Relation between soluble interleukin 2 receptor and clinical findings in patients with systemic lupus erythematosus

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SUMMARY The concentration of soluble interleukin 2 receptor (IL-2R) was determined in the serum of 54 patients with systemic lupus erythematosus (SLE) by an enzyme linked immunosorbent assay (ELISA) using two monoclonal antibodies with the IL-2R. Concentrations of soluble IL-2R in the serum of the patients with SLE (study group) were significantly higher than in 20 normal subjects (control group). The relation between concentrations of soluble IL-2R and clinical findings was investigated. The concentration of soluble IL-2R showed no particular relation with the clinical manifestations and did not correlate with the level of anti-DNA antibody or CH₅₀. Significant correlation between the concentration of soluble IL-2R and disease activity did exist, however. Furthermore, the concentration of soluble IL-2R in some cases changed simultaneously with the disease activity. Thus the concentration of soluble IL-2R may serve as a new clinical indicator of disease activity in patients with SLE.

It is known that patients with systemic lupus erythematosus (SLE) have various immunological abnormalities. Thus patients with SLE have B cell abnormalities, such as polyclonal B cell activation, and T cell abnormalities have also been reported—for example, change in CD4/8 ratio,¹ decrease in the response to various mitogens (phytohaemagglutinin, concanavalin A, etc), non-response to autologous mixed lymphocyte reaction,^{2,3} and decrease in the production of interleukin 2.^{4,5} Furthermore, other studies have shown that patients with SLE have activated T cells, as indicated by Ia positive T cells (HLA-DR positive T cells).^{6,7}

It is known that interleukin 2 receptor (IL-2R) is detected on the cellular surface of T cells after stimulation by mitogens⁸ and on tumour cells in adult T cell leukaemia.⁹ Recent studies have also reported that the soluble form of IL-2R has been detected in the supernatant of T cell cultures by mitogens or antigens^{10,11} and in serum of patients with adult T cell leukaemia,¹² acquired immune

deficiency syndrome,¹² Sézary syndrome,¹³ and Hodgkin's disease.¹³ Thus soluble IL-2R may serve as an index of activated T cells or T cell malignancy.

In this study we determined the concentration of soluble IL-2R in the serum of patients with SLE, who were said to have activated T cells, and correlated the results obtained with the clinical findings. We found that the patients with SLE had significantly higher concentrations of soluble IL-2R than normal controls and that the concentration correlated with disease activity. Therefore, soluble IL-2R may serve as a new clinical indicator in patients with SLE.

Patients and methods

PATIENTS

Serum samples were obtained from 54 patients with SLE (50 women, four men; aged 21–49 years), according to the American Rheumatism Association criteria (1982), and from 20 normal subjects matched in age and sex with the patients.

The patients with SLE varied in the degree of severity of their serological indices and clinical symptoms. All patients, apart from two, had been

Accepted for publication 3 February 1989.
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receiving steroid treatment (10–30 mg of prednisolone a day) for from one to 26 years.

DETERMINATION OF SOLUBLE INTERLEUKIN 2 RECEPTOR

The concentration of soluble IL-2R was determined by enzyme linked immunosorbent assay (ELISA) as previously reported.¹⁰ To each well of a 96 well multiplate was added 100 μ l of a 1:20 dilution of anti-Tac antibody (T Cell Sciences Inc, Cambridge) in 0.01 M phosphate buffered saline pH 7.2, followed by incubation at room temperature for 48 hours. Blocking was carried out with 1% bovine serum albumin in phosphate buffered saline at 37°C for two hours, and then 100 μ l/well of serum, dilution 1:4, was added and the wells incubated at 37°C for an additional two hours. After washing, 100 μ l/well of peroxidase conjugated with anti-IL-2R (7G7/B6; T cell Science Inc, Cambridge) was added and incubated at 37°C for a further two hours. After washing, 100 μ l/well of *o*-phenylenediamine (in citrate buffer pH 5.0 and H₂O₂) was added and left for 30 minutes at room temperature. Finally, 100 μ l/well of 2.5 M H₂SO₄ was added to terminate the reaction and the optical density at 490 nm was determined. The same procedure was applied to the reference reagent, which was prepared from the culture supernatant of normal interleukin 2 dependent human T cell line four days after stimulation with 10% interleukin 2. The undiluted reagent was assigned a concentration of 3200 units/ml, and serial dilutions of 1:1, 1:2, 1:4, and 1:16 were used to formulate a reference curve. The concentration of soluble IL-2R in the serum samples from the patients with SLE was determined by comparison with this reference curve.

DETERMINATION OF ANTI-DNA ANTIBODIES AND CH₅₀

Determination of anti-DNA antibodies was by the Farr assay¹⁴ and of CH₅₀ by 50% haemolysis.¹⁵

DISEASE ACTIVITY OF SLE

The disease activity of each patient was assessed by the activity score described by Lahita *et al*,¹⁶ which was graded as follows: 0=none; 1=false positive serology, raised erythrocyte sedimentation rate, leucopenia, arthritis, rash, low grade fever, mild cutaneous vasculitis; 2=haemolytic anaemia, thrombocytopenia without bleeding, pleuropericarditis, nephritis without azotaemia, neurological symptoms, severe anaemia, or significantly raised blood pressure; 3=more severe nephritis (creatinine $\leq$ 177 μ mol/l), neurological symptoms, mild central nervous system symptoms, and peripheral vasculitis with gangrene; 4=life threatening (creatinine >265

μ mol/l), coma or obtundation, pulmonary haemorrhage, and central nervous system vasculitis.

STATISTICAL ANALYSIS

The statistical significance of the difference in concentration of soluble IL-2R between the patients

Fig. 1 Concentrations of soluble interleukin 2 receptor (IL-2R) of 54 patients with systemic lupus erythematosus (SLE) and 20 normal controls. The concentrations of soluble IL-2R are plotted as U/ml; the unit values were determined by a reference curve formulated with the use of reference agents (see text). The central horizontal bar indicates the mean value and the upper and lower horizontal bars the standard deviation. The statistical significance was determined with the Wilcoxon rank sum test.

with SLE and the normal subjects was obtained with the Wilcoxon rank sum test. Correlation between the concentration of soluble IL-2R and the titre of anti-DNA antibodies, CH_{50} , or disease activity was evaluated with Student's *t* test. Difference in the incidence of the clinical manifestations was evaluated with the χ^2 test.

Results

CONCENTRATIONS IN SOLUBLE INTERLEUKIN 2 RECEPTOR

Figure 1 shows the concentrations of soluble IL-2R, assessed by an ELISA, in the serum of 54 patients with SLE and 20 normal subjects. It can be seen that the patients with SLE had significantly higher concentrations of soluble IL-2R, ranging from 80 to 960 U/ml (345.2 (SD 222.59)) than the normal subjects, whose range was between 90 and 310 U/ml (155.05 (48.77)) ($p<0.05$). About 30% of the patients with SLE had concentrations of soluble IL-2R more than three SD above the mean concentration of the normal subjects. The two patients who were not receiving steroid treatment also had higher concentrations of soluble IL-2R (650 and 840 U/ml).

CLINICAL MANIFESTATION AND SEROLOGICAL CHARACTERISTICS

Correlation between the concentration of soluble IL-2R and the increase of clinical manifestations

Table 1 Incidence of clinical manifestations in patients with systemic lupus erythematosus with high concentrations of soluble interleukin 2 receptors and normal concentrations. Number (%) of patients is shown

	Soluble interleukin 2 receptor	
	High concentration (n=18)	Normal concentration (n=32)
Malar rash	15 (83)	17 (53)
Discoid	3 (17)	6 (19)
Photosensitivity	5 (28)	9 (28)
Oral ulcer	10 (56)	13 (41)
Joint involvement	17 (94)	26 (81)
Serositis		
Pleuritis	3 (17)	3 (9)
Pericarditis	7 (39)*	2 (6)
Central nervous system involvement		
Seizure	3 (17)	1 (3)
Psychosis	2 (11)	2 (6)
Haemolytic anaemia	5 (28)	8 (25)
Leucopenia	4 (22)	7 (22)
Lymphopenia	14 (78)	26 (81)
Thrombocytopenia	4 (22)	4 (13)
Renal involvement	10 (56)	10 (31)

* $p<0.05$, comparison with patients with normal concentration of interleukin 2 receptor (χ^2 test).

Fig. 2 Correlation between the concentration of soluble interleukin 2 receptor (IL-2R) and the disease activity score. The disease activity score was obtained as described by Lahita *et al.*¹⁶

Fig. 3 Longitudinal evaluation of the concentrations of soluble interleukin 2 receptor (IL-2R) relative to those of anti-DNA antibodies, CH_{50} , and proteinuria, and relative to treatment with prednisolone of patient No 1 with systemic lupus erythematosus and lupus nephritis. The concentration of soluble IL-2R increased with an increase in the amount of urine protein and the concentration of anti-DNA antibodies, and with a decrease in the level of CH_{50} . With treatment these changes improved.

(according to the criteria of the American Rheumatism Association) throughout the clinical course was investigated. The patients were classified into two groups: patients with high concentrations of soluble IL-2R (group 1) and those with normal concentrations (group 2). The patients in group 1 had concentrations more than three SD above the mean concentration of the normal subjects, whereas those in group 2 had concentrations less than three SD above the concentration of the normal subjects. As shown in Table 1 the incidence of pericarditis was the only clinical manifestation that was greater in patients with high concentrations of soluble IL-2R than in those whose concentrations were normal ($p<0.05$). Pleuritis, central nervous system involvement, and renal involvement also showed a greater incidence in patients with high concentrations of soluble IL-2R, but the differences did not reach statistical significance. No particular correlation was noted with the skin and mucosa (malar rash, discoid, photosensitivity, and oral ulcer), joint and haematological involvement (haemolytic anaemia, lymphocytopenia, and thrombocytopenia).

Fig. 4 Longitudinal evaluation of the concentrations of soluble interleukin 2 receptor (IL-2R) relative to those of anti-DNA antibodies, CH₅₀, proteinuria, and fever, and relative to treatment with prednisolone of patient No 2 with systemic lupus erythematosus and lupus nephritis. The concentration of soluble IL-2R increased with fever, with an increase in the amount of urine protein, and with a decrease in CH₅₀. With treatment these changes improved. There was no change in the concentration of anti-DNA antibodies during the clinical course.

Fig. 5 Longitudinal evaluation of the concentrations of soluble interleukin 2 receptor (IL-2R) relative to those of anti-DNA antibodies, CH₅₀, proteinuria, and pneumonitis, and relative to treatment with prednisolone of patient No 3 with systemic lupus erythematosus, lupus nephritis, and pneumonitis. The concentration of soluble IL-2R increases with fever, with an increase in the amount of urine protein, and with a worsening of the pneumonitis. With treatment these changes improved. There were no changes in the concentrations of anti-DNA antibodies and CH₅₀.

Correlation between the concentration of soluble IL-2R and the serological characteristics was investigated by using anti-DNA antibody and the CH₅₀ which were considered to be the main laboratory variables of SLE. There was no significant correlation between the concentration of soluble IL-2R and anti-DNA antibodies (data not shown), possibly because some patients with high concentrations of soluble IL-2R have low concentrations of anti-DNA antibodies. Similarly, there was no significant correlation between the concentration of soluble IL-2R and CH₅₀ (data not shown).

DISEASE ACTIVITY

As the patients with high concentrations of soluble IL-2R tended to have an increased incidence of serositis, central nervous system involvement, and renal involvement the possible correlation between concentration of soluble IL-2R and the disease activity at the time when the serum was prepared was further investigated by applying the activity score described by Lahita *et al.*¹⁶ Figure 2 shows that there was significant correlation between the concentration of soluble IL-2R and the disease activity ($p<0.05$). Of the 18 patients with high concentrations of soluble IL-2R, 11 belonged to the more severe

disease activity group (score >2). The degree of severity in these patients was directly related to the degree of renal involvement (eight cases), serositis (two cases), central nervous system involvement (one case), and peripheral vasculitis (one case).

We also monitored the change in the concentration of soluble IL-2R in four patients during their clinical course. Figures 3–6 show that the concentrations of soluble IL-2R in all cases increased as the amount of urine protein increased, but with treatment these increases and proteinuria were reversed. In some patients pneumonitis, pleuritis, central nervous system involvement, and peripheral vasculitis (skin ulcer) also related to the concentration of

soluble IL-2R. Levels of anti-DNA antibodies and CH_{50} also varied with the disease activity in most patients, but there were no changes in the levels of anti-DNA antibodies or CH_{50} during the clinical course in some patients (Figs 4, 5, and 6).

Discussion

Although cellular surface IL-2R, the receptor for transferrin, or class II antigen (HLA-DR) on T cells are believed to be markers of activated T cells,^{8 17 18} recent studies have reported that IL-2R also appears in the culture supernatant or in the serum, or both, during T cell proliferation,^{10 11} which suggests that soluble IL-2R may also be a marker of activated T cells. The concentration of soluble IL-2R was determined by an ELISA using two standard monoclonal antibodies.¹⁰ Soluble IL-2R was easily distinguished from the other markers of activated T cells (cellular surface IL-2R, the receptor for transferrin, or class II antigen) by this method as serum is used in an ELISA and other markers are determined by flow cytometry.

Previous reports have stated that patients with SLE have activated T cells,^{6 7} and therefore we determined the concentration of soluble IL-2R in patients with SLE as another indicator of T cell activation. It was found that the concentrations of soluble IL-2R in patients with SLE were significantly higher than in normal subjects. This result agrees with other studies^{19 20} and further supports the existence of activated T cells in patients with SLE. Even though the patients in our study had been receiving steroid treatment, we believe that the result does not reflect this use of steroids because patients who were not receiving steroids also had increased concentrations of soluble IL-2R; the concentration of soluble IL-2R in the lupus mouse was also increased.²¹

We further investigated the correlation between concentration of soluble IL-2R and the clinical findings. Although we were unable to find any statistically significant correlation between the concentration of soluble IL-2R and specific clinical manifestations, the incidence of pericarditis was significantly greater ($p < 0.05$) in patients with high concentrations of soluble IL-2R than in patients within the normal range. Even though there was no statistically significant correlation with pleuritis, central nervous system involvement, and renal involvement, it is important to point out that these conditions tended to prevail among patients with a high concentration of soluble IL-2R. Further, as the serum was obtained randomly from the patients with SLE instead of during the active and inactive phases of the disease and we were unable to monitor the

Fig. 6 Longitudinal evaluation of the concentrations of soluble interleukin 2 receptor (IL-2R) relative to those of anti-DNA antibodies, CH_{50} , proteinuria, central nervous system (CNS) involvement, and peripheral vasculitis, and relative to treatment with prednisolone of patient No 4 with systemic lupus erythematosus, lupus nephritis, CNS lupus, and peripheral vasculitis. The concentration of soluble IL-2R increased with an increase in the amount of urine protein and the concentration of anti-DNA antibodies, and with worsening of the CNS involvement and peripheral vasculitis. With treatment these changes improved. There was no change in the level of CH_{50} .

concentration of soluble IL-2R throughout the disease course of all patients, these results suggest that even patients within the normal range might have had increased concentrations during the active phase of pleuritis, central nervous system involvement, and renal involvement. Therefore, we investigated the correlation between the concentration of soluble IL-2R and disease activity at the time when the serum was obtained and prepared. From these results we found that the concentration of soluble IL-2R correlated with disease activity, especially with renal involvement, serositis, central nervous system involvement, and peripheral vasculitis. In addition, we also noticed that the concentration of soluble IL-2R increased with an increase in the activity of the disease and decreased with treatment in some patients, thus supporting a correlation between concentrations of soluble IL-2R and disease activity. Although another study reported similar results in a patient with lupus nephritis,¹⁹ it is interesting that the correlation between concentration of soluble IL-2R and disease activity was also found in patients with serositis, central nervous system involvement, and peripheral vasculitis. There was no significant correlation between the concentration of soluble IL-2R and anti-DNA antibodies and CH₅₀. Although anti-DNA antibodies and CH₅₀ are usually used as indices of disease activity in patients with SLE, especially lupus nephritis, it is known that a correlation is not shown in all cases. Furthermore, the titre of anti-DNA antibodies and CH₅₀ did not change in some patients monitored during the clinical course. Thus these results suggest that soluble IL-2R may be a new marker of disease activity independent of anti-DNA antibodies or CH₅₀.

The mechanism regulating the secretion or biological role of soluble IL-2R is not clearly understood and this poses some problems. Firstly, cellular surface IL-2R of the peripheral blood lymphocytes does not increase in our patients with high concentrations of soluble IL-2R (data not shown), whereas both types of IL-2R increased in patients with adult T cell leukaemia.⁹⁻¹² The reason for this is not clear, but we believe that it may be due to the shedding of cellular surface IL-2R as previously reported²² or an increase of cellular surface IL-2R in the lymphoid organ. The second problem is whether soluble IL-2R has an immunoregulatory role or not. A previous report suggested that soluble IL-2R competed with cellular surface IL-2R for the interleukin 2 and therefore reduction of activated T cells occurred.¹⁰ Soluble IL-2R in patients with SLE may also serve the same purpose as a recent report indicates that the concentration of interleukin 2 in the serum of patients with SLE increases with T cell

activation.²⁰ We think that this theory should be carefully evaluated, however, because it has been reported that soluble IL-2R binds with low affinity.²³ The third problem is whether the soluble IL-2R of patients with SLE is derived from the T cells only or not. Other studies have reported that soluble IL-2R is derived not only from T cells but also from B cells,²¹⁻²⁴ and it is known that B cell abnormality exists in patients with SLE. Therefore we cannot disregard the possibility that soluble IL-2R is also derived from B cells. As the concentration of soluble IL-2R derived from B cells is minute in comparison with that from T cells,²⁴ however, we believe that the soluble IL-2R in the serum of patients with SLE is derived mostly from the T cells. Although the role of soluble IL-2R is not yet clear, it may be an important clue in the investigation of the pathogenesis of SLE.

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