A long term immunological study of childhood onset systemic lupus erythematosus

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Abstract

Immunological dysregulation is an important cause of the development of systemic lupus erythematosus (SLE). Serological evaluation has been useful in the clinical management of patients and as a prognostic indicator.

Sixteen patients who developed SLE as children were followed up for more than three years and immunological data collected. The results showed that (a) complement C3 concentration was lower in the active stage of SLE, especially during a major clinical exacerbation, but rarely preceded a major flare up. The concentration was often normal during the mildly to moderately active stage. In contrast, a low complement C4 concentration often preceded a major clinical exacerbation and could be of longer duration, sometimes persisting regardless of disease activity. (b) A T cell subset distribution study showed persistently low CD4 positive T cells in the peripheral blood of patients with SLE during the long term follow up, strongly suggesting that the intrinsic defect is mainly localised in T helper/inducer cells. These abnormal cellular defects did not tend to return to normal even in long term remission. (c) The persistently higher serum interleukin 2 and interleukin 2 receptor concentrations in SLE strongly suggested that the T cells were preactivated in vivo and that these phenomena might persist even in remission. (d) The best single parameter for predicting active SLE was anti-dsDNA. It was highly correlated with disease activity in most patients, and the asymptomatic increase of anti-dsDNA (≥60 U/ml, radioimmunoassay) was often followed by a major clinical exacerbation, especially in patients with a simultaneously low complement C4 concentration, suggesting that it might be an important warning sign of a major flare up. High dose steroids are indicated in this group of patients.

Patients and methods

Sixteen patients (13 female, three male), who met the 1982 American Rheumatism Association revised criteria for SLE and had an age of disease onset below 15, were enrolled in this study. Their age at enrolment ranged from 12 to 27 years (mean 15·5). Blood samples were taken every one to three months from each patient, and disease activity, indicated by the LACC, recorded prospectively from the serological data. During the long term follow up an evaluation was made of the serum complement components C3 and C4, antinuclear factor, anti-dsDNA, the T cell subpopulation distribution in peripheral blood mononuclear cells, serum interleukin 2 and interleukin 2 receptor, and in vitro interleukin 2 production by phytohaemagglutinin stimulated cultures of peripheral blood mononuclear cells.

Systemic lupus erythematosus (SLE) is a well known autoimmune disorder. Its pathogenesis, studied extensively, shows multiple immune dysregulation, which includes defective lymphokine production (interleukins 1 and 2) and decreased interleukin 2 responsiveness of T cells, defective antigen presenting cell-like macrophages and monocytes, increased spontaneous B cell activation and autoantibody production, decreased suppressor T cell function and defective serum immune complex clearance, and decreased CR1 in red blood cells. Although the anti-dsDNA-DNA complex may have an important role in the pathogenesis of SLE, there are conflicting data about the relation between anti-dsDNA level and disease activity. It is generally agreed that patients with no symptoms should not be given special treatment, regardless of their abnormal serological data. Until the present, however, no single serological parameter has been able accurately to predict the disease activity. Although several scoring systems have been proposed for SLE activity, the lupus activity criteria count (LACC) system was used in this study because of its high sensitivity and specificity. There are few reports of long term immunological data in SLE. At the rheumatology clinic, paediatric department of the National Taiwan University Hospital we analysed data from 16 patients with SLE over a period of at least three years. Multiple blood samples were taken at both active and inactive stages of the disease in an attempt to obtain a long term immunological profile of patients with childhood onset SLE.

Preparation of plasma and culture supernatants

Heparinised whole blood was drawn from each patient. After centrifugation, plasma was collected and stored at −70°C. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation. After washing three times with RPMI-1640 (Gibco, NY, USA) mononuclear cells were adjusted to a concentration of 2×10⁶ cells/ml in complete culture medium (RPMI-1640 supplemented with 10% heat inactivated fetal calf serum, 100 U/ml,
streptomyein 100 µg/ml, and 2 mM L-glutamine) containing phytohaemagglutinin (1%). Cultures were done in a humidified 5% CO₂ incubator at 37°C, and the supernatants containing interleukin 2 were harvested at 24 hours and stored at −70°C.

T cell subset distributions were enumerated by indirect immunofluorescence using OKT3, OKT4, OKT8, and OKIal monoclonal antibodies purchased from Orthoclone (Raritan, NJ, USA). In some patients interleukin 2 receptor expression of mononuclear cells was also determined using a monoclonal antibody to Tac.

MEASUREMENT OF INTERLEUKIN 2 IN PLASMA
Interleukin 2 in plasma was determined with a human interleukin 2 enzyme linked immunosorbent assay (ELISA) test kit (Genzyme, Boston, MA, USA). The principle of the method was a solid phase enzyme immunoassay based on the 'dual antibody immunometric sandwich' reported by Ferrua et al.,18 using two distinct antibodies to interleukin 2 derived from two different species. The first antibody, mouse antibody specific to interleukin 2, was coated on to a polystyrene 96 well immunoplate, and the wells were incubated with samples and appropriate standards. Interleukin 2 present in the test sample was 'captured' and bound to the solid phase. Unbound material was removed by washing. Next, the rabbit polyvalent antibody to human interleukin 2 was incubated in the wells and bound to the captured interleukin 2 at multiple epitopes. Unbound second antibody was removed by washing. A third enzyme labelled goat antirabbit antibody (conjugated with alkaline phosphatase) was added to the wells. Unbound labelled antibody was removed by washing, and a substrate, p-nitrophenyl phosphate, was then added to each well. The bound specific antibody to interleukin 2 was measured by an enzymatic reaction resulting in a detectable colour change with an ELISA reader at an absorbance of 405 nm. A standard curve was constructed using standards of known concentration provided by the manufacturer. The interleukin 2 concentrations of tested samples and controls were then determined from the standard curve.

MEASUREMENT OF INTERLEUKIN 2 RECEPTOR
INTERLEUKIN 2 RECEPTOR IN PLASMA
Interleukin 2 receptor concentration in plasma was determined with a cell free interleukin 2 receptor test kit (T Cell Sciences, Cambridge, MA, USA). The principle of the method was also a sandwich enzyme immunoassay, based on the report of Rubin et al.,19 using two murine monoclonal antibodies to interleukin 2 receptor, which recognised different epitopes of the interleukin 2 receptor molecule. The first antibody to interleukin 2 receptor was absorbed on to a polystyrene 96 well microtitre plate. Interleukin 2 receptor present in the samples or standards bound to the antibody coated wells; unreacted sample components were removed by washing. A horseradish peroxidase conjugated second monoclonal antibody to interleukin 2 receptor was then added, which bound to the interleukin 2 receptor captured by the first antibody and completed the sandwich reaction. After removal of unbound horseradish peroxidase conjugated anti-interleukin 2 receptor by washing a substrate (o-phenylenediamine) solution was added to the wells. A coloured product was formed in proportion to the amount of interleukin 2 receptor present in the sample. The reaction was stopped with 1 M sulphuric acid and absorbance at 490 nm was measured. A standard curve was prepared from four interleukin 2 receptor standards. Unknown values were calculated from the standrd curve.

MEASUREMENT OF INTERLEUKIN 2 IN CULTURE SUPERNATANTS
Interleukin 2 in culture supernatants was determined by a standard bioassy method using an interleukin 2 dependent murine cytotoxic T cell line, as reported by Gillis et al.,20 results were double checked with an interleukin 2 ELISA test kit (Genzyme).

Anti-dsDNA was measured by a radioimmunoassay test kit (Amersham, UK) and C3 and C4 concentrations by rate nephelometry with C3 and C4 test kits (Beckman, Bera, CA, USA).

STATISTICAL METHODS
Data were analysed by a weighted mean, Wilcoxon signed rank test as a transformed rank test,23 Mann–Whitney U test, linear regression analysis, ϕ coefficient test, and χ² test.

Results
The 16 patients studied visited the clinic often and were available for serial follow up, and thus, possibly, were not completely representative of those with SLE in the general population; this was unavoidable in carrying out a long term serial immunological study. Sequential blood samples were taken every one to three months and disease activity for each patient noted. Eighty two blood samples were taken during the three years—43 from patients with inactive disease and 39 from those with active SLE. More serum samples were taken from some patients than others, and thus a weighted mean was used to analyse changes of the immune parameters. Wilcoxon's signed rank test was used to compare patients with active and inactive disease and the Mann–Whitney U test to compare patients with normal controls. Table 1 gives details of the patients. Most patients had renal disease of varying duration and severity. Mean age of onset was 12 years; follow up period ranged from three to 16 years.

SEROLOGICAL PARAMETERS
Table 2 shows that anti-dsDNA in patients with active SLE was significantly higher than in the group with inactive disease (p<0.05, Wilcoxon signed rank test). The antinuclear antibody titre
was also significantly higher in the active group. Both C3 and C4 concentrations were lower in active than in inactive serum samples, but neither was significantly low when compared with normal controls (Mann–Whitney U test). The data also showed that most patients with childhood onset SLE had a low concentration of C4 regardless of disease activity. Frequency analysis (data not shown) showed that the cumulative frequency of C3 below 600 mg/l (mean±2SD) in the groups with active and inactive disease was 23-1% and 14-3% respectively. For a C4 concentration below 200 mg/l (mean±2SD) the cumulative frequency was 55-2% and 41-8% in those with active and inactive disease respectively.

**T cell subset distribution**

Table 2 shows the distribution of T cell subsets. For both active and inactive status the weighted mean percentages of CD4+ T cells were lower than those for normal controls but those for CD8+ T cells were higher than for controls; the CD4/CD8 ratio showed the reverse pattern (<1-0), which was statistically different from the normal distribution. T cell subset distribution was similar in those with active and inactive disease. The long term follow up of CD4+ and CD8+ T cell subset distribution (fig 1) confirmed that for most cases (n=15) there were fewer CD4+ T cells (mean±2SD) 31±9 (10%) during the study, regardless of disease activity, whereas, in contrast, the level of CD8+ T cells was higher (36±7 (10%) during most of the disease course.

![Sequential follow-up of CD4+ cells](http://ard.bmj.com/)

**Figure 1** The distribution of CD4+ in peripheral blood mononuclear cells of patients with systemic lupus erythematosus was sequentially enumerated every one to three months during the long term follow up, which included both active and inactive stages. Each curve represents one patient's long term follow up data. The mean (SD) percentage of CD4+ T cells in normal controls was 41 (6%). The percentage of CD4+ T cells was less than 35% at most time points.

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**Table 1 Details of the patients with systemic lupus erythematosus**

<table>
<thead>
<tr>
<th>Case No</th>
<th>Sex</th>
<th>Age of onset (years)</th>
<th>Present age (years)</th>
<th>Follow up period (years)</th>
<th>Renal disease</th>
<th>Carditis history</th>
<th>Pleurisy history</th>
<th>Seizure history</th>
<th>Hypertension</th>
<th>Gastro-intestinal bleeding</th>
<th>Associated syndrome</th>
<th>Present status</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>12</td>
<td>17</td>
<td>4</td>
<td>++/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Polyarteritis</td>
<td>Remission</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>11</td>
<td>15</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Low C4</td>
<td>Died</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>14</td>
<td>17</td>
<td>13</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Mitral R</td>
<td>Died</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>6</td>
<td>Died 1985</td>
<td>14</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Died of CHF*</td>
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</tr>
<tr>
<td>5</td>
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<td>14</td>
<td>13</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Died of RF*</td>
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</tr>
<tr>
<td>6</td>
<td>F</td>
<td>13</td>
<td>18</td>
<td>12</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Heavy proteinuria</td>
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</tr>
<tr>
<td>7</td>
<td>F</td>
<td>14</td>
<td>30</td>
<td>16</td>
<td>++/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Heavy proteinuria</td>
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</tr>
<tr>
<td>8</td>
<td>F</td>
<td>13</td>
<td>19</td>
<td>6</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Remission and married</td>
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</tr>
<tr>
<td>9</td>
<td>F</td>
<td>13</td>
<td>26</td>
<td>13</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Complete remission</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>13</td>
<td>19</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Remission</td>
<td></td>
</tr>
<tr>
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<td>M</td>
<td>9</td>
<td>14</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Died of CNS haemorrhage</td>
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<td>19</td>
<td>6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Heavy proteinuria</td>
<td></td>
</tr>
<tr>
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<td>F</td>
<td>9</td>
<td>13</td>
<td>3</td>
<td>++/</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IDDMM*+</td>
<td>Low PL*</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>8</td>
<td>20</td>
<td>12</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Low PL</td>
<td>Hypertension and RF</td>
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<tr>
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<td>F</td>
<td>15</td>
<td>21</td>
<td>6</td>
<td>+++</td>
<td>PE+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>RF</td>
<td></td>
</tr>
<tr>
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<td>F</td>
<td>12</td>
<td>15</td>
<td>3</td>
<td>+++</td>
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<td>PE+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PE=pericardial effusion; RF=Raynaud's phenomenon; IDDMM=insulin dependent diabetes mellitus; PL=platelets; CHF=congestive heart failure; RF=renal failure; CNS=central nervous system.

**Table 2 Weighted means of various immunological parameters in patients with active and inactive systemic lupus erythematosus during long term follow up. Values are given as means (SEM)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Weighted mean Active</th>
<th>Wilcoxon p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 (%)</td>
<td>70 (2.5)</td>
<td>0.51</td>
</tr>
<tr>
<td>CD4 (%)</td>
<td>31 (1.2)</td>
<td>0.13</td>
</tr>
<tr>
<td>CD8 (%)</td>
<td>39 (1.6)</td>
<td>0.75</td>
</tr>
<tr>
<td>C1q (%)</td>
<td>17 (7)</td>
<td>0.05</td>
</tr>
<tr>
<td>C3 (mg/l)</td>
<td>890 (43)</td>
<td>2.4</td>
</tr>
<tr>
<td>C4 (mg/l)</td>
<td>220 (8)</td>
<td>4.0</td>
</tr>
<tr>
<td>Anti-dsDNA (U/ml)</td>
<td>418 (71)</td>
<td>0.93</td>
</tr>
<tr>
<td>Serum IL2 (U/ml)</td>
<td>33 (5.2)</td>
<td>1.29</td>
</tr>
<tr>
<td>Serum IL2R (U/ml)</td>
<td>758 (37)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mitogen response (cpm)</td>
<td>5697 (470)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Wilcoxon signed rank test as a rank transformation test.
†ANA=antinuclear antibody; IL2=interleukin 2; IL2R=interleukin 2 receptor.
STEROID EFFECT ON T CELL SUBSET DISTRIBUTION
To understand further the reason for the persistently lower level of CD4+ T cells and higher level of CD8+ T cells during the long term follow up in most of the patients with childhood onset SLE the effect of steroids was examined. In both active and inactive disease groups there was no difference in the percentage of CD4+ T cells between patients taking a low dose (<10 mg/day) of prednisolone or none at all and those taking a higher dose (≥10 mg/day) (table 3).

SERUM INTERLEUKIN 2 AND INTERLEUKIN 2 RECEPTOR CONCENTRATIONS
Table 2 shows that the weighted mean (SEM) serum interleukin 2 concentrations in the active (20.8 (1.7) U/ml) and inactive stages (33 (5.2) U/ml) were higher than that of normal subjects (1.0 (0.5) U/ml, n=35); the weighted mean serum interleukin 2 receptor concentrations in both active (371 (77) U/ml) and inactive (558 (47) U/ml) disease were also higher than that of normal subjects (300 (89) U/ml, n=23). There was no difference, however, in serum interleukin 2 concentrations between the groups with active and inactive disease. In contrast with the serum data, no interleukin 2 was detected in culture supernatants from patients with active or inactive disease (<0.05 U/ml), whereas a small amount of interleukin 2 was detected in normal subjects (2.0 (0.5) U/ml). Figure 2 shows the changes of serum interleukin 2 concentration in eight patients with SLE. There were marked individual variations, and some patients had a persistently high serum interleukin 2 concentration even after long term clinical remission.

SENSITIVITY, SPECIFICITY, AND PREDICTIVE VALUE OF PARAMETERS FOR ACTIVE SLE
The four most commonly used serological parameters (C3, C4, anti-dsDNA, antinuclear antibody) were analysed further to evaluate their sensitivity, specificity, and predictive value for SLE disease activity. The result (data not shown) showed that anti-dsDNA (≥30 U/ml) was the most sensitive and most useful parameter for detecting active SLE (LACC ≥2); a low C3 concentration (<600 mg/l) was the least sensitive marker for active SLE, but it showed the highest specificity.

An attempt was made to define the level of anti-dsDNA showing the highest predictive value, sensitivity, and specificity for active SLE. It was found that although a cut off point of ≥60 U/ml gave the highest specificity and predictive value for active SLE, the sensitivity was too low (0.48). If the cut off point was set at ≥30 U/ml, however, the sensitivity was increased significantly, and the predictive value and specificity were still acceptable. Thus an anti-dsDNA value of ≥30 U/ml was most suitable for managing SLE.

RELATION BETWEEN SLE AND VARIOUS IMMUNOLOGICAL PARAMETERS
Table 4 analyses the relation between a number of laboratory parameters and disease activity during the long term follow up. The results

![Figure 2](http://ard.bmj.com/) Long term follow-up of serum interleukin 2 in patients with systemic lupus erythematosus. The numerals below the x axis represent the sequence of blood sampling carried out every one to three months. The * indicates those patients who died during the follow up.

Table 3  Steroid effect on T cell subset distribution both in patients with active and inactive systemic lupus erythematosus. Values are given as means (SD)

<table>
<thead>
<tr>
<th></th>
<th>CD3 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active*</td>
<td>A (n=4)</td>
<td>66.5 (8.8)</td>
<td>30.3 (11.9)</td>
</tr>
<tr>
<td></td>
<td>B (n=27)</td>
<td>64.4 (8.2)</td>
<td>32.1 (10.5)</td>
</tr>
<tr>
<td>Inactive*</td>
<td>A (n=15)</td>
<td>64.7 (9.8)</td>
<td>29.1 (12.8)</td>
</tr>
<tr>
<td></td>
<td>B (n=21)</td>
<td>68.9 (16.2)</td>
<td>31.5 (11.6)</td>
</tr>
<tr>
<td>Normal (n=30)</td>
<td>69.0 (7.7)</td>
<td>41.0 (6.0)</td>
<td>24.0 (6.1)</td>
</tr>
</tbody>
</table>

*Active*=lupus activity criteria count (LACC) ≥2; inactive=LACC <2.
†A=prednisolone <10 mg/day; B=prednisolone ≥10 mg/day. The numbers of CD4 cells both in patients with active and inactive disease were lower than those of normal controls, whereas the numbers of CD8 cells were higher than in normal subjects.

Table 4  Linear regression analysis between systemic lupus erythematosus activity and various immunological parameters

<table>
<thead>
<tr>
<th>Serum IL-2*</th>
<th>Serum IL-2R*</th>
<th>Anti-dsDNA (RIA*, U/ml)</th>
<th>Antinuclear antibody (HEP–2)</th>
<th>C3</th>
<th>C4</th>
<th>T cell subsets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4</td>
</tr>
<tr>
<td>t</td>
<td>-2.4</td>
<td>-0.04</td>
<td>4.6</td>
<td>0.13</td>
<td>-3.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>25</td>
<td>25</td>
<td>0.001</td>
<td>0.89</td>
<td>0.003</td>
</tr>
<tr>
<td>p</td>
<td>0.02</td>
<td>0.97</td>
<td>0.0001</td>
<td>0.89</td>
<td>0.003</td>
<td>0.16</td>
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</table>

*IL–2=interleukin 2; IL–2R=interleukin 2 receptor; RIA=radioimmunoassay.
showed that anti-dsDNA had the best linear
correlation with the LACC (n=25, t=4.6, p<0.0001). Both the serum interleukin 2 and C3 concentrations had a negative correlation with the LACC (p<0.02 and p<0.003 respectively). Antinuclear antibody and serum interleukin 2 receptor showed the least correlation with SLE disease activity during the long term follow up. There was no correlation between serum interleukin 2 and interleukin 2 receptor (data not shown).

Discussion

Serum complement concentrations are often used as prognostic indicators and diagnostic aids in patients with SLE.\textsuperscript{16-22} Data analysis showed that a low C3 concentration had high specificity but low sensitivity as a marker for the active status of SLE. Whether renal disease was present or not, only nine (23.1%) blood samples taken during the active stage (n=39) had a low C3 concentration (below 600 mg/l). Variation in the degree of disease activity (from mild to severe exacerbation) might be the reason for the low percentage of samples with a depressed C3 concentration in patients with active SLE. In most individual long term observations the C3 concentration fell only close to the time of a major clinical exacerbation and with proper treatment returned to a higher concentration in weeks or months; in most cases the fall in C3 did not precede the major exacerbation. In contrast, as noted in table 2, the C4 concentration was low even during the inactive stage of SLE (cumulative frequency of 41.8% for a C4 concentration below 200 mg/l). Thus C4 alone is not a good predictor for disease activity. Previous studies had shown that 71% of patients with a congenital deficiency of C4 developed SLE.\textsuperscript{26} In this study we measured the serum C4 concentration in family members of patients with persistently low C4 despite clinical remission, but we were unable to find congenital C4 deficiency in our series. In individual cases (fig 3) the C4 concentration may be continuously low for several months before a major clinical exacerbation; the low concentration may persist for one year or longer despite drug treatment, and even after clinical remission. Even in patients with no renal disease a persistently low C4 concentration was seen; its delayed recovery might be due to continued consumption and decreased production.\textsuperscript{29,30}

Cellular dysregulation and autoantibody overproduction have been extensively studied,\textsuperscript{2,4-5} but the exact pathogenesis is still unknown. Animal models have suggested a genetics based pathogenesis.\textsuperscript{31,32} Our study enabled observation of the distribution of T cell subpopulations during long term follow up. The results showed that most patients with childhood onset SLE had persistently lower CD4+ T cell levels and higher CD8+ T cell levels than normal controls (tables 2 and 3; fig 1), regardless of their disease activity and whether their steroid dose was high, low or even zero. Possibly, the persistently low number of helper/inducer T cells might be related to the intrinsic defect of CD4+ T cells, which did not tend to return to normal. On the other hand, although the CD8+ T cell numbers remained higher, they were not necessarily functionally normal, especially for regulation of autoantibody overproduction and B cell hyperactivity. Finally, linear regression analysis showed no significant correlation between T cell subset distributions and lupus activity. All this evidence supports the hypothesis that regulatory T cell imbalance does exist in patients with SLE but that enumeration of T cell subsets cannot be used as a clinical parameter.

Our patients with SLE also had higher serum interleukin 2 and interleukin 2 receptor concentrations than normal subjects, as shown by other studies,\textsuperscript{33-35} but these concentrations did not correlate with disease activity. Figure 2 shows the marked variation in serum interleukin 2 concentration in patients with SLE during long term follow up. Some patients had persistently high serum interleukin 2 concentration despite long term clinical remission (cases 1, 7, and 8), while two patients (cases 4 and 12) showed sequentially low serum interleukin 2 concentration during the last few months before death. Thus although linear regression analysis showed an inverse correlation between serum interleukin 2 and LACC, serum interleukin 2 cannot be used as a clinical predictor. Finally, the increased serum interleukin 2 concentration strongly suggests an in vivo activation of T or B cells, or both, in patients with SLE, or decreased activity of serum interleukin 2 inhibitors, as reported recently by Honda et al\textsuperscript{36} and Djeu et al.\textsuperscript{37} Decreased in vitro production and responsiveness to interleukin 2 of mononuclear cells from patients with SLE has been reported previously.\textsuperscript{1-3,38} The defect seemed to reside mainly in CD4+ cells.\textsuperscript{39-42} The lack of a relation between these functions and disease activity provides further evidence for the intrinsic defect of CD4+ T cells in patients with SLE. In this study the absence of interleukin 2 in the culture supernatants (table 2) and lack of responsiveness to interleukin 2 in two out of

Figure 3 Follow up of patient with systemic lupus erythematosus, aged 13 years. Serological tests were carried out every one to three months and their relation with drug dosage, disease activity (lupus activity criteria count, LACC), and clinical symptoms were depicted. It was found that high anti-dsDNA and low complement C4 concentrations preceded the major clinical flare up by one to four months.
### Table 5 Relation of the interval between asymptomatic increase of anti-dsDNA (≥60 U/ml) and clinical flare up with the dose of prednisolone used. Number of flare ups is shown.

<table>
<thead>
<tr>
<th>Interval (months)</th>
<th>Low dose steroid (&lt;0.3 mg/kg daily)</th>
<th>Medium dose steroid (0.3-0.6 mg/kg daily)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1-3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>≥1-3</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

ϕ Coefficient analysis showed the flare ups were significantly related to steroid dose. χ² Distribution t=6, p=0.025.

three cases tested (data not shown) were consistent with results of other investigators.1–3 39–42

The decreased in vitro interleukin 2 production in patients with SLE might be due to in vivo activation and thus exhaustion of T or B cells, or both.35

We found that anti-dsDNA is the most useful parameter for the management of patients with SLE, in agreement with other reports.10 11 43 but there are exceptions. For example, central nervous system lupus and certain haematological manifestations of SLE might have normal anti-dsDNA during clinical major flare ups, and some patients might have raised anti-dsDNA for months to a year but remain free from symptoms. Although during our study at least 10 patients continued to have a moderately raised anti-dsDNA level (≥60 U/ml) while remaining asymptomatic or with only minimal symptoms, like mild malar rash, mild proteinuria, or haematuria, it should be noted that most of the major clinical flare ups occurred in patients with persistently high anti-dsDNA (≥60 U/ml) who continued to take an insufficient dose of steroid (fig 3, table 5). Thus patients with SLE who have few or no symptoms may still have a major clinical flare up if the anti-dsDNA level is already moderately raised and no attempt is made to increase the drug dose. We thus recommend that all patients with SLE should have their serum anti-dsDNA and C4 concentrations determined every one to three months. Drugs should be adjusted if anti-dsDNA is higher than 60 U/ml, especially when serum C4 concentration is simultaneously low; management of disease in this situation is still controversial.13 44

Finally, as low platelet count with frank bleeding tendency is often found in patients with SLE who have no clinical and serological abnormalities, but who respond to steroid treatment, we recommend that thrombocyte count should be included as one of the lupus activity criteria.

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A long-term immunological study of childhood onset systemic lupus erythematosus.

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