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Changes in plasma levels of interleukin-2 receptor in relation to disease exacerbations and levels of anti-dsDNA and complement in systemic lupus erythematosus

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SUMMARY

Interleukin-2 receptor (IL-2R) is expressed and released predominantly by activated T cells. In order to investigate whether disease exacerbations of systemic lupus erythematosus (SLE) are preceded by T cell activation, we prospectively measured levels of IL-2R once a month, from 6 months prior to exacerbations until 1 month afterwards. To assess the temporal relation between T cell activation and B cell activation, we measured, in addition, levels of anti-dsDNA, complement C3/C4, and total IgG. During a mean follow-up period of 23 months, 40 exacerbations occurred in 21 out of the 71 participating patients. For the present study one exacerbation per patient was evaluated. During exacerbation levels of IL-2R were increased in 18 out of the 21 cases and correlated with levels of anti-dsDNA ($P < 0{\cdot}02$), C3 ($P < 0{\cdot}02$), and C4 ($P < 0{\cdot}01$), but not with the score of the disease activity index. Levels of IL-2R rose prior to the excerbation ($P < 0{\cdot}02$) and fell afterwards following treatment ($P < 0{\cdot}05$). Even in the absence of disease activity or during minor disease symptoms IL-2R levels were higher ($P < 0{\cdot}01$) than in healthy controls. Sixteen out of the 21 exacerbations (76%) were preceded by a significant increase in IL-2R. Changes in levels of anti-dsDNA and complement C3/C4 tended to precede changes in levels of IL-2R. We conclude that increased levels of IL-2R, compatible with T cell activation, are present in SLE already during inactive disease. These levels further increased prior to exacerbations of disease. As such, IL-2R is an indicator of disease activity in SLE. Serial measurement of IL-2R is a sensitive test for predicting disease exacerbations of SLE.

Keywords interleukin-2 receptor systemic lupus erythematosus exacerbations T cell activation

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by multiple B and T cell abnormalities (Theofilopoulos & Dixon, 1985). Generalized B cell hyperactivity is thought to be the hallmark of active SLE (Klinman & Steinberg, 1987). This B cell hyperactivity might be explained by direct, T cell-independent B cell activitation, or by (excessive) production of B cell growth and differentiation factors by regulatory T cells. *In vivo* studies in SLE have demonstrated increased percentages of activated T lymphocytes (Inghirami *et al.*, 1988), whereas *in vitro* increased T helper cell activity as well as depressed T suppressor cell function have been found (Fauci *et al.*, 1978). Only a few studies indicate that B cell hyperactivity in SLE depends on T (helper) cell activation (Huang *et al.*, 1988). T cell abnormalities in SLE may, however, also be a sequel of disease activity rather than have a primary role.

Shortly after activation of lymphocytes, interleukin-2 receptors (IL-2R) are expressed on their surface, and subsequently released in proportion to the state of activation. The soluble form of IL-2R can be detected in blood. The function of soluble IL-2R is uncertain but an immunoregulatory role has been suggested by binding IL-2 (Rubin *et al.*, 1985). Increased serum levels of IL-2R have been found in rheumatic conditions characterized by activation of the immune system such as rheumatoid arthritis (Campen *et al.*, 1988; Semenzato *et al.*, 1988), Behçet's disease (Hamzaoui & Ayed, 1989), primary Sjögren's syndrome (Manoussakis *et al.*, 1989) and SLE (Campen *et al.*, 1988; Semenzato *et al.*, 1988; Wolf & Brelsford, 1988). Serum levels of IL-2R have been found to correlate strongly with (indicators of) disease activity in patients with SLE (Campen *et al.*, 1988; Semenzato *et al.*, 1988; Wolf & Brelsford, 1988). Currently, measurement of anti-dsDNA and complement components is most commonly used to monitor

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disease activity in patients with SLE (Lloyd & Schur, 1981). Regarding anti-dsDNA, we found that exacerbations of SLE were frequently heralded by a significant rise in these antibodies (ter Borg *et al.*, 1990).

Here we prospectively measured plasma levels of IL-2R in addition to anti-dsDNA, complement C3/C4, and total IgG prior to, during and after exacerbations of SLE. The aim of the study was to establish whether disease exacerbations of SLE are indeed preceded by T cell activation, and if so, whether T cell activation occurs prior to or following B cell activation. In addition, we investigated whether particular disease manifestations during exacerbation were related to plasma levels of IL-2R.

SUBJECTS AND METHODS

Subjects

Seventy-one unselected patients (59 women and 12 men) fulfilling the revised criteria for establishing the diagnosis of SLE were included (Tan *et al.*, 1982). Their mean age at the start of the study was 39·2 years (range 15–70). A diagnosis of SLE was made a mean of 7·7 years (range 0–25 years) before the start of the study. The total follow-up time was 1610 patient months (mean 22·7 months/patient; range 6–47 months).

Assessment of disease activity

Disease activity was scored at every visit to the Outpatient Clinic and at least once a week during admission according to a previously reported and validated disease activity index (ter Borg *et al.*, 1990). This index contains objective items and results of standard laboratory examinations only and was calculated without prior knowledge of the study parameters (Table 1).

Exacerbations (major and minor) were defined according to a previous report (ter Borg *et al.*, 1990). Immunological variables are not included in the index nor in the criteria for exacerbations.

Blood samples

Blood samples were drawn in EDTA at least once a month and during active disease more frequently. Plasma was stored at −80°C.

Study design

All patients were seen at at least 3-month intervals at the Outpatient Clinic. During active disease the patients were seen as frequently as was judged clinically necessary. At every visit to the clinic, and during admission to the hospital at least once a week, a history taking and a physical examination as well as standard laboratory tests were performed according to a protocol. Patients were treated for major exacerbations with prednisolone (1 mg/kg per day), in some cases in conjunction with cyclophosphamide or azathioprine. Minor exacerbations were treated with lower dosages of prednisolone (0·5 mg/kg per day), sometimes in combination with anti-malarials. After disease symptoms had declined, the dosage of prednisolone was tapered off slowly. From 6 months prior to the exacerbation until 1 month afterwards, the following parameters were measured serially in plasma once a month: IL-2R, anti-dsDNA, C3, C4 and total IgG.

Table 1. Disease activity index

	Points*
Kidneys	
Proteinuria:	
newly developed (> 0·5 g/day) or doubling within 4 months (basal proteinuria > 0·5 g/day)	2
Erythrocyturia:	
newly developed (> 5 E/HPF) or doubling	1
Erythrocyte and/or granular casts:	
presence	1
newly developed	2
Creatinin clearance: decrease of > 25% (within 4 months)	2
Central nervous system†	
Cerebral vascular accident	4
Seizure	4
Psychosis	4
Choreathetosis	4
Transverse myelitis	4
Motoric nerve palsy	4
Skin/mucosa	
Alopecia*	1
Active discoid rash	1
Malar rash	2
Other active rash	1
Active oral and/or nasal ulcerations	2
Blood	
Haemolytic anaemia:	
Hb < 100 g/l	1
Hb < 80 g/l	2
Leucopenia:	
$< 4{\cdot}0 \times 10^9/l$	1
$< 3{\cdot}0 \times 10^9/l$	2
Thrombocytopenia:	
$< 100 \times 10^9/l$	1
$< 50 \times 10^9/l$	2
$< 25 \times 10^9/l$	3
Musculoskeletal system	
Arthralgia and/or myalgia†	1
Arthritis (≥ 2 joints) and/or tendinitis	1
Serosa	
Pleural and/or pericardial pain†	1
Pleural and/or pericardial rub	2
Abnormalities on chest X-ray, EKG, and/or echocardiogram	2
Vessels	
Minor vasculitis (purpura, periungual infarction)	2
Major vasculitis (ulcerations, mononeuritis)	4
Miscellaneous	
Uveitis and/or chorioretinitis	3
Myositis:	
rising CPK > 150 U/l	2
rising CPK > 500 U/l	3

† Only scored when occurring within 2 weeks of a visit to the Outpatient Clinic, or admission under consideration.

* When an item is related, in all likelihood, to medication or an unrelated condition, no points are given.

E/HPF, erythrocytes/high-power field; Hb, haemoglobin; CPK, creatine phosphokinase.

Interleukin-2-receptor plasma levels

These were measured by sandwich ELISA according to the manufacturer (T Cell Sciences, Cambridge, MA). In brief, microtitre plates were coated with a murine monoclonal IgG antibody recognizing one epitope on human IL-2R. After incubation of plasma, horseradish peroxidase-conjugated murine monoclonal IgG antibody recognizing a second epitope on IL-2R was added. After colour reaction the plates were read at 490 nm. Normal value of IL-2R levels in our laboratory is below 650 arbitrary units/ml (U/ml). Intra- and interassay variation are less than 5% and 15%, respectively. A significant rise in plasma IL-2R was defined as a rise of $\geq 25\%$ and at least 200 U/ml.

Anti-dsDNA antibody levels

Antibody levels were measured by the Farr assay, using ^{125}I-labelled recombinant dsDNA (Diagnostic Products Corporation, Los Angeles, CA) which is free from contamination with ssDNA. The assay was performed according to the manufacturer and positive samples were measured at different dilutions to obtain measurements within the range of the assay. Normal value of this assay in our laboratory is ≤ 26 U/ml; intra- and interassay variation are both less than 10%. Results were expressed in U/ml using Wo/80 as the ultimate standard (Feltkamp *et al.*, 1988). A significant rise in anti-ds DNA was defined as a rise of $\geq 25\%$ and exceeding 30 U/ml.

Complement C3 and C4 levels

C3/C4 levels were measured by nephelometry (normal values $\geq 0{\cdot}64$ g/*l* and $\geq 0{\cdot}11$ g/*l*, respectively). Intra- and interassay variation of C3 and C4 measurements were less than 10%. A significant fall in complement levels was defined as a decrease in C3 of $\geq 25\%$ and at least 0·10 g/*l* and as a decrease in C4 $\geq 25\%$ and at least 0·05 g/*l*.

Total IgG levels

These were measured by nephelometry (normal value 8·5–15·0 g/*l*). The criteria for significant changes of the tests used were partly based on the values of intra- and interassay variation of these tests. All these significant changes had to occur within a period of 4 months.

Statistical analysis

Spearman's rank sum test was applied for detecting correlations between the different study parameters. Differences of parameters between groups were evaluated with Wilcoxon's rank sum test (two sample). Changes of parameters within a group were evaluated with Wilcoxon's signed rank test (paired). For comparison of differences in prevalence between groups, χ^2 analysis was applied. $P < 0{\cdot}05$ was considered significant.

RESULTS

Exacerbations

During follow up, a total of 40 exacerbations (17 major and 23 minor) occurred in 21 out of the 71 participating patients. For this study only one exacerbation (the clinically most severe exacerbation) per patient ($n = 21$; 10 major and 11 minor exacerbations) was evaluated. Only exacerbations without evidence of an infection at the time of maximal disease activity were included.

Table 2. Correlation coefficients* between levels of IL-2R and the score of disease activity index, levels of anti-dsDNA, complement C3/C4, and IgG during 21 exacerbations of systemic lupus erythematosus

	Disease† activity index	Anti-dsDNA	C3	C4	Total IgG
r	0·22	0·53	−0·54	−0·62	0·06
P	NS	<0·02	<0·02	<0·01	NS

† Spearman's rank sum test.

† Disease activity index was significantly correlated with levels of C3 ($r = -0{\cdot}60$, $P < 0{\cdot}01$) but not with levels of anti-dsDNA, C4 or total IgG.

Levels of IL-2R at the time of maximal disease activity during exacerbation

At the time of maximal disease activity during exacerbation IL-2R plasma levels were increased (median level 1260 U/ml, range 425–5020 U/ml) in 18 out of the 21 cases (86%). At that time, before immunosuppressive treatment was started or intensified, 12 out of the 21 patients used prednisolone and, in addition to prednisolone, three patients used cytostatics. Although the four patients with the highest IL-2R levels during exacerbation did not use prednisolone, no significant difference was found in IL-2R levels during exacerbations between patients using prednisolone ($n = 12$) and those not using prednisolone ($n = 9$).

Significant correlations were found between the levels of IL-2R and the levels of anti-dsDNA ($P < 0{\cdot}02$), C3 ($P < 0{\cdot}02$) and C4 ($P < 0{\cdot}01$) during the exacerbation but not between the levels of IL-2R and the score of the disease activity index or the levels of total IgG at that time (Table 2). Levels of IL-2R were higher during the exacerbations with decreased C3 levels ($n = 11$; median value 1400 U/ml, range 540–5020 U/ml) than during the exacerbations with normal C3 levels ($n = 10$; median value 1040 U/ml, range 425–1950 U/ml; $P < 0{\cdot}05$). This was also the case for the exacerbations with decreased C4 levels ($n = 6$; median value 2230 U/ml, range 1130–5020 U/ml) compared with the exacerbations with normal C4 levels ($n = 15$; median value 1030 U/ml, range 425–1950 U/ml; $P < 0{\cdot}01$). No difference was found between IL-2R levels during the anti-dsDNA positive exacerbations ($n = 17$) compared with the anti-dsDNA negative exacerbations ($n = 4$). The score of the disease activity index at the time of the exacerbation correlated with levels of C3 ($P < 0{\cdot}01$) but not with levels of C4, anti-dsDNA and IgG. No correlation was found between the levels of IL-2R and any particular disease manifestation at the time of the exacerbation.

Serial measurements of IL-2R prior to, during and after the exacerbation

To evaluate changes in IL-2R in relation to the exacerbations, IL-2R levels were assessed at five points in time: P6, 6 months prior to the exacerbation; P3, three months prior to the exacerbation; P1, 1 month prior to the exacerbation; M, at the time of maximal disease activity during the exacerbation; and A, 1 month after the exacerbation (Fig. 1, Table 3). IL-2R plasma levels at each of these five points in time were higher than the levels of IL-2R in healthy controls ($P < 0{\cdot}01$). Between points P6

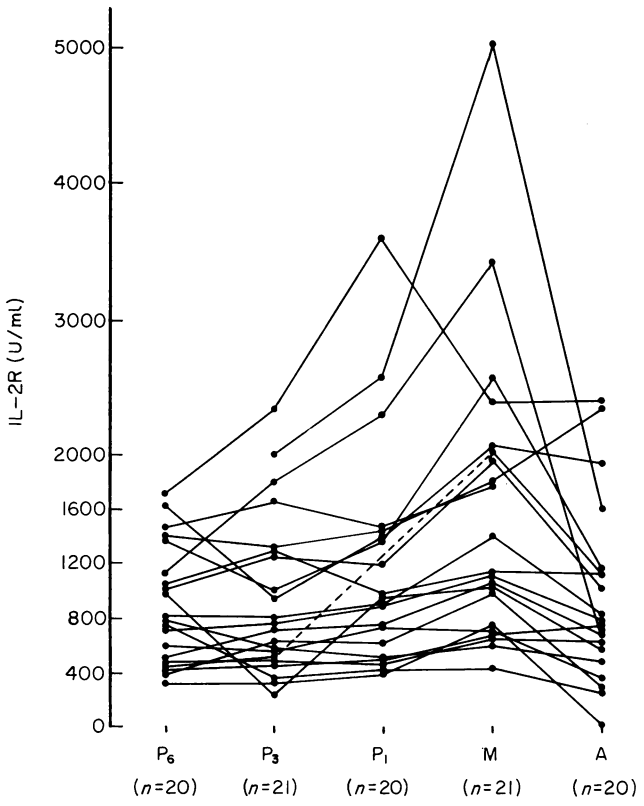

Fig. 1. Levels of IL-2R in patients with systemic lupus erythematosus at time points P6 (6 months prior to exacerbation); P3 (3 months prior to exacerbation); P1 (1 month prior to exacerbation); M (at the time of maximal disease activity of the exacerbation); and A (1 month after the exacerbation). In one patient, the IL-2R level at point P1 is lacking and is denoted by a dashed line.

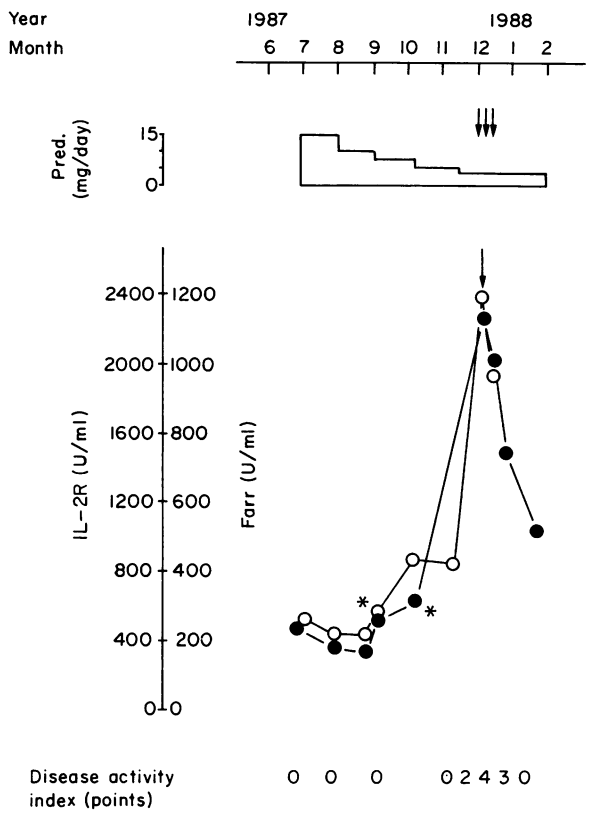

Fig. 2. Course of IL-2R (●) and anti-dsDNA antibody levels (○, Farr test) prior to, during, and after a major exacerbation of systemic lupus erythematosus. Pred., prednisolone; arrows, 1 g i.v. pulses. * Significant rise; arrow, major exacerbation (nephritis, vasculitis).

Table 3. Plasma levels of IL-2R and daily dosages of prednisolone at five defined points in time

	P6 ($n=20$)	P3 ($n=21$)	P1 ($n=20$)	M ($n=21$)	A ($n=20$)
Levels of IL-2R (U/ml)					
Median	790	750	940*	1125†	780‡
Range	320–1725	235–2350	415–3600	425–5020	10–2400
Daily dosage of prednisolone (mg/day)					
Median	7·5	3·8	4·4	3·8	20§
Range	0–30	0–30	0–22·5	0–22·5	0–60

P6, 6 months prior to the exacerbation; P3, 3 months prior to the exacerbation; P1, 1 month prior to the exacerbation; M, at the time of maximal disease activity of the exacerbation; A, 1 month after the exacerbation.

* $P<0·02$ *versus* P3; † $P<0·01$ *versus* P1; ‡ $P<0·05$ *versus* M; § $P<0·01$ *versus* M.

and P3 no significant change in IL-2R levels was observed. However, between points P3 and P1 ($P<0·02$) as well as between points P1 and M ($P<0·01$), IL-2R levels rose. After the exacerbation (between points M and A), IL-2R levels fell again ($P<0·05$). Although the mean dosage of prednisolone used at the time-point P6 was higher than at time-point P3, these differences were not statistically different. Also, the dosages used at the points P3 and P1, and P1 and M, did not differ (Table 3). Dosages of prednisolone were higher at point A compared with point M ($P<0·01$). Two representative examples of changes in IL-2R levels prior to, during and after the exacerbation are given in the Figs 2 and 3. Serial measurements of IL-2R in an SLE patient with minor (but stable) disease activity and in a healthy control showed no significant change.

Comparison of serial measurements of IL-2R, anti-dsDNA, C3/C4, and total IgG during the period prior to the exacerbation

At the time of the exacerbation a positive test for anti-dsDNA was found in 17 out of the 21 cases, a decreased level of C3 and C4 in 11 and six cases, respectively, and an increased level of IgG in 18 cases.

During the time period of 6 months prior to the exacerbation, significant changes in IL-2R levels occurred in 16 out of the 21 cases (76%), in anti-dsDNA in 14 out of the 17 cases with a positive test for anti-dsDNA (82%), in C3 in 11 cases (52%), in C4 in eight cases (38%), and in total IgG in only three cases. The sensitivity of a significant change in IL-2R for an ensuing

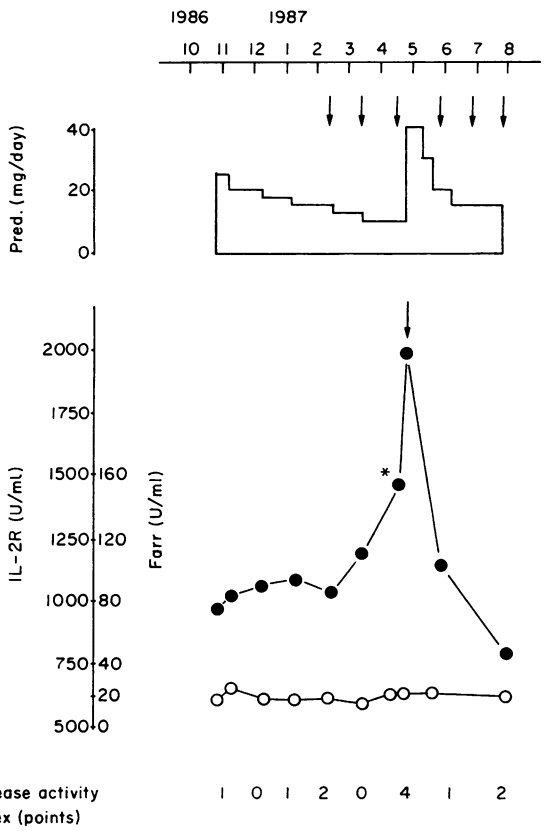

Fig. 3. Course of IL-2R (●) and anti-dsDNA antibody levels (○, Farr test) prior to, during, and after a minor exacerbation of systemic lupus erythematosus. Pred., prednisolone; arrows, 900 mg i.v. cyclophosphamide pulses. * Significant rise; arrow, minor exacerbation (pleuropericarditis; fever, arthralgia).

exacerbation was higher than that of C4 ($P < 0{\cdot}05$) but was not statistically different from that of anti-dsDNA or C3. Three out of the five exacerbations not preceded by a significant rise in IL-2R were preceded by a significant rise in anti-dsDNA and one by a significant decrease in C3. None of these five exacerbations was preceded by a significant change in C4 or total IgG. Five out of the seven exacerbations not preceded by a significant rise in anti-dsDNA were preceded by a significant rise in IL-2R, including all four exacerbations with a negative test for anti-dsDNA. The percentual rise in anti-dsDNA prior to the exacerbation (median rise 102%, range 29–635%) far exceeded the rise in total IgG (median rise 9%, range 0–37%, $P < 0{\cdot}01$).

Although the point at which a significant change was observed prior to the exacerbation occurred later for IL-2R (median 3·5 weeks, range 0–18) than for anti-dsDNA (median 9 weeks, range 5–24), or C3 (median 9·5 weeks, range 5–18) and C4 (median 10 weeks, range 5–18), these differences were not statistically significant. The significant rises in IL-2R ($n = 16$) occurred in seven cases prior to, in four cases simultaneously with, and in five cases after the rise in the disease activity index.

DISCUSSION

B cell hyperactivity is thought to be the hallmark of active SLE (Klinman & Steinberg, 1987). In lupus mice (Datta, 1989; Gleichman, van Elven & van der Veen, 1982) and in human SLE (Gharavi, Chu & Elkon, 1988) the production of autoantibodies such as anti-dsDNA, however, has been suggested to result from a more selective B cell response. The results of the present study are indeed in favour of a restricted B cell response prior to the exacerbation as the rise in anti-dsDNA far exceeded the rise in total IgG. This might indicate that in SLE T cell-dependent, antigen-driven B cell stimulation is operative rather than T cell-independent polyclonal B cell activation. In accordance, activated T lymphocytes from the peripheral blood of SLE patients have been found to provide effective help for B cells in the production of immunoglobulins (Inghirami *et al.*, 1988). Recently, increased T cell activation has been observed in SLE and has been suggested to play a role in B cell hyperactivity (Huang *et al.*, 1988).

IL-2R is expressed on and released by activated T lymphocytes (Rubin *et al.*, 1985). In the present prospective study, plasma levels of IL-2R rose prior to disease exacerbations of SLE. This finding is in favour of T cell activation occurring prior to exacerbations of SLE. Recently, in a serial study on three SLE patients, IL-2R levels were reported to be an earlier indicator for fluctuations in disease activity than C3 (Wolf & Brelsford, 1988). In our study significant rises in IL-2R prior to exacerbation tended to occur later than changes in C3/C4 and anti-dsDNA. This difference was not statistically significant, probably due to small sample size. Our findings, however, do not necessarily argue against a primary role of T lymphocyte activation in the production of autoantibodies such as anti-dsDNA. Alternatively, T cell activation prior to an exacerbation of SLE, as demonstrated by a rise in IL-2R, may also be a sequel of a general immune activation. Even in the absence of disease activity or during minor disease activity, IL-2R levels were higher in our SLE patients than in healthy controls, indicating persistent T cell activation in SLE which agrees with a previous study (Wolf & Brelsford, 1988).

The release of IL-2R appears to be a characteristic of T lymphocyte activation (Rubin *et al.*, 1985). However, in SLE patients and in lupus mice evidence has been found for the expression (Balderas *et al.*, 1987; Wigvall *et al.*, 1988) and subsequent release (Balderas *et al.*, 1987) of IL-2R by activated B lymphocytes. Thus, rises in IL-2R prior to exacerbations of SLE may at least in part originate from activated B cells. The same applies to killer cells that have been shown to get activated by IL-2 (Rosenberg *et al.*, 1885). As such, a rise in IL-2R may only be a sequel of a general immune activation preceding an exacerbation of SLE. Longitudinal FACS analysis of activated T and B cells may probably give a more direct answer to the question whether T cell activation precedes B cell activation prior to an exacerbation of SLE. Whatever their origin, IL-2R levels might be used to assess disease activity or to predict disease exacerbations in SLE. In our study, a significant rise in IL-2R was sensitive for predicting an exacerbation of SLE. For predicting exacerbations, serial measurement of IL-2R proved at least as sensitive as serial measurement of anti-dsDNA and C3. Interestingly, five out of the seven exacerbations not preceded by a significant rise in anti-dsDNA, were preceded by a significant rise in IL-2R, including all four anti-dsDNA-negative exacerbations. As to the specificity of rises in IL-2R for ensuing exacerbations, preliminary results showed that IL-2R levels did not change in patients without or with stable disease activity.

A negative correlation has been reported between the dosage

of prednisolone and IL-2R levels during a serial study in SLE patients (Wolf & Brelsford, 1988). In the present study, however, rises in IL-2R occurred prior to the exacerbation although no significant reduction of the dosage of prednisolone took place during that period. Moreover, IL-2R levels were not different between the exacerbations with and without prednisolone. Serum levels of IL-2R in SLE have been reported to correlate strongly with clinical disease activity (Campen *et al.*, 1988; Semenzato *et al.*, 1988) and levels of complement C3/C4 (Campen *et al.*, 1988; Wolf & Brelsford, 1988). In our study, levels of IL-2R during exacerbation related well to the levels of anti-dsDNA and C3/C4, but not to the score of the disease activity index. In contrast to IL-2R, levels of C3 correlated with the score of the disease activity index at the time of the exacerbation. Levels of C3 may therefore be a more direct reflection of the pathophysiological processes resulting in the disease manifestations of an exacerbation in SLE than levels of IL-2R.

Exacerbations of SLE are preceded by a rise in IL-2R, probably reflecting T cell activation. The role of T cell activation in the production of autoantibodies, such as anti-dsDNA, remains unclear since rises in IL-2R tended to occur later than rises in anti-dsDNA. Serial measurement of IL-2R seems to be a sensitive test for predicting exacerbations of SLE and seems valuable especially in anti-dsDNA negative patients.

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