Changes in levels of soluble T-cell activation markers, sIL-2R, sCD4 and sCD8, in relation to disease exacerbations in patients with systemic lupus erythematosus: a prospective study

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Abstract

Objectives—To assess serial activation of T-cell subsets in relation to auto-antibody production and the occurrence of disease exacerbations in patients with systemic lupus erythematosus (SLE).

Methods—To study the possible role of T-cells in the pathophysiology of the disease, 16 consecutive exacerbations were prospectively studied in a cohort of patients with SLE, and serial plasma levels of sIL-2R, sCD4, and sCD8 preceding and during these exacerbations were determined. Levels of these molecules were related to total IgM and IgG, and anti-dsDNA.

Results—During major disease exacerbations (n = 6), levels of sIL-2R increased significantly (p < 0.001). Levels of sCD4 were predominantly in the normal range, whereas levels of sCD8 were frequently increased. No change in levels of both molecules could be detected in the period before the exacerbation. During minor exacerbations (n = 10), levels of sIL-2R remained stable. Levels of sCD4, however, tended to drop, whereas levels of sCD8 tended to rise. No correlations were found between sIL-2R, sCD4 or sCD8 on the one hand, and total IgM, IgG, or anti-dsDNA on the other.

Conclusions—Levels of sIL-2R are increased, and rise before major exacerbations of SLE. Levels of sCD4 and sCD8, however, are not related to levels of sIL-2R, and do not reflect B-cell activation, nor disease activity during exacerbations of SLE. Thus for the clinical follow up of SLE measurement of levels of sCD4 or sCD8 is of limited value.


Systemic lupus erythematosus (SLE) is characterised by the production of a wide range of autoantibodies. Among these, antibodies to double stranded DNA (anti-dsDNA) and antibodies to the so called Sm-antigen are considered most specific for the disease. Anti-dsDNA antibodies fluctuate with disease activity, can be detected at high levels during active disease, and are thought to play an important role in the pathogenesis of SLE by the formation of immune-complexes that are involved in the development of tissue injury. The rise in levels of anti-dsDNA, in the period before clinical exacerbations of the disease, exceeds by far the rise in levels of total IgG. This suggests a selective B-cell proliferation, possibly T-cell dependent and antigen-driven. Involvement of T-cells, either providing help for B-cells or acting as effector cells, is also suggested by increased expression of activation markers on both CD4+ and CD8+ peripheral blood lymphocytes in non-active SLE. Furthermore, T-lymphocytes isolated from broncho-alveolar lavage fluid of patients with SLE are activated. T-cells also play an important role in glomerulonephritis in lupus prone mice. During T-cell activation soluble IL-2 receptor (sIL-2R) is released. Levels of this molecule have been found increased in patients with SLE. Recently, it has been shown that soluble markers of lymphocyte activation other than sIL-2R, that is, soluble CD4 (sCD4) and soluble CD8 (sCD8), can be detected in plasma. Levels of these molecules have been found increased in patients with Kawasaki disease, rheumatoid arthritis (RA), systemic sclerosis, Wegener’s granulomatosis, and SLE. To assess serial activation of T-cell subsets in relation to autoantibody production and clinical disease expression, consecutive plasma samples of SLE patients before, and during disease exacerbations were analysed for levels of sIL-2R, sCD4 and sCD8, and these values were related to levels of total IgM, total IgG, and anti-dsDNA.

Patients and methods

PATIENTS
This study concerns a cohort of 71 outpatients (59 females, 12 males) fulfilling the 1982 revised ARA criteria for the diagnosis of SLE who participate in longterm clinical follow up according to a protocol. All 71 patients are seen at the clinic at least once every three months, and more frequently if clinical symptoms suggest disease activity. A validated disease activity index is calculated at every outpatient visit from signs and symptoms and results of laboratory tests (table 1). Doses of prednisolone are recorded. Blood samples are
Table 1  SLE disease activity index

<table>
<thead>
<tr>
<th>Kidneys</th>
<th>points**</th>
</tr>
</thead>
<tbody>
<tr>
<td>• proteinuria: newly developed (&gt;0.5 g/day) or doubling within 4 months (basal proteinuria &gt;0.5 g/day)</td>
<td>2</td>
</tr>
<tr>
<td>• erythrocyturia: newly developed (&gt;5 ery/hpf) or doubling</td>
<td>1</td>
</tr>
<tr>
<td>• erythrocye and/or granular casts: presence newly developed</td>
<td>2</td>
</tr>
<tr>
<td>• creatinine clearance: decrease of &gt;25% (within 4 months)</td>
<td>2</td>
</tr>
</tbody>
</table>
| Major exacerbation: erythematosus* | | }

Central nervous system*

• cerebral vascular accident 4
• seizure 4
• psychosis 4
• chorea 4
• transverse myelitis 4
• motoric nerve palsy 4

Skin/mucosa

• alopecia* 1
• active discoid rash 2
• malar rash 2
• other active rash 2
• active oral and/or nasal ulcerations 2

Blood

• haemolytic anaemia: Hb <100 g/l | 1 |
• Hb <80 g/l | 2 |
• leucopenia: <4 × 10^9/l | 3 |
• lymphopenia: <10 × 10^9/l | 1 |
• thrombocytopenia: <100 × 10^9/l | 2 |
• <50 × 10^9/l | 2 |
• <25 × 10^9/l | 3 |

Musculoskeletal system

• arthralgia and/or myalgia* 1
• arthritis (>2 joints) and/or tendinitis 1

Serena

• pleural and/or pericardial pain* 1
• pleural and/or pericardial rub | 2 |
• abnormalities on chest x-ray, EKG, and/or echocardiogram | 2 |

Vessels

• minor vascularitis (pupura, perianginal infarction) | 2 |
• major vascularitis (ulcerations, mononeuritis) | 4 |

Miscellaneous

• uveitis and/or chorireetinitis | 3 |
• myositis: rising CPK >150 U/l | 3 |
• rising CPK >500 U/l | 3 |

*only scored when occurring within two weeks of the time of the outpatient visit or admission under consideration.
**when an item is related, possibly to medication or an unrelated condition, no points are given.

Table 2  Criteria for major and minor disease exacerbations in systemic lupus erythematosus

Major exacerbation: fulfilling one or more of the following*!
1  Severe renal disease;
   a) recent renal biopsy showing active proliferative lupus nephritis (>50% of glomeruli affected), and/or
   b) decrease of creatinine clearance of >25% within 4 months, accompanied by an active sediment (>5 ery/hpf, and/or casts) and by proteinuria of >0.5 g/day
2  Severe central nervous system disease;
   Seizures, cerebral vascular accidental, coma, transverse myelitis, psychosis, chorea, arthralgia, central nerve palsy
3  Haemorheological disease;
   haemolytic anaemia (Hb <60 g/l) and/or thrombocytopenia (<50 × 10^9/l)
4  Severe serositis;
   pericarditis with (impending) tamponade and/or massive pleural effusion
5  Uveitis and/or retinitis
6  Myocarditis with arrhythmia and/or congestive heart failure
7  Severe myositis with proximal muscle weakness
8  Lung involvement with hemoptysis
9  Major vasculitis;
   with ulcerations and/or mononeuritis multiplex
10 Miscellaneous;
   fever (>38°C rectally), serositis, haemolytic anaemia (>60 g/l), or thrombocytopenia (>50 × 10^9/l), all without improvement after prednisolone in a maximum dosage of 30 mg/day for at least one week

Minor exacerbation: fulfilling all of the following items
1  increase of activity index by >2 points within 6 months, with a minimal activity index of 3 points, accompanied by:
2  the clinically judged necessity to start prednisolone at a dose of at least 10 mg/day, or to increase the prednisolone dose with ≥5 mg/day, or to start with animalarials, or immuno-suppressive drugs, and;
3  not fulfilling the criteria for a major exacerbation

*only features present within 2 weeks of the moment of the outpatient clinic or the admission under consideration are taken into account.

For this study the first 16 consecutive exacerbations (6 major and 10 minor) in different individuals (14 women and 2 men) were evaluated. In this group of patients the mean age at the start of the study was 31.2 years (range 21–47). SLE was diagnosed at a mean of 4.9 years (range 0–25) before the start of the study.

METHODS

Monthly plasma samples from four months before, and at the moment of maximum disease activity, were used for analysis of levels of sIL-2R, sCD4, sCD8, total IgM and IgG, and anti-dsDNA. Levels of sIL-2R, sCD4, and sCD8 were measured by ‘sandwich’ ELISA technique according to the manufacturer (T-cell Sciences, Cambridge, USA). In brief, microtitre plates were coated with a murine monoclonal IgG antibody recognising one epitope on human sIL-2R, sCD4, and sCD8, respectively. After incubation of patient plasma or standards, a horseradish peroxidase conjugated murine monoclonal IgG antibody recognising a second epitope on sIL-2R, sCD4, and sCD8, respectively, was added. After colour reaction, the plates were read at 490 nm. Normal value of sIL-2R levels in our laboratory is below 517 arbitrary units/ml (U/ml). Normal values of sCD4 and sCD8, according to the manufacturer, are below 48 U/ml and 435 U/ml, respectively. Levels of total IgM and IgG were measured by nephelometry (Behring). Anti-dsDNA antibody levels were determined by Farr assay (Diagnostic Products Corporation, Los Angeles, USA), using 125I-labelled recombinant ds-DNA which is free from contamination with ss-DNA. Farr assay was performed according to the manufacturer’s instruction and positive samples were measured at different dilutions to obtain measurements within the range of the assay. C1q does not interfere with this assay. Results of this assay were expressed in IU/ml, using Wo/80 as the ultimate standard. Normal value of this Farr assay in our laboratory is ≤26 IU/ml.

STATISTICS

Analysis was carried out using the SYSTAT statistical package. Differences in parameters between groups were evaluated with the paired t test. Pearson’s test was applied for detecting correlations between different study parameters. If normal distribution could not be assumed, Wilcoxon’s paired rank-sum test and Spearman’s test were applied respectively. A p value <0.05 (two-sided) was considered significant.

Results

Clinical characteristics of the 16 exacerbations that are analysed in this study are shown in table 3. Only exacerbations without evidence of an infection in the study period before these exacerbations are included. At the time of

The table shows the criteria for major and minor disease exacerbations in systemic lupus erythematosus. The main criteria include severe renal disease, severe central nervous system disease, haemorheological disease, severe serositis, uveitis and retinitis, myocarditis with arrhythmia or congestive heart failure, severe myositis with proximal muscle weakness, lung involvement with hemoptysis, major vasculitis with ulceration and/or mononeuritis multiplex, and miscellaneous symptoms such as fever, serositis, haemolytic anaemia, and thrombocytopenia. Minor exacerbations are defined as a change in activity index by more than 2 points within 6 months, accompanied by clinical judgment to start prednisolone at a dose of at least 10 mg/day, or to increase the prednisolone dose by 5 mg/day, or to start with immunosuppressive drugs.

The data were analyzed using SYSTAT statistical software. Differences between groups were evaluated using the paired t test. Pearson’s correlation was used to detect correlations between different study parameters. Normal distribution was assumed for the results, and Wilcoxon’s paired rank-sum test and Spearman’s test were applied respectively. A p value of less than 0.05 (two-sided) was considered significant.

Results

Clinical characteristics of the 16 exacerbations that are analyzed in this study are shown in Table 3. Only exacerbations without evidence of an infection in the study period before these exacerbations are included. At the time of the exacerbation, patients were evaluated for their symptoms and signs, including possible viral or bacterial infections.
Changes in levels of soluble T-cell activation markers, sIL-2R, sCD4 and sCD8 in exacerbations in SLE

Table 3  Characteristics of disease exacerbations in 16 patients with systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Type of exacerbation</th>
<th>Disease activity score</th>
<th>Renal involvement</th>
<th>Central nervous system involvement</th>
<th>Skin/mucosal involvement</th>
<th>Haematological abnormalities</th>
<th>Musculoskeletal involvement</th>
<th>Serositis</th>
<th>Vasculitis</th>
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<tbody>
<tr>
<td>Major</td>
<td>7</td>
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<td>Minor</td>
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*Disease activity index and type of exacerbation as described previously.*

the exacerbation, before immunosuppressive therapy was started, 10 of the 16 patients used prednisolone, although in low doses (median 5.6 mg/day, range 0–22.5 mg/day). No correlation was found between levels of T-cell activation markers and the dose of prednisolone therapy.

Levels of sIL-2R at four months before the exacerbation (median 725, range 235–3800 U/ml) were higher than in normal controls (median 368, range 200–660 U/ml, p < 0.001), and rose significantly during the study period up to the moment of maximal disease activity (median 1100, range 425–2375 U/ml, p < 0.01). In contrast, levels of sCD4 and sCD8 did not change significantly in this period. The rise in levels of sIL-2R was mainly due to changes during major exacerbations (n = 6). Levels of sIL-2R (median 1037, range 425–2025 U/ml) had significantly increased compared with four months before these exacerbations (median 348, range 20–800 U/ml) in all six cases (p < 0.001, fig 1). In three cases this rise was paralleled by a rise in sCD4 levels, although the normal range of sCD4 levels was exceeded in only one case. No pattern could be detected in the change of sCD8 levels in relation to those exacerbations, although levels of sCD8 were raised in the patients both before, and at the time of

Figure 1  Changes in levels of soluble interleukin-2 receptor (sIL-2R, U/ml) before major exacerbations in six patients with SLE. Shaded area represents the normal range. The x-axis represents the time points before the exacerbations (t = 0).

Figure 2  Changes in levels of soluble CD4 (sCD4, U/ml) (left side) and soluble CD8 (sCD8, U/ml) (right side) before minor exacerbations in 10 patients with SLE. Dotted lines indicate the upper limit of normal values of sCD4 (48 U/ml) and sCD8 (435 U/ml). H indicates the difference between P1 and P3, and P2,1–5H, respectively. *denotes a value higher than the upper hinge, but lower than P1; + 3H. O denotes a value higher than P1; + 3H. The x-axis represents the time points before the exacerbation (t = 0).
exacerbations. During minor exacerbations (n = 10), levels of sIL-2R did not change significantly. Levels of sCD4 tended to be lower (median 7, range 2–39 U/ml) at the time of those exacerbations than at four months before the exacerbation (median 20, range 4–58 U/ml, p < 0.1) although levels remained predominantly in the normal range (fig 2A). In contrast, levels of sCD8 tended to be higher at the time of minor exacerbations (median 425, range 32–1563 U/ml) compared with four months before those exacerbations (mean 506, range 30–1322 U/ml, p < 0.1), and were predominantly above the range of normals (figure 2B). Fluctuations of levels of sCD4, sCD8, and sIL-2R, including the rise in sIL-2R observed in relation to major exacerbations, could not be explained by impaired renal function (data not shown).

T cell activation markers in relation to levels of immunoglobulins and anti-dsDNA
Levels of sCD4 and sCD8 were not related to levels of total IgG, total IgM, or anti-dsDNA. Changes in levels of sCD4 and sCD8 were not interrelated. Levels of sIL-2R rose significantly, predominantly in relation to major exacerbations of the disease, but were not related to changes in levels of total IgG, total IgM, or anti-dsDNA. Rises in levels of anti-dsDNA were detected in relation to both major and minor disease exacerbations. Levels of anti-dsDNA were higher at the time of the exacerbation (median 395, range 11–1500 IU/ml) than at four months before the exacerbation (median 113, range 46–730 IU/ml, p < 0.02), correlated with levels of total IgG (p < 0.02), and rose every month with the exception of the one month period preceding the exacerbation.

Disease activity and type exacerbation
Although levels of T-cell activation markers changed over time before disease exacerbations, these changes were not consistent. Consequently, changes in levels of those markers did not interrelate nor correlate with the score of the disease activity index. Patterns of changes in levels of T-cell activation markers over time were not related to the organ systems involved during the exacerbations.

Discussion
SLE is characterised by persistent B cell activation resulting in the presence of a wide range of autoantibodies. Their production might be T-cell dependent. In addition, T-cells might be involved in the effector phase of the immune response. To gain more insight in the T-cell subsets involved, we measured soluble T-cell activation markers during exacerbations in SLE. To our knowledge, this is the first study that reports changes in levels of sCD4 and sCD8 over time before lupus disease exacerbations. For the total group of exacerbations levels of sIL-2R rose significantly, whereas no significant changes could be observed for levels of sCD4 or sCD8. No relation in time between soluble T-cell activation markers of B-cell activation, that is, total IgM and IgG, and anti-dsDNA, could be demonstrated. Although rises in levels of both sIL-2R and anti-dsDNA were detected in relation to the exacerbations studied, the rises in levels of sIL-2R occurred predominantly during major exacerbations, whereas rises in anti-dsDNA levels were detected in both minor and major disease exacerbations.

Levels of anti-dsDNA rise significantly before exacerbations in most cases of SLE. This rise exceeds by far the rise in total IgG levels, thus suggesting a selective T-cell dependent B-cell activation. The present findings do not support the assumption that B-cell activation occurs in dependence of T-cell activation, as rises of sIL-2R levels were not related to changes in levels of anti-dsDNA and levels of sCD4 remained predominantly in the normal range. The increased levels of sIL-2R in SLE, also during periods without clinical disease activity, suggest T-cell activity. T-cell involvement is further suggested by the finding that both CD4⁺ and CD8⁺ peripheral blood lymphocytes are already activated in clinically quiescent SLE. Activated CD4⁺ lymphocytes have also been found in bronchoalveolar lavage fluid in SLE. The presence of activated circulating CD4⁺ cells, however, reflected in increased levels of sCD1. Levels of sCD8 were increased before and at the time of the exacerbation, suggesting the involvement of CD8⁺ T-cells. This is supported by findings of others reporting increased levels of sCD8 during active SLE in cross-sectional studies. In the present study, a tendency towards rising sCD8 levels was observed in relation to minor exacerbations only, while no significant changes occurred during major exacerbations. Levels of sCD4 and sCD8 are in the normal range, and do not change during a quiescent phase of SLE (manuscript in preparation), suggesting that the changes observed are indeed related to changes in disease activity. Carpenter et al described normal serum levels of sCD8 in active rheumatoid arthritis patients, while synovial fluid levels of this molecule were significantly increased. This might be due to changes in the renal clearance of sCD8, as has been reported for sIL-2R. In our study, renal impairment could not explain the lack of change in sCD8 levels.

In conclusion, levels of sIL-2R are increased, and rise before major exacerbations of SLE. Levels of sCD4 remain predominately in the normal range, whereas levels of sCD8 are frequently elevated. Levels of sCD4 and sCD8, however, are not related to levels of sIL-2R, and do not reflect B-cell activation as assessed by levels of total IgM, IgG, and anti-dsDNA, nor disease activity during exacerbations of SLE. Thus for the clinical follow up of SLE measurement of levels of sCD4 or sCD8 is of limited value.

This study was financed by the Dutch League against Rheumatism (grant 90/296). We are indebted to Dr V Fidler for valuable discussions concerning statistical analysis.
Changes in levels of soluble T-cell activation markers, sIL-2R, sCD4 and sCD8 in exacerbations in SLE

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