Effect of cyclosporin A on interleukin-6 and soluble interleukin-2 receptor in patients with rheumatoid arthritis

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Abstract

Objective—To investigate the effect of cyclosporin A (CyA) therapy on circulating concentrations of interleukin-6 (IL-6) and soluble interleukin-2 receptor (sIL-2R) in patients with rheumatoid arthritis (RA).

Methods—Twenty four RA patients with active disease were studied. Plasma was collected before and after 16 weeks of CyA treatment. IL-6 was measured by B9 bioassay and sIL-2R by enzyme linked immunosorbent assay (ELISA).

Results—The initial median IL-6 concentration of 165 IU/ml decreased significantly to 71 IU/ml after 16 weeks ($p < 0.05$). Similarly, the initial median plasma sIL-2R value of 665 U/ml decreased significantly to 570 U/ml ($p < 0.05$). This decrease was accompanied by an improvement in clinical parameters of disease activity. Some association between sIL-2R, IL-6, haemoglobin, and platelets was also observed.

Conclusions—This study has demonstrated that, in vivo, CyA therapy in RA can significantly reduce circulating concentrations of IL-6 and sIL-2R. Modulation of both T and non-T cell derived cytokines may be one mechanism by which CyA improves rheumatoid disease. Whether this is a direct effect of CyA on the cells within the rheumatoid joint producing these cytokines or an indirect effect mediated by other cytokines which can influence IL-6 and Il-2R values remains to be determined.

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Cyclosporin A (CyA) is a novel immunosuppressant which reversibly inhibits T cell function.[1] Expression of the CD4 T cell derived cytokines, interleukin-2 (IL-2), interleukin-4 and interferon gamma are specifically affected.[2] Despite the finding that T cell derived cytokines are underexpressed in rheumatoid arthritis (RA),[3] CyA is more effective than placebo in patients with persistently active rheumatoid synovitis.[4] Unlike other disease modifying drugs, CyA does not significantly affect erythrocyte sedimentation rate although C reactive protein (CRP) concentrations are persistently reduced.[5] Hepatic synthesis of CRP is mediated primarily by the non-T cell derived cytokines tumour necrosis factor α (TNFα) and interleukins-1 and 6 (IL-1, IL-6).[6] In vitro, CyA predominantly affects expression of IL-2.[2] It has been shown in vitro that IL-2 induces IL-6 secretion.[7]

This study investigated the effect of CyA treatment in RA patients on plasma concentrations of soluble interleukin-2 receptor (sIL-2R), a marker of lymphocyte activation[8] and IL-6 which is primarily a product of activated macrophages and fibroblasts in the RA joint.[9]

Patients and methods

PATIENTS

The patients were among a prospective cohort of RA patients treated with CyA at a single centre. All patients had RA as defined by the American College of Rheumatology criteria and had active articular disease requiring disease modifying therapy. No patient had a coexisting disease or was receiving concomitant medication other than simple analgesics, non-steroidal anti-inflammatory drugs and prednisolone. Only those patients in whom paired stored plasma samples (n = 24) were available were included.

CYA DOSAGE

CyA (Sandimmun, Sandoz) was administered as the drink solution as advised by the manufacturer. The initial dose was 5 mg/kg/day and was adjusted (1 mg/kg/day) every four weeks for efficacy and every two weeks according to its toxicity.

EFFICACY ASSESSMENT

Clinical parameters assessed included duration of morning stiffness, Ritchie articular index (RAI) and pain score (PS) rated on a 10 cm visual analogue scale, and number of swollen joints. Laboratory assessments included CRP (laser nephelometry), erythrocyte sedimentation rate (Westergren), haemoglobin, and platelets. Plasma from an unselected cohort was stored at −20°C before and at 16 weeks of treatment.

MEASUREMENT OF IL-6

IL-6 was assessed in plasma using the B9 bioassay as previously described.[10] The assay was carried out in 96 well flat bottomed tissue

culture plates. Samples were heat inactivated (56°C, 30 minutes). Five thousand B9 cells were added to twofold serially diluted plasma giving a final volume of 100 μl. Plates were cultured for 72 hours at 37°C in 5% carbon dioxide in air and cell proliferation assessed by a colorimetric method.[11] IL-6 values were calculated against a preparation of recombinant IL-6 (88/1540, National Institute of Biological Standards and Control, UK, kindly donated by Dr A Meager) and expressed in IU/ml, 1 unit being equivalent to the amount of IL-6 required to yield 50% proliferation of the cells. Assay specificity was assessed by neutralisation studies. The bioassay inter and intra assay variation were 19% and 14%, respectively, with a detection limit of 0·15 IU/ml.

MEASUREMENT OF SOLUBLE IL-2 RECEPTOR
A commercially available enzyme linked immunoassay using two non-competing murine monoclonal antibodies to Tac protein of the human IL-2 receptor was used (T Cell Sciences Inc., Lab-Impex, UK). The inter and intra assay variations were 5% and 3%, respectively. The limit of detection was 50 U/ml.

STATISTICAL METHODS
Paired data were analysed using a Wilcoxon test and Spearman rank correlation coefficients were calculated. Median values and ranges are shown, with $p < 0.05$ taken as significant.

Results

PATIENTS
Twenty four patients (22 female) were included in this study. The median patient age was 58 (range 28–69) years and median disease duration was 13 (2–47) years. Median initial prednisolone dose was 8·5 (0–12·5) mg and median subsequent dose was 7 (0–12·5) mg.

EFFICACY
CyA treatment resulted in a significant improvement in three of four clinical indices of disease activity (table). Platelet count and CRP decreased significantly. The median dose of CyA used at 16 weeks was 5 (2–7·5) mg/kg/day.

Clinical and laboratory indices of disease activity before and after 16 weeks of cyclosporin A therapy

	Before	After	p
Pain (mm)	65·5 (0–96)	60 (7–87)	NS
MS (min)	90 (0–360)	30 (0–360)	0·001
RAI	13 (7–23)	7 (2–26)	0·0004
SJ (No)	12 (3–20)	6 (0–19)	0·001
ESR (mm/1st h)	40 (8–85)	37·5 (8–90)	NS
CRP (mg/ml)	29 (3–116)	15 (3–118)	0·005
Hb (g/dl)	11·8 (10–12)	11·6 (10·8–12·2)	NS
Platelets (×1000/mm³)	420 (221–924)	315 (217–886)	0·002

Median values and ranges. $p < 0.05$ taken as significant. MS = Morning stiffness; RAI = Ritchie articular index; SJ = swollen joints; ESR = erythrocyte sedimentation rate; CRP = C reactive protein; Hb = haemoglobin.
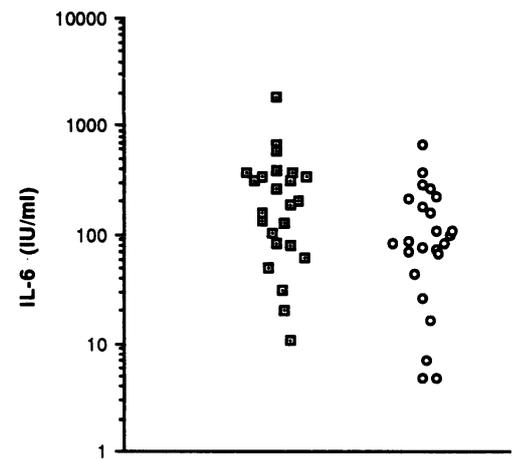

Figure 1   Plasma IL-6 concentrations before (left) and after (right) 16 weeks of cyclosporin A therapy. Median IL-6 concentrations were significantly reduced (from 165 (9–1567) IU/ml to 71 (4–559) IU/ml; p = 0·02) after the 16 weeks of therapy. n = 24.

SERUM IL-6 CONCENTRATION
IL-6 concentrations decreased by a median value of 57% after treatment (fig 1). The median concentration of IL-6 before treatment (165 (9–1567) IU/ml) decreased significantly (to 71 (4–559) IU/ml) (p = 0·02). Of the 24 patients included in this study, 11 had a greater than 50% reduction in their plasma IL-6. Those with a greater than 50% decrease in plasma IL-6 concentrations did not differ from the remainder in their clinical indices.

SERUM SOLUBLE IL-2 RECEPTOR
The median concentration before treatment of 665 (50–3200) U/ml decreased significantly to 570 (170–2160) U/ml (p = 0·001) (fig 2). CyA treatment resulted in a median decrease of 29% in sIL-2R concentrations after 16 weeks. Of the 24 patients, only three had greater than 50% reduction in their sIL-2R concentrations and these patients did not differ from the others in their clinical indices.
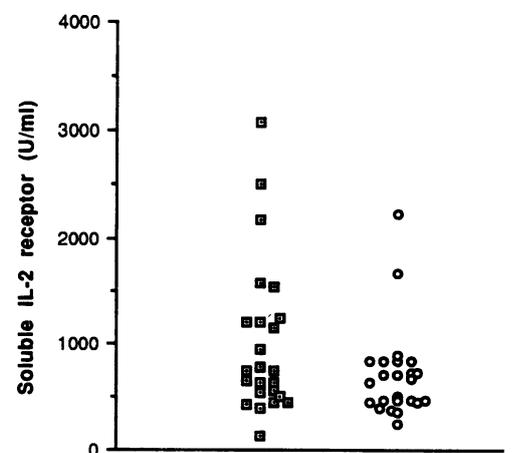

Figure 2   Plasma soluble IL-2 receptor concentrations before (left) and after (right) 16 weeks of cyclosporin A therapy. Median soluble IL-2 receptor concentrations were significantly reduced (from 665 (50–3200) U/ml to 570 (170–2160) U/ml; p = 0·001) after the 16 weeks therapy. n = 24.

CORRELATION OF IL-6 AND SOLUBLE IL-2
RECEPTOR WITH OTHER INDICES

There was some association between initial IL-6 concentrations and sIL-2R (r_{Sp} = 0·48, p = 0·02), but no significant correlation between percentage change in sIL-2R and percentage change in IL-6. No significant correlation was observed between IL-6 and any other clinical indices. Some association between initial concentrations of sIL-2R and haemoglobin (r_{Sp} = –0·4, p = 0·049) and platelets (r_{Sp} = 0·52, p = 0·009) was observed.

Discussion

The fundamental immunological event in RA is the presentation of processed antigen by antigen presenting cells to CD4 T cells—a process which is modulated by cytokines from both cell types. We have shown that CyA in vivo not only affects products of T cell activation, but also suppresses IL-6, a cytokine predominantly derived from intra-articular macrophages and fibroblasts in RA.[9]

Soluble IL-2R is thought to be generated by proteolytic cleavage of the α chain of the IL-2R.[8] The effect of CyA on the α chain or Tac molecule of the IL-2R which gives rise to the sIL-2R is not clear, reported results being conflicting.[2] Although our study has demonstrated a reduction in circulating concentrations of sIL-2R in RA patients receiving CyA, the study has not investigated if this is a direct effect on the expression of the Tac molecule or if it reflects an effect on IL-2 production which influences sIL-2R release.

Previous studies have found sIL-2R concentrations to be increased in active RA.[12] We and others have shown that other second line drugs reduce IL-6[13][14] but do not significantly affect sIL-2R concentrations.[15] These second line drugs are thought to target macrophages specifically, although their mechanism of action is not totally clear.

Although we showed a reduction in sIL-2R concentrations, we could not correlate the percentage change with the improvement observed in clinical indices. However, a correlation of initial sIL-2R concentrations with initial IL-6, haemoglobin and platelets was observed, the significance of which is not totally clear. It may suggest a role for sIL-2R in the pathology of RA, as the associations were found with indices used to follow disease activity.

In common with other disease modifying drugs, CyA significantly reduced IL-6 concentrations. This is in keeping with the decrease in CRP which was observed. Previous studies have reported a decrease in CRP with CyA treatment,[5] but we did not find any significant correlation between CRP and IL-6. We believe our results reflect the fact that, in vivo, the acute phase response is under the influence of several mediators.

Studies in vitro have demonstrated a synergistic effect between IL-6 and other cytokines in the induction of acute phase proteins.[6] It is not clear if the decrease in IL-6 concentrations we observed was a direct effect of CyA on IL-6 production or reflected a reduction in IL-2 and sIL-2R expression; in vitro, the latter affects IL-6 secretion.[7] Similarly, modulation by CyA of other inflammatory cytokines may be involved.

CyA caused a significant improvement in three indices of clinical disease. The absence of any effect on pain score despite a clear improvement in the extent of clinical joint inflammation is not easily explained except on the basis of sample size.

In conclusion this study showed a reduction in plasma sIL-2R and IL-6 after CyA therapy. Unlike other second line agents which appear to have a more specific effect, CyA appears capable of targeting both T and non-T cells. It is not clear if the reduction in IL-6 is a direct effect on macrophages or fibroblasts or caused by a reduction in other cytokines which modulate IL-6.

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