

Plasma interleukin-2 and a soluble/shed interleukin-2 receptor in serum of patients with Crohn's disease. Effect of cyclosporin

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Abstract

Circulating concentrations of interleukin-2 (IL-2) and a soluble or shed form of the IL-2 receptor (sIL-2R) were determined by enzyme-linked immunosorbent assays (ELISA) in 61 patients with chronic active Crohn's disease (CD) initially and during a three month placebo controlled trial of cyclosporin 5-7.5 mg/kg/day. The baseline median (25-75% range) plasma IL-2 concentration was 0.6 ng/ml (0.3-2.85 ng/ml) in patients who did not receive prednisolone, 0.5 ng/ml (0.23-3.4 ng/ml) in patients who did (not significant), and 0 ng/ml (0-0.07 ng/ml) in control subjects ($p < 0.00001$). The corresponding median serum sIL-2R concentrations were 747 U/ml (580-1287 U/ml), 540 U/ml (422-616 U/ml) respectively in CD patients ($p = 0.006$) and 320 U/ml (268-406 U/ml) in control subjects ($p < 0.00001$). Increased concentrations of plasma IL-2 and serum sIL-2R were seen in 66% and 81% of the patients, respectively. A fall in serum sIL-2R was only seen in patients who improved with cyclosporin treatment ($p = 0.006$). At month 3 the median serum sIL-2R concentration was 440 U/ml (400-668 U/ml) v 801 U/ml (534-1067 U/ml) in patients not responding to cyclosporin ($p = 0.003$). No changes occurred in the placebo group. These results suggest that the IL-2 dependent pathway of immune activation is upregulated in vivo in CD and that cyclosporin may interfere with this process.

Interleukin-2 (IL-2) functions as an obligatory signal for T cell growth, and this ligand effect is mediated through a specific T cell surface receptor (IL-2R). IL-2 production and IL-2R expression are determined by antigen recognition or mitogen stimulation.¹ In vitro studies on isolated mononuclear cells, using bioassays, suggest that the production of IL-2 may be impaired in active Crohn's disease (CD),^{2,4} even though T cells appear to be activated in this disease.⁵ Accordingly, we have recently shown that cyclosporin, a potent inhibitor of IL-2 production by T cells,⁶ has a beneficial therapeutic effect on chronic active CD.⁷

Specific enzyme-linked immunosorbent assays (ELISAs) are now available for the determination of IL-2 and a soluble/shed form of IL-2R (sIL-2R) in vivo, using plasma or serum.⁸ Clinical studies have shown that conditions characterised by enhanced T cell mediated immunity in vivo, such as transplant rejection, are associated with high concentrations of IL-2 or sIL-2R, or both.⁹⁻¹¹ Therefore, it seemed of interest to determine circulating IL-2 and sIL-2R

concentrations as measures of continuing T cell activation in CD. Furthermore, in longitudinal studies we investigated whether these measurements might be a means of monitoring cyclosporin treatment.

Methods

Serial serum and heparin-stabilised plasma samples were collected at five of six centres participating in a three-month placebo controlled trial of cyclosporin 5-7.5 mg/kg/day in patients with chronic active CD.⁷ Serum samples were available for 59 of 63 eligible patients and plasma samples for 61 patients. Single samples were missing at baseline and later. There were 22 men and 39 women, with a median age of 33 years (range 16-68 years) and a median disease duration of five years (range 1-22 years). Twenty patients (33%) had ileal disease, 10 (16%) colonic disease, and 31 (51%) ileocolic disease. Eighteen patients (30%) received fixed doses of prednisolone during the study (median 10 mg/day, range 5-20 mg/day) and 41 patients (67%) received fixed doses of sulphasalazine or 5-aminosalicylic acid (median 3 g/day, range 1-3 g/day) alone or in combination with corticosteroids. All patients had active CD according to the Crohn's Disease Activity Index¹² (median 268, range 151-540). The median plasma orosomucoid concentration was 35 μ mol/l (range 15-82 μ mol/l); reference interval 11-30 μ mol/l. There were no significant differences in baseline characteristics between patients treated with cyclosporin and those receiving placebo. The patients were studied before treatment, after two weeks of treatment, and then monthly and were classified as either improved or not improved according to a modified clinical grading score.^{7,13}

Nineteen healthy subjects (median age 38 years, range 28-61 years) served as controls for the IL-2 assay and another 21 healthy subjects (median age 37 years, range 21-60 years) served as controls for the sIL-2R assay.

ELISA

Serum and heparin-stabilised plasma samples were stored at -80°C until use. The ELISAs were performed in one setting at the end of the study using previously unfrozen samples. Serial samples from one patient were analysed on the same ELISA plate.

IL-2 ASSAY

Plasma IL-2 concentrations were determined by

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solid phase, triple layer ELISA (Intertest-2, Genzyme Corp, Boston MA). Fibrin was removed by incubation of CaCl_2 and plasma 1:9 for two hours at 37°C followed by centrifugation. Plasma samples were diluted 1:2 by phosphate buffered saline before assay. Firstly, a specific monoclonal anti-IL-2 antibody was absorbed onto 96-well polystyrene microtitre plates by incubation at 37°C for 18 hours. Plasma samples were added in duplicates together with known recombinant IL-2 standards for each plate and incubation was continued for 18 hours. Secondly, a polyvalent rabbit anti-IL-2 antibody recognising a different epitope on the human IL-2 was added and bound to captured IL-2 in the solid phase. Thirdly, an alkaline phosphate-conjugated goat antirabbit immunoglobulin antibody was added. A substrate solution (para-nitrophenyl phosphate) was finally added, and the colour reaction was read against a substrate blank using a microtitre ELISA reader (Titertek, Flow Laboratories, USA). The amount of IL-2 in each sample was derived from the optical density curve of the known standards. Previous studies have shown that in the case of fresh recombinant IL-2, 1 ELISA unit (equivalent to 0.33 ng) corresponds to about 0.8–1.2 units (0.27–0.4 ng) defined by the CTLL bioassay.¹⁴ Reproducibility in our laboratory was within 10%.

SIL-2R ASSAY

Serum sIL-2R concentrations were determined by solid phase, double layer ELISA (T Cell Sciences Inc, Cambridge MA) according to Rubin *et al.*⁸ Firstly, a specific monoclonal antibody recognising the Tac-epitope on the human IL-2R¹⁵ was absorbed onto 96-well microtitre plates at 4°C for 18 hours. Serum samples were added in duplicates together with known IL-2R standards for each plate and incubated at 37°C for two hours. The second antibody layer consisted of a specific horseradish peroxidase-conjugated monoclonal antibody (7G7/B6)¹⁶ that binds to an epitope on the human IL-2R distinct from those of anti-Tac and IL-2, and incubation was continued for two hours. A substrate solution (*o*-phenylenediamine) was added and the colour reaction was read against substrate blank using the ELISA reader. The amount of sIL-2R in each sample was derived from the optical density curve of the known standards. Previous studies have shown that 1 ELISA unit sIL-2R is equivalent to about 0.3 pg purified receptor.¹⁷ The reproducibility in our laboratory was again within 10%.

STATISTICS

Unpaired observations were tested by the Kruskal-Wallis and the Mann-Whitney test; paired observations were tested by Page's test for trend. Two sided tests were used (except for Page's test, which is one tailed). For Page's test, single missing values were replaced by the nearest or the mean of nearest values. Values of premature withdrawals were carried over to later time points of evaluation. The level of significance was $p < 0.05$.

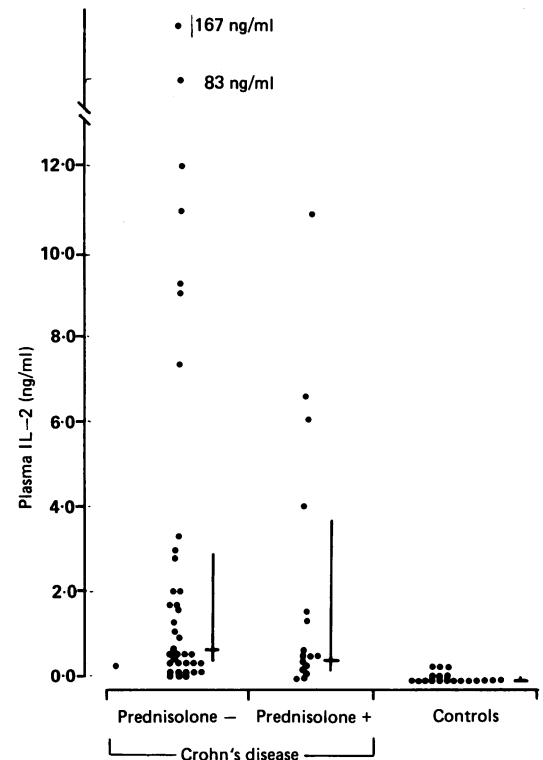


Figure 1: Plasma interleukin-2 (IL-2) concentrations in 53 patients with Crohn's disease and 19 matched controls. Patients are stratified according to baseline treatment with prednisolone (+/-). Bars indicate medians and the 25th and 75th percentiles ($p < 0.00001$, Kruskal-Wallis test).

Results

The baseline median (25–75% range) plasma IL-2 concentration was 0.6 ng/ml (0.3–2.85 ng/ml) in patients not receiving prednisolone, 0.5 ng/ml (0.23–3.4 ng/ml) in patients who did receive prednisolone (not significant), and 0 ng/ml (0–0.07 ng/ml) in control subjects ($p < 0.00001$) (Fig 1). There was no correlation between plasma IL-2 levels and plasma orosomucoid levels or Crohn's Disease Activity Index values. Plasma IL-2 concentrations above the total range observed in healthy controls (0.3 ng/ml) were seen in 66% of the patients, and very high concentrations in two patients.

The corresponding median serum sIL-2R concentrations were 747 U/ml (580–1287 U/ml) and 540 U/ml (422–616 U/ml) in CD patients ($p = 0.006$) without and with steroid medication respectively, and 320 U/ml (268–406 U/ml) in control subjects ($p < 0.00001$) (Fig 2). There was a significant correlation between serum sIL-2R and plasma orosomucoid concentrations ($r(S) = 0.55$, $p = 0.00008$), but not between sIL-2R concentrations and Crohn's Disease Activity Index values. By contrast, significant correlations were found among changes in sIL-2R concentrations and grading score totals, which measured changes in disease activity, at month 3 ($r(S) = 0.429$, $p = 0.01$) as well as at earlier points of evaluation. Increased serum sIL-2R concentrations (> 470 U/ml) were seen in 81% of the patients. There was no correlation between plasma IL-2 and serum IL-2R concentrations.

EFFECTS OF TREATMENT

No significant changes were observed in plasma IL-2 either within or between treatment groups

TABLE I Interleukin-2 concentrations (ng/ml) in 61 patients with Crohn's disease during a placebo controlled trial of cyclosporin treatment (median and 25-75% range)

Groups	Month		No of patients
	0	3	
Cyclosporin	0.5 (0.2-1.9)	0.5 (0.2-2.1)	32
Placebo	1.0 (0.4-6.3)	1.0 (0.3-6.2)	29
Improved with cyclosporin		0.5 (0.1-1.0)	19
Not improved with cyclosporin		0.8 (0.3-3.7)	13
Improved with placebo		0.9 (0.3-7.4)	9
Not improved with placebo		1.4 (0.3-7.6)	20

All comparisons: not significant.

(Table I). In addition to the two patients with a high baseline plasma IL-2, high concentrations were encountered again during treatment in three patients. These five patients showed no specific features apart from raised serum sIL-2R (970-3019 U/ml) and plasma orosomucoid (32-78 μmol/l) concentrations.

By contrast, median serum sIL-2R concentrations were significantly lower in the cyclosporin group compared with the placebo group from week two and later on (Table II). Stratification of patients according to treatment and clinical outcome showed a highly significant trend towards a decrease in sIL-2R, but only in patients who improved with cyclosporin treatment (Table II).

Discussion

This study showed high circulating concentrations of IL-2 and sIL-2R in patients with chronic active CD compared with healthy control subjects. Although the majority of the CD patients showed only slightly raised plasma IL-2 concentrations, the fact that about two thirds of these values were above the range found in healthy subjects (Fig 1) seems to contrast with previous in vitro findings. Here decreased or absent IL-2 production by blood or mucosal mononuclear cells, or both, have been found in active inflammatory bowel disease (IBD) using biological assays.²⁻⁴ It has also been suggested that an insufficiency of IL-2 may hamper the development of a normal immune response, perhaps contributing to the chronicity of IBD.⁴ Mucosal lymphocytes, however, produce high concentrations of IL-2 after challenge with a strong antigen-unspecific stimulus, such as combined phorbol-12-myristate-13-acetate and phytohaemagglutinin.⁴ Moreover, production of IL-2 by blood mononuclear cells is normal in

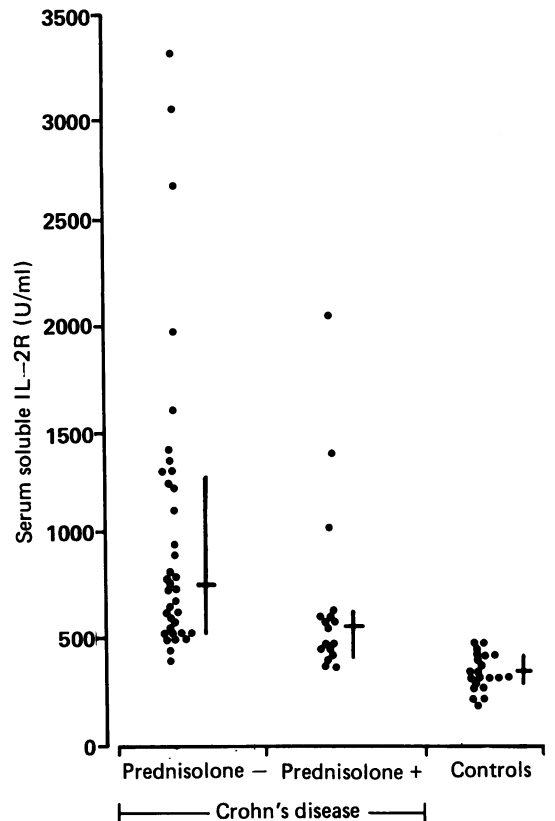


Figure 2: Serum soluble interleukin-2 receptor concentrations (sIL-2R) in 53 patients with Crohn's disease and 21 matched controls. Patients are stratified according to baseline treatment with prednisolone (+/-). Bars indicate medians and the 25th and 75th percentiles (p<0.00001, Kruskal-Wallis test).

patients with inactive IBD, which suggests that a deficiency of this lymphokine is not a primary factor in IBD.¹⁸ This assumption is supported by the fact that cyclosporin, a potent inhibitor of IL-2 production and T cells,⁶ has a beneficial therapeutic effect in chronic active CD.⁷

It was Rubin *et al*⁸ who showed that activated peripheral T cells¹⁹ released/shed a soluble form of cellular IL-2R in vitro. Increased serum or plasma sIL-2R concentrations have subsequently been shown by ELISA in clinical conditions characterised by enhanced T cell activity in vivo, such as kidney and liver transplant rejection,⁹⁻¹¹ T cell leukaemia,²⁰ systemic lupus erythematosus,²¹ and viral infections of the liver.²² The present finding of significantly increased serum sIL-2R concentrations in patients with CD suggests that T cells also are activated in this condition. Moreover, this result is in good agree-

TABLE II Serum sIL-2R concentrations (U/ml) in 59 patients with Crohn's disease during a placebo controlled trial of cyclosporin treatment (median and 25-75% range)

Groups	Month					No of patients	p†
	0	0.5	1	2	3		
Cyclosporin	603 (501-885)	563 (460-852)	597 (416-792)	534 (416-788)	566 (420-828)	32	NS (0.06)
Placebo	792 (586-1388)	974 (614-1600)	903 (561-1442)	1042 (654-1590)	962 (654-1442)	27	NS
p*	NS (0.058)	0.002	0.002	0.0003	0.005		
Improved with cyclosporin		558 (460-844)	580 (413-766)	479 (358-804)	440 (400-668)	19	0.006
Not improved with cyclosporin		569 (465-912)	616 (411-801)	672 (542-798)	801 (534-1067)	13	NS
p*		NS	NS	NS	0.003		
Improved with placebo		966 (562-1602)	1037 (749-1675)	1245 (899-1727)	1175 (1004-1565)	9	NS
Not improved with placebo		974 (645-1665)	819 (496-1242)	764 (575-1442)	816 (511-1337)	18	NS
p*		NS	NS	NS	NS		

* Mann-Whitney test for unpaired data.

† Page's test for trend towards a decrease at month 3 (one tailed).

NS=not significant.

ment with previous studies showing an increased T cell surface expression of early activation markers such as 4F2, OKT9, and HLA-DR as well as IL-2R in CD.²³⁻²⁴

The dimeric high affinity receptor complex that mediates the proliferative signal for T cells consists of an intermediate affinity beta chain and a low affinity alpha chain, called Tac.²⁵⁻²⁸ The density of Tac receptors increases during immune stimulation and may function as a down regulator of the T cell response by covering the high affinity binding sites and by trapping free IL-2.²⁹ Since sIL-2R binds free IL-2, the latter function may also be operative in vivo.³⁰

This study suggests that serum sIL-2R may be a marker of disease activity in CD, in contrast to plasma IL-2. The finding, however, of a significantly decreased median serum sIL-2R concentration only in patients who improved with cyclosporin treatment at month 3 as well as a strong significant trend throughout the treatment period (Table II) may be of even greater interest. A simple explanation would be that serum sIL-2R is a marker of an acute-phase response, but a tendency towards an increase was seen in patients who improved with placebo (Table II). Another possibility is that suppression of IL-2 production itself leads to decreased sIL-2R release since the ligand regulates the expression of IL-2R.²⁹ The finding, however, of unchanged serum sIL-2R in patients who failed with cyclosporin makes this less likely. The effect of cyclosporin on IL-2R expression is obscure. Conflicting results have been obtained in vitro, and the expression seems to depend on the type of stimulation,⁶ suggesting that cyclosporin exerts no direct effect on IL-2R expression.

Finally, the decline in serum sIL-2R concentrations in patients who improved with cyclosporin treatment may reflect a drug induced diminished T cell activation. Hence Baker and Jewell³¹ have recently reported that cyclosporin clears the expression of HLA-DR antigens on colonic epithelial cells, normally for responsible activation of T-helper cells. These findings are in keeping with the prevailing concept that activated immunological effector mechanisms occur in CD as a result of an increased antigen stimulation.⁵ But, owing to the considerable variation in sIL-2R suppression, this measure cannot be used to monitor cyclosporin treatment in individual patients.

Taken together the results suggest that the IL-2 dependent pathway of immune activation is upregulated in vivo in CD and that cyclosporin treatment may interfere with this process. Further studies are, however, needed to detail the effects of cyclosporin in CD.

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Correction Abnormal gastric adaptive relaxation in patients with gastroesophageal reflux by M N Hartley, S J Walker, C R Mackie. May 1990 issue, pp 500–3. In this article several lines of text in the Methods section were transposed. The corrected text appears below.

Methods

SUBJECTS

Fifteen normal healthy volunteers were studied. These were 13 men and two women, with a median age of 30 years (range 22–41) and a median body weight of 70 kg (range 50–79) kg. Twelve patients with symptomatic gastroesophageal reflux were studied. They were eight men and four women, with a median age of 48 years (range 23–65) and a median body weight of 77 kg (range 60–92 kg). All subjects in the control group were asymptomatic. Among patients in the reflux group, eight were complaining of regurgitation, 11 of heartburn, and four of dysphagia.

The presence of pathological gastroesophageal reflux was confirmed in all patients in the reflux group by 24 hour ambulatory oesophageal pH monitoring. Pathological gastroesophageal reflux was defined as the presence of one or more of the following criteria: total reflux time greater than 6%, erect reflux time greater than 8%, supine reflux time greater than 2%; more than two reflux episodes longer than five minutes. A reflux episode was defined as a drop in pH to 4 or less.

Endoscopy showed macroscopic oesophagitis in seven patients, associated with a hiatus hernia in four patients. Five patients had no abnormality at endoscopy. None of the subjects in the control group was on any medication and all medication was withheld for 12 hours before the study in all patients. After an overnight fast, subjects swallowed, *via* the nose a 10F Ryle's tube with a collapsed, plastic bag (800 ml) sealed over the end, the side holes of the tube being within the bag. Within the lumen of the tube, positioned near its tip, was a pressure microtransducer (Gaeltec, Dingwall, Rosshire, Scotland) which was calibrated against a water manometer to provide a full scale deflection of

50 cm water pressure. The transducer was interfaced with an amplifier and pen recorder (Gould Electronics Limited, Coventry, England) to record pressure changes against time. The bag was positioned manometrically in the corpus-fundus region of the stomach. A baseline recording was obtained during a 15 minute rest period. Air from a compressed air cylinder (British Oxygen Company, Guildford, Surrey, England) was delivered at a constant rate through an air flow meter and a safety valve to the Ryle's tube. This constant flow of air was used to distend the bag at a rate of 15 ml/second for 30 seconds during which the pressure rise was recorded (dynamic recording). The recording was continued at this end volume for a further 150 seconds (static recording). The air was then aspirated with a syringe and the volume noted. This procedure was repeated at 15 minute intervals to provide four recordings for each subject, two each with the subjects sitting and supine. For each recording a dynamic pressure index was derived from the area under the curve during distension and a static pressure index was derived similarly, during the postinsufflation period of 150 seconds. These measurements were ascribed units in cm H₂O (Fig 1). A dynamic and static pressure index was derived for each subject, sitting and supine, based on the mean of the two values for each. These data were used to assess the effect of posture on gastric adaptive relaxation and also to compare the dynamic and static methods. Statistical analysis was by the Wilcoxon's signed-rank sum test for the former and the Mann Whitney U test for the latter. The reproducibility of the technique was assessed in duplicate studies on separate days among eight healthy volunteers, seven of whom were drawn from the control group for this study. The eighth subject gave a history of reflux symptoms and was therefore excluded from the control group for the main study. Reproducibility was expressed as the Spearman rank correlation coefficient calculated from the pressure indices obtained from paired studies. Finally, in view of the differences in ages and body weights between the two groups, a multiple regression model was created to see if there was any effect of these two variables on pressure index (Statistical Analysis System package, SAS Institute Inc, NC 27511, USA).