesis; #: defined as one or more positive skin-prick tests to a range of 12 common aeroallergens in the presence of a positive histamine control and a negative vehicle control. IgE: immunoglobulin E; PEFR: peak expiratory flow rate; FEV_1: forced expiratory volume in one second; VC: vital capacity; M: male; F: female.

18–65 yrs, who had a forced expiratory volume in one second (FEV_1) and/or PEFR below 75% of the predicted value and greater than 20% reversibility to β_2-agonist, were considered for the study if they required long-term maintenance treatment with 5–20 mg oral prednisolone daily in addition to maximal tolerated and effective additional therapy, including high dose inhaled glucocorticoids (table 1). Written informed consent was obtained from each patient, and the study was approved by the Ethics Committee of the Royal Brompton National Heart and Lung Hospitals.

Study design

After a 4 week run-in period, subjects were randomized to receive cyclosporin (initial dose 5 mg·kg^{-1}·day^{-1}) or matching placebo for 12 weeks, in addition to their usual treatment. Following a 2 week wash-out period, subjects crossed over to placebo or cyclosporin medication for a further 12 weeks. The cross-over design allows direct intrasubject comparisons, with each patient acting as his/her own control. For this reason, all treatment except inhaled bronchodilator usage was kept constant during the trial, although during exacerbations oral prednisolone dosage was increased if required. Exacerbations were defined as worsening of asthma symptoms in association with decreased PEFR, and were treated by increasing the prednisolone dosage. As patients served as their own controls, those in whom prednisolone dosage had been increased during a disease exacerbation, and at the time of blood sampling were receiving prednisolone at a greater dosage than maintenance, were excluded from analysis. Patients attended the clinic weekly for the first 4 weeks of each treatment period and, thereafter, every 2 weeks. Whole blood trough concentrations of cyclosporin were measured using a specific monoclonal radioimmunoassay (Cyclo-Trac SP, Incstar Corp., Stillwater MN, USA). Mean concentrations for the last 4 weeks of cyclosporin therapy were 154 ng·ml^{-1}.

Isolation of serum

Peripheral venous blood samples were obtained from each patient at the same time of day at a clinic visit during the last 4 weeks of both the cyclosporin and placebo treatment periods. Venesection was performed a mean of 17 (SEM 0.4) h after the last cyclosporin dose. Blood was allowed to clot on glass for 1 h at room temperature. After centrifuging the clotted blood at 1,000 ×g for 10 min at 4°C, serum was removed into sterile polystyrene containers and stored at -80°C pending analysis.

Soluble IL-2R enzyme immunoassay

Soluble IL-2R concentrations in serum were measured using a two-site sandwich enzyme immunoassay ("Cellfree", T Cell Diagnostics, Cambridge, MA, USA). All samples were analysed in duplicate in the same assay, and multiple samples from the same patient were always measured in the same 96-well plate. Standard curves were constructed using a reference preparation of supernatant from phytohaemagglutinin-stimulated peripheral blood lymphocytes supplied by the manufacturer. The coefficient of intra-assay variation was estimated as 2.2%, and inter-assay variation as 5.6%. The lower limit of sensitivity of the assay was 50 U·ml^{-1}.

Statistical analysis

Analysis of the clinical trial has been reported previously [6]. Briefly, daily morning prebronchodilator PEFR data for each patient were summarized as the mean for the last 4 weeks of each treatment period. Clinical improvement in mean PEFR was determined as percentage difference between cyclosporin therapy and placebo, *i.e.* ((cyclosporin/placebo) × 100) - 100.

Paired serum samples were obtained during therapy with cyclosporin and placebo for 29 of the 30 patients completing the clinical trial. At the time of blood sampling, seven patients (three during cyclosporin and four during placebo treatment) were receiving prednisolone at an increased dosage during a disease exacerbation, and were unable to act as their own controls. Data from 22 patients were therefore available for analysis.

Serum concentrations of sIL-2R during placebo and cyclosporin therapy (fig. 1) were found to be normally distributed and equality of variance was established using the F-test. Differences in serum concentrations of sIL-2R in individual patients during therapy with cyclosporin and placebo were compared using a paired t-test, and subgroup analysis of atopic status and theophylline usage using a two sample t-test. The changes in serum concentrations of sIL-2R in individual patients and the

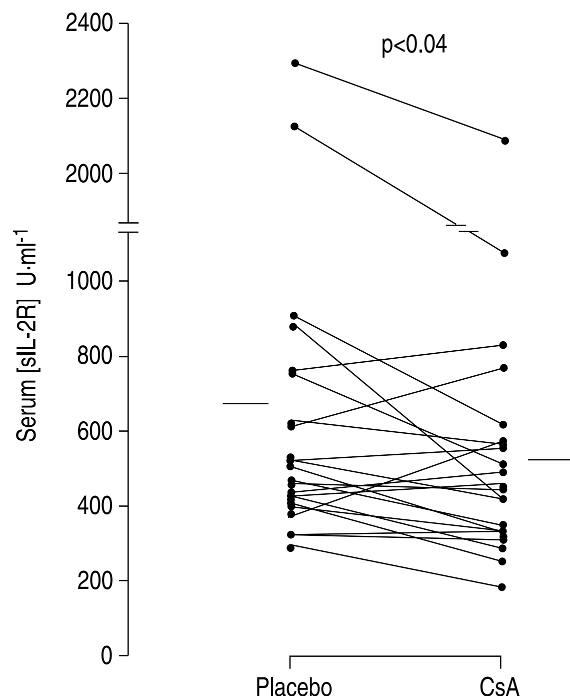

Fig. 1. – Serum soluble interleukin-2 receptor (IL-2R) concentrations in each patient with chronic severe asthma during cyclosporin A (CsA) and placebo treatment periods. Horizontal bars represent mean concentrations for each treatment period.

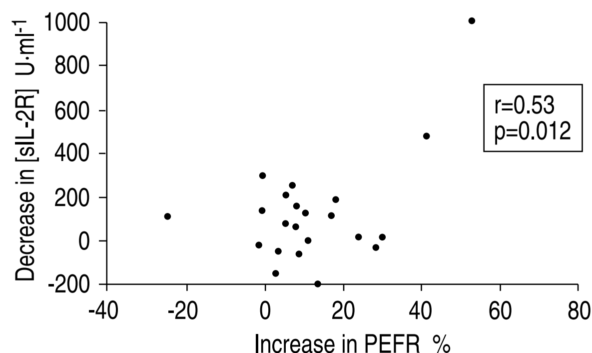

Fig. 2. – Correlation between the change in serum concentration of soluble interleukin-2 receptor (IL-2R) and the corresponding percentage change in peak expiratory flow rate (PEFR) (mean morning prebronchodilator) during cyclosporin therapy as compared with placebo.

corresponding percentage changes in morning PEFR during cyclosporin therapy as compared with placebo were found to be normally distributed, and Pearson's test was used to calculate the correlation coefficient between these two variables (fig. 2). Analysis was performed using the Minitab statistical software package (State College, Pennsylvania, USA).

Results

Soluble IL-2R was detected in all samples at a concentration range of 191–2,297 U·ml^{-1}, considerably above the lower limit of sensitivity of the assay. Figure 1 compares the serum concentrations of sIL-2R for each patient during the cyclosporin and placebo treatment periods. Taking the patients as a group, the mean serum concentrations of sIL-2R were significantly lower during cyclosporin therapy as compared with placebo (560 *vs* 676 U·ml^{-1}; $p<0.04$; 95% confidence limits 6.3–225.0).

In figure 2 the changes in the serum concentrations of sIL-2R between the placebo and cyclosporin treatment periods are plotted against the corresponding changes in mean morning prebronchodilator PEFR expressed as percentage increase during cyclosporin therapy as compared with placebo. A significant correlation was observed between the changes in serum concentrations of sIL-2R and clinical response as measured by changes in PEFR with cyclosporin therapy ($p=0.012$).

Subgroup analysis revealed no significant differences in serum concentrations of sIL-2R during placebo therapy, or changes in concentration compared with cyclosporin therapy according to atopic status or theophylline usage. No associations were observed between changes in serum sIL-2R concentrations and mean whole blood cyclosporin concentrations, disease severity or duration, maintenance prednisolone dose or duration of glucocorticoid dependence. No correlation was observed between the serum concentrations of sIL-2R during the placebo treatment period and the maintenance prednisolone dosage.

Discussion

In this study, we have been able to document serum concentrations of sIL-2R in severe, oral glucocorticoid-dependent asthmatic patients. Despite the potential variability introduced by short-term alterations in disease severity in this type of study, we were able to observe, using patients as their own controls, a reduction in serum sIL-2R concentrations in association with cyclosporin therapy which correlated with clinical improvement. This suggests that activation of T-lymphocytes is a feature of chronic severe asthma, and that changes in serum sIL-2R concentration reflect disease severity and response to therapy.

Soluble IL-2R is a 45 kD glycosylated protein generated on T-lymphocyte activation by the proteolytic cleavage of the p55 subunit of cell-surface IL-2R [8–10]. The serum concentration is a sensitive and quantitative marker of circulating T-lymphocyte activation, and may also reflect T-lymphocyte activation in other sites, such as allograft rejection in transplant recipients [11]. It has been shown to be a marker of disease activity in various diseases thought to be mediated by T-lymphocytes, such as Crohn's disease [12], atopic dermatitis [13], and rheumatoid arthritis [14], in many of which cyclosporin therapy has been shown to be efficacious.

Elevated serum and plasma concentrations of sIL-2R have been described in association with asthma [1, 15, 16], but have not always been found to correlate with disease activity [15]. In one study, serum concentrations of sIL-2R were elevated in patients with acute severe asthma as compared with mild asthmatics or normal controls [1], but in another, both the acute and mild asthmatics had plasma concentrations of sIL-2R greater than in the normal controls [15]. Another group [16] reported

elevated plasma concentrations of sIL-2R in oral glucocorticoid-dependent asthmatics as compared with patients controlled without oral glucocorticoids. These glucocorticoid-dependent patients have severe disease despite maintenance prednisolone therapy, which may reflect ongoing T-lymphocyte activation despite systemic glucocorticoid therapy.

In view of these discrepancies, it is perhaps not surprising that, in the present study, changes in serum concentrations of sIL-2R did not correlate more strongly with changes in PEFR. In contrast to a previous study of patients admitted to hospital with acute severe asthma [1], in which many patients showed considerable improvement, there was wide variability in the present study in the clinical response to cyclosporin therapy, with many patients showing small or negligible increases in PEFR [6]. This group of severe glucocorticoid-dependent asthmatics is clinically heterogeneous, with a wide range of age, disease severity and duration, and maintenance prednisolone dosage. Furthermore, patients may also vary in their sensitivity to the effects of glucocorticoids [17, 18], and in their rate of clearance of sIL-2R from peripheral blood. Finally, the absence of strong correlation may be due to the fact that inhibition of T-lymphocytes within the bronchial mucosa, which might be more relevant to the improvement of clinical asthma, is not directly reflected by changes in the peripheral blood. It is also possible that cyclosporin exerts its beneficial effect in asthma through actions on other cells, in addition to its inhibitory actions on T-lymphocytes.

Obviously, conclusions based on single measurements during each treatment period should be drawn with caution. Little is known about the day-to-day variability of serum sIL-2R concentrations. Nevertheless, taking the asthmatic patients as a group, cyclosporin therapy was associated with a consistent improvement in asthma severity (as measured by PEFR) throughout the entire 12 weeks of treatment [6]. We have hypothesized in this study that this improvement reflects, at least partly, a reduction in ongoing T-lymphocyte activation; and, since this implies a chronic process, there is reason to believe that a single measurement may reflect this process adequately.

In this cross-over trial, where patients acted as their own control, we considered it important, in view of the previously described effects of oral prednisolone therapy on serum sIL-2R concentrations [1], to compare only those patients whose dosage of oral prednisolone was the same at the time of blood sampling during both the cyclosporin and placebo treatment periods. Patients whose baseline dose of prednisolone had been increased for disease exacerbation at the time of blood sampling during either arm of the trial were therefore excluded from analysis. Although we observed no linear correlation between prednisolone dosage and serum sIL-2R concentrations in individual patients during placebo therapy, this does not exclude the possibility that increased prednisolone dosage, as well as disease exacerbation, may have variable effects on serum sIL-2R concentrations in individual patients [1]. We wished to examine the effects of cyclosporin on serum sIL-2R concentrations in the absence of these unknown variables. The lack of correlation between prednisolone dosage and serum sIL-2R concentrations during the placebo treatment period may reflect interindividual variability arising from other influences, such as disease severity and rates of clearance of sIL-2R from peripheral blood.

Although serum concentrations of sIL-2R are known to be elevated in the presence of impaired renal function [9], and cyclosporin therapy resulted in a mild reversible decrease in renal function in these patients [6], any increase in serum concentrations would act to decrease the observed difference between the placebo and cyclosporin treatment periods. Cyclosporin is known to accumulate in tissues at concentrations greater than in blood [19], and whole blood concentrations do not necessarily reflect the concentrations to which T-lymphocytes in peripheral blood or in lung are exposed. This may explain, at least partly, why no simple relationship was observed between whole blood cyclosporin concentrations and either the changes in serum concentrations of sIL-2R, or the clinical response as measured by changes in PEFR [6] in the asthmatic patients during cyclosporin therapy.

Preliminary data from two controlled trials have shown that cyclosporin is efficacious as a steroid-sparing agent in asthma therapy [20, 21], but there are as yet no studies investigating its mechanism of action in asthma. Cyclosporin is believed to exert its immunosuppressive action principally by inhibition of T-lymphocyte activation [5, 22]. It is known to inhibit IL-2R expression *in vitro* [5, 23], and T-lymphocytes are the major, if not the only, source of serum sIL-2R [10]. The decrease in serum concentrations of sIL-2R in the patients in this study in association with cyclosporin therapy, suggests that cyclosporin is inhibiting T-lymphocyte activation *in vivo*, and the correlation of these decreases in sIL-2R concentration with clinical response is compatible with the hypothesis that cyclosporin ameliorates asthma, at least partly, by inhibition of T-lymphocyte activation. This does not exclude the possibility that cyclosporin may exert some of its anti-asthma activity through inhibition of other inflammatory cells, such as mast cells [24] and eosinophils [25].

Longitudinal studies of serum cytokines and cell phenotypic markers in chronic severe asthma are in progress in our laboratory in order to investigate this matter further, to observe the effects of glucocorticoid and cyclosporin therapy, and to determine whether changes in serum sIL-2R concentrations are related to changes in disease severity and may be predictive of disease exacerbations.

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