Significance of low molecular weight C1q in systemic lupus erythematosus

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Abstract
The significance of high serum concentrations of low molecular weight C1q (LMW-C1q) in patients with systemic lupus erythematosus (SLE) was studied. Concentrations of LMW-C1q were increased in SLE, but not in rheumatoid arthritis or acute poststreptococal glomerulonephritis. Concentrations of LMW-C1q in SLE serum samples correlated with titres of anti-dsDNA and were inversely related to concentrations of normal C1q and C3. Serial studies in six patients, who had rising anti-dsDNA titres and who developed a major exacerbation requiring admission to hospital, showed that LMW-C1q increased in parallel with anti-dsDNA, reaching peak values of more than 2000% of normal just before or at the time of clinical relapse and decreasing during convalescence. Most marked increases in LMW-C1q were noted in the three patients in whom C1q concentrations remained normal, whereas increases were less in the three patients who had strongly depressed concentrations of normal C1q. A study of C1q biosynthesis by macrophages cultured from patients with SLE and high serum concentrations of LMW-C1q did not show impaired secretion of normal C1q in favour of LMW-C1q, but indicated that serum concentrations of LMW-C1q may reflect the synthetic rate of C1q in vivo. The results show that increased serum concentrations of LMW-C1q may be helpful in diagnosing SLE and suggest that serial determination of LMW-C1q in serum may have predictive value in monitoring patients with SLE.

In a previous study we reported increased concentrations of low molecular weight C1q (LMW-C1q) in serum samples from 54 patients with SLE who had not been selected for their disease activity. This antigenically defective molecule, which does not participate in complement activation, was originally found in homozygous subjects with an inherited deficiency of functional C1q and a tendency to develop SLE-like syndromes. Low molecular weight C1q was also found at lower concentrations in serum samples from normal controls. Furthermore, recent studies with cultured blood monocytes from normal donors and subjects genetically deficient in C1q indicate that in vitro LMW-C1q is an important by-product of C1q synthesis. These observations raise the following questions about the significance of LMW-C1q in SLE: Are high serum concentrations of LMW-C1q specific for SLE or a common finding in immune complex mediated disorders? Do they reflect increased or impaired synthesis of C1q? Are serum concentrations of LMW-C1q related to serological or clinical indices of disease activity and does LMW-C1q itself behave as such an index? The aim of the work described here was to answer these questions.

Patients and methods
PATIENTS AND SERUM SAMPLES
Eighty two patients with SLE fulfilling the revised American Rheumatism Association (ARA) criteria were entered into the study. Blood samples from 71 of these patients were obtained within six weeks after the appearance of one or more of the following symptoms: skin lesions, including rashes, palpable purpura, and digital infarctions (present in 34 patients); arthralgia or arthritis (in 32 patients); haematological abnormalities—that is, haemolytic anaemia, thrombocytopenia or leucopenia (in 38 patients); pleuritis (in 22 patients); renal symptoms manifested by a decrease of glomerular filtration rate of more than 20%, appearance of or an increase in red cell casts in the urinary sediment, or the appearance of or an increase in proteinuria (in 33 patients); central nervous system disease, in most cases manifested by the occurrence of psychotic episodes, and in occasional patients by aseptic meningitis or convulsions (in 21 patients). Anti-dsDNA antibodies, measured by the Farr assay, in these 71 serum samples were increased. In addition, blood samples were obtained from the other 11 patients with SLE, who had no symptoms of SLE at that time. In addition, we studied 38
patients with polyarthritis, all with classic or definite rheumatoid arthritis according to the ARA criteria,\textsuperscript{11} and 21 children (ages 2–18 years) with acute glomerulonephritis, whose diagnosis was based on a combination of clinical criteria and laboratory findings. Raised anti-streptolysin titres were present in 15 of these children. From all these patients one serum or plasma sample was tested. Serial samples were also tested from six other patients with SLE who fulfilled the revised ARA criteria. Samples of serum, citrate, or EDTA plasma from patients and from 60 healthy donors were frozen at $-70^\circ\text{C}$ until use.

**MONOCYTES**

Blood (30 ml) from patients with SLE was collected in siliconised vacutainer tubes (Becton and Dickinson, Meylan, France) containing trisodium citrate at a final concentration of 0.38\% (w/v). Within four hours after collection plasma was separated from cells by centrifugation (five minutes, 4000 g at room temperature) and stored at $-70^\circ\text{C}$. Monocytes were purified from the cell pellets essentially as described by de Boer et al.,\textsuperscript{12} with the exception that Ficoll was replaced by Percoll (Pharmacia Fine Chemicals A/B, Uppsala, Sweden). The purity of monocytes obtained by this procedure ranged from 60 to 90\% as determined by size analysis differentiation (Coulter counter with Channelizer) and yields ranged from 5 to 20 x 10$^7$ monocytes. The cells were cultured in 24-well multishishes (Nunclon, Roskilde, Denmark) at 5 x 10$^5$ cells/well in Iscove’s modified Dulbecco’s medium, supplemented with 10\% (v/v) heat inactivated (one hour, 56\°C) fetal calf serum (Sera-Lab, Sussex, UK), 10 $\mu$g/ml of L(+)-ascorbic acid (Merck, Darmstadt, FRG), penicillin, and streptomycin. Culture supernatant was harvested and medium replenished after 24 hours and then twice a week.

Each time that cells were obtained from patients, blood was also collected from healthy donors with similar sex and age (the difference in age between patient and donor was not more than four years). The monocytes from these donors were studied for Clq production at the same time as those from the patients.

**MONOCLONAL ANTIBODIES AGAINST C1q**

The following two monoclonal antibodies (MAbs) against Clq were used in this study: MAb 130, which is directed against an epitope on the fibril-like strands (arms) of Clq close to the globular heads,\textsuperscript{13} and which can bind normal Clq, peptic digested Clq, and also collagenase digested Clq,\textsuperscript{13} but cannot bind LMW-C1q produced by cultured monocytes,\textsuperscript{8} nor the dysfunctional LMW-C1q found in some patients with a hereditary Clq defect:\textsuperscript{8} MAb 101, which hardly binds normal Clq, but does bind the LMW-C1q produced by cultured monocytes.\textsuperscript{9} Monoclonal antibody 101 also binds the LMW-C1q that is present in some patients with a genetic Clq deficiency (unpublished observations).

**COMPLEMENT ASSAYS**

**Low molecular weight Clq**

Concentrations of LMW-C1q were measured by a three step radioimmunoassay: (1) Normal Clq was removed from samples of serum or plasma (25 $\mu$l) or monocyte culture supernatant (800 $\mu$l) by immunoaerosorption with 3 mg of CNBr activated Sepharose 4B (Pharmacia) to which 60 $\mu$g of MAb 130, specific for normal Clq, had been coupled; the Sepharose beads were suspended in 1 ml of phosphate buffered saline containing 0.1 (v/v) Tween 20, 10 mM EDTA, and 800 mM NaCl, pH 7.4 (PETS) and incubated with the samples for 16 hours at room temperature in rotating polystyrene tubes (Thovadex, Nieuwkoop, The Netherlands). (2) The Sepharose beads were removed by centrifugation of the tubes, and serial dilutions of the supernatants were prepared in PETS, again in polystyrene tubes. These samples were incubated (five hours, room temperature) with 1 ml of PETS and 1.5 mg of Sepharose containing 30 $\mu$g of MAb 101 that recognises LMW-C1q. (3) The Sepharose MAb 101 beads were washed extensively to remove unbound protein and incubated (16 hours, room temperature) with 50 $\mu$l of $^{125}$I labelled rabbit antibodies against Clq (2 ng=20 000 cpm)$^7$ in 0.5 ml of PETS containing 0.1\% (w/v) normal rabbit IgG (Miles, Naperville, IL, USA). The beads were washed again and bound radioactivity was measured by a gammacounter.

**Normal C1q**

Concentrations of normal Clq were determined by two different assays: (1) In monocyte culture supernatants functional Clq was measured by a Clq haemolytic assay described previously.\textsuperscript{7} (2) In serum and plasma normal Clq was measured by Behring Nephelometer Analyzer (Behringwerke, Marburg, FRG) with the use of a monospecific antiserum (SH4-3-3-N02) obtained from our institute (department of immune reagents). In both assays the results were expressed as a percentage of the amount of Clq present in pooled normal human serum.

**Other complement components**

Concentrations of C3 and C4 in serum or plasma were measured by Behring Nephelometer Analyzer with the use of monospecific antisera (department of immune reagents) and expressed as a percentage of concentrations in normal human serum.

**ANTI-DNA ASSAYS**

The Farr assay and the indirect immunofluorescence technique on *Cristidna luciella* for the measurement of antibodies specific for DNA were performed as described by Aarden and Smeenk.\textsuperscript{14}

**STATISTICAL METHODS**

Correlations between indices were calculated by linear regression analysis. The statistical significance of differences between patients and healthy...
controls was determined by the Wilcoxon-Mann-Whitney test.

**Results**

**CHARACTERISTICS OF RADIOIMMUNOASSAY FOR LMW-Clq**

Although MAb 10l used for the LMW-Clq radioimmunoassay preferably binds LMW-Clq, binding of normal Clq does occur to some extent (Hoekzema et al., unpublished data). Therefore, all sera and monocyte culture supernatants were first depleted of Clq by an incubation with another MAb (130), specific for normal Clq, which had been coupled to Sepharose beads as described under ‘Patients and methods’. Conditions of immunoadsorption were chosen in such a way that all normal Clq was removed from 50 µl of normal human serum. Low molecular weight Clq in serum from a patient with a genetic Clq deficiency was not removed by this adsorption procedure (data not shown). Figure 1 shows that this radioimmunoassay procedure resulted in a reproducible dose-response curve for LMW-Clq in normal human serum. When a solution of purified normal Clq (250 µg/ml) was used as starting material, no significant binding was found. The antigen detected in this radioimmunoassay had the physicochemical properties of LMW-Clq—that is, a sedimentation coefficient of 4S in sucrose gradient ultracentrifugation and an apparent molecular weight of 130 000 in gel filtration (not shown).

In the following experiments concentrations of LMW-Clq in serum samples and monocyte culture supernatants were calculated by reference to standard curves of normal human serum as in fig 1, arbitrarily defined as containing 100% LMW-Clq.

**SERUM CONCENTRATIONS OF NORMAL AND LMW-Clq IN PATIENTS AND IN NORMAL DONORS**

Concentrations of normal and LMW-Clq were determined as described under ‘Patients and methods’ in serum samples from 82 patients with SLE, and compared with concentrations in serum samples from 38 patients with rheumatoid arthritis, 21 patients with acute glomerulonephritis, and 60 healthy controls (fig 2). In comparison with controls (median 102.5%), concentrations of LMW-Clq were increased in SLE (median 163.5%, p < 0.0001) but not in rheumatoid arthritis (median 110%, p = 0.7565). In acute glomerulonephritis serum concentrations of LMW-Clq were decreased (median 78%, p = 0.0116). Normal Clq was decreased in SLE (median 47.5%, p < 0.0001) and acute glomerulonephritis (median 92%, p = 0.0002) but showed a tendency towards increased concentrations in rheumatoid arthritis (median 125%, p = 0.0502) when compared with controls (median 113.5%).

Seventy one patients with SLE had had one or more clinical symptoms within six weeks before the time of blood sampling. Low molecular weight Clq concentrations in these patients were not particularly associated with one or more clinical manifestations. Low molecular weight Clq concentrations in patients with these symptoms were: 174% (101–667%) (median (range)) in patients with pleuritis; 167% (96–667%) in patients who had haematological abnormalities; 160% (96–421%) in patients with skin lesions; 154% (96–400%) in patients who
had arthritis or arthralgia; 143% (96–667%) in patients with central nervous system disease; and 160% (96–667%) in patients with renal disease.

**RELATION OF LMW-Clq TO OTHER SEROLOGICAL INDICES**

In the 60 healthy controls highly significant correlations were found between concentrations of LMW-Clq and Clq (r=0-6611, p<0-001), C3 (r=0-5279, p<0-001), and C4 (r=0-5137, p<0-001), indicating that in the absence of complement activation the synthetic rates of these components are probably coupled (table 1).

In contrast, in SLE serum samples concentrations of LMW-Clq were inversely correlated with concentrations of Clq (n=82, r=0-2239, p<0-05) and C3 (n=69, r=0-3375, p<0-01). When concentrations of LMW-Clq or Clq were compared with titres of anti-dsDNA it was found that LMW-Clq but not normal Clq correlated with anti-dsDNA measured by the Farr assay (n=71, r=0-2481, p<0-05) or by the immunofluorescence technique (n=71, r=0-2688, p<0-05). In serum samples from patients with rheumatoid arthritis and acute glomerulonephritis, no correlation was found between concentrations of LMW-Clq and Clq. A negative correlation was again observed between LMW-Clq and C3, however, both in patients with RA (n=38, r=0-4731, p<0-01) and in those with acute glomerulonephritis (n=18, r=0-6234, p<0-01). The presence or absence of disease related antibodies in serum from patients with rheumatoid arthritis or acute glomerulonephritis did not influence the concentration of LMW-Clq. Rheumatoid arthritis sera containing rheumatoid factors did not have higher concentrations of LMW-Clq, whereas in acute glomerulonephritis serum concentrations of LMW-Clq were not related to titres of antistreptolysin antibodies (not shown).

Table 1 summarises the relations between LMW-Clq and other serological indices in patients and controls. In contrast with LMW-Clq, normal Clq did not correlate significantly with C3 in sera from patients with rheumatoid arthritis or acute glomerulonephritis. In SLE sera a positive correlation was found between Clq and C3 (n=69, r=0-7126, p<0-002).

**LMW-Clq Profiles during Exacerbation**

Concentrations of normal and LMW-Clq were also measured in serum samples obtained at one to eight week intervals from six patients who, according to rising serum titres of anti-dsDNA, were expected to experience a clinical exacerbation of SLE.2-4 Indeed, according to previously described criteria for disease activity,2 15 these patients all developed major flares that required treatment in hospital. Furthermore, in all patients studied titres of anti-dsDNA peaked *in or just before* the period of severe relapse: this moment is indicated as ‘time zero’ (t=0) in figs 3 and 4.

### Table 1  Correlation between serum concentrations of low molecular weight Clq and other serological indices

<table>
<thead>
<tr>
<th>Indices correlated with LMW-Clq*</th>
<th>Control</th>
<th>RA*</th>
<th>AGN*</th>
<th>SLE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clq 0-66 (n=60) p&lt;0-001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C3 0-53 (n=60) p&lt;0-001</td>
<td>NT*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C4 0-51 (n=60) p&lt;0-001</td>
<td>NT*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-dsDNA (Farr)</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Anti-dsDNA (IFT)*</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

*AGN=acute glomerulonephritis; IFT=immunofluorescence technique; LMW-Clq=low molecular weight Clq; NT=not tested; RA=rheumatoid arthritis; SLE=systemic lupus erythematosus.
Figure 3 shows the longitudinal profile of normal and LMW-Clq in serum of patients D, S, and P, who developed a severe deterioration of renal function during a major flare. In these patients the exacerbation was heralded not only by rising anti-dsDNA titres (not shown in fig 3), but also by markedly depressed serum Clq, starting several months before clinical signs of nephritis were apparent. Concentrations of Clq returned to normal during convalescence except in patient S who died of sepsis as a complication of severe skin ulcers. Serum concentrations of LMW-Clq fluctuated in a manner opposite to those of normal Clq and more similar to titres of anti-dsDNA: they ‘peaked’ in the period of severe relapse and decreased during convalescence.

When profiles of LMW-Clq were studied in three other patients who had normal serum concentrations of Clq in the period of rising anti-dsDNA that preceded major flares, unexpected results were obtained: the increase in LMW-Clq was much more pronounced in these patients, reaching values of more than 20 times the normal concentration (fig 4). None of these patients had severe renal disease, though patients H and W had mild renal symptoms about two weeks after the anti-dsDNA peak. In

Table 2 Correlation between concentrations of Clq and low molecular weight Clq (LMW-Clq) in serum and monocyte cultures of patients with systemic lupus erythematosus (SLE)

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Serum</th>
<th>Clq*</th>
<th>Culture (day 20)</th>
<th>R²</th>
<th>Clq*</th>
<th>Culture (day 20)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>116</td>
<td>0.86</td>
<td></td>
<td>8.20</td>
<td>0.21</td>
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<tr>
<td>2</td>
<td>180</td>
<td>100</td>
<td>1.80</td>
<td></td>
<td>8.20</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>98</td>
<td>87</td>
<td>1.13</td>
<td></td>
<td>5.30</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>311</td>
<td>166</td>
<td>1.87</td>
<td></td>
<td>5.10</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>103</td>
<td>110</td>
<td>0.84</td>
<td></td>
<td>2.40</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>121</td>
<td>1.49</td>
<td></td>
<td>5.70</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>259</td>
<td>140</td>
<td>1.85</td>
<td></td>
<td>7.30</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>246</td>
<td>174</td>
<td>1.41</td>
<td></td>
<td>5.40</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>321</td>
<td>162</td>
<td>1.98</td>
<td></td>
<td>7.00</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>355</td>
<td>182</td>
<td>1.95</td>
<td></td>
<td>5.60</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage of the concentration in normal human serum.
†Expressed as percentage of the concentration in normal human serum, secreted in 24 hours.
‡R=ratio LMW-Clq/Clq.
both patients this was accompanied by moderate depression in Clq concentrations (fig 4).

SECRETION OF NORMAL AND LMW-Clq BY CULTURED MONOCYTES
To investigate whether a dysfunction of Clq synthesis is present in Clq producing cells from patients with SLE, blood monocytes were cultured from 10 patients with SLE and 10 healthy controls matched for sex and age, as described under 'Patients and methods'. None of the patients had a major relapse or depressed serum Clq, but seven of the 10 patients had a LMW-Clq serum concentration ≥180% of normal, and serum concentrations of LMW-Clq and Clq correlated significantly (p<0.001) in these patients (table 2). During the period of in vitro maturation into Clq synthesising macrophages samples were taken from the culture supernatants and tested for functional Clq (by haemolytic assay) and LMW-Clq (by radioimmunoassay). Figure 5 indicates that impaired synthesis of Clq could not be shown in SLE monocytes by comparison with controls. On the contrary, the monocyte derived macrophages from patients tended to secrete more Clq than cells from controls. Table 2 shows the significant correlation (p<0.001) between amounts of functional and LMW-Clq that had been secreted by cells from patients with SLE as well as the excessive production of LMW-Clq, as judged by LMW-Clq/Clq ratios in comparison with sera. No correlations were present between concentrations of LMW-Clq or Clq in serum and those in culture supernatant or between ratios in serum and those in culture supernatant. Similar results were obtained with cultures from healthy controls matched for age and sex (not shown).

Discussion
In a previous study we reported that serum samples from patients with SLE contain increased concentrations of LMW-Clq when compared with samples from healthy donors. The possibility was considered that serum concentrations of LMW-Clq reflect the rate of biosynthesis of normal Clq in these patients. At that time it was not clear whether LMW-Clq represents a product of Clq-synthesising cells, however, and the study on patients with SLE did not include serial measurements of LMW-Clq at varying stages of disease activity.

In this study we used a particular radioimmunoassay to measure LMW-Clq concentrations in serum. LMW-Clq was bound to MAb 101 and measured by a subsequent incubation with 125I labelled purified rabbit antibodies to Clq. Although normal Clq hardly bound to MAB 101, at higher concentrations it interfered with the assay. Therefore, we removed normal Clq from serum samples by absorption with MAb 130 before determining LMW-Clq concentrations. Although MAb 130 neither binds the LMW-Clq produced by cultured monocytes nor the LMW-Clq found in some patients with a genetic Clq deficiency, it can bind other low molecular weight forms of Clq, in particular those forms generated by enzymatic degradation of normal Clq. We did not analyse the composition of the Clq molecules removed from sera by MAb 130. Therefore, the possibility still remains that in addition to the normal Clq and the LMW-Clq described in this study, other forms of Clq, generated by enzymatic degradation, may circulate in patients with SLE. In a recent study we showed that the LMW-Clq detected by MAB 101 is produced by cultured monocytes and contains normal A, B, and C chains as well as an abnormal AC dimer. This LMW-Clq is indistinguishable from the serum LMW-Clq present in normal subjects and at higher concentrations in patients with SLE. Therefore, we believe that the LMW-Clq described in this study is formed as a by-product of Clq synthesis and that serum LMW-Clq concentrations reflect Clq synthesis in vivo.

The results shown here confirm our previous finding—that is, that high serum concentrations of LMW-Clq seem to be specific for SLE: concentrations higher than 170% of normal were not found in patients with rheumatoid arthritis or in those with poststreptococcal acute glomerulonephritis. Although LMW-Clq will have to be measured in other disorders, these data indicate that the determination of LMW-Clq in serum may prove a helpful addition to the revised ARA criteria in diagnosing SLE. The correlation (p<0.05) between concentrations of LMW-Clq and anti-dsDNA titres in SLE sera suggested to us that they may fluctuate in a comparable manner—that is, rise in periods of active disease and decrease during convalescence. This was confirmed by a prospective study in six patients who experienced a major flare: in these patients serum concentrations of LMW-Clq started to rise several weeks or even months before admission into hospital was required. Similar fluctuations were found for serum titres of anti-dsDNA and the rise in LMW-Clq and anti-dsDNA occurred simultaneously.

Remarkable were the differences of LMW-Clq profiles in patients with SLE with and without disease, especially in relation to concentrations of normal Clq. It is well known that serum Clq is lower in patients with nephritis, which is usually attributed to increased catabolism of this complement component. Indeed, experiments in vivo and in vitro showed that after binding to aggregates of IgG (as a model for circulating immune complexes) Clq is rapidly cleared and degraded by cells of the mononuclear phagocyte system, suggesting that the fate of Clq and immune complexes in vivo may be closely connected. If the turnover of Clq is accelerated in SLE, one would expect increased catabolic and synthetic rates, as found in other disorders with depressed serum Clq, such as paraproteinaemia. The few studies on the metabolism of Clq in SLE do not confirm this, however, as they report normal or only slightly accelerated catabolism. Therefore, the possibility has to be considered that impaired biosynthesis of Clq contributes to the low serum concentrations of Clq in renal SLE, as has been shown for
complement component C3.\textsuperscript{24–27} The high peak values (up to more than 2000%) of LMW-Clq with normal or slightly depressed Clq found in patients G, H, and W, who had no renal disease, (fig 4) would then be compatible with a greater ability of these patients to accelerate biosynthesis of Clq than patients D, S, and P with renal disease (fig 3), who had very low or undetectable Clq and a less pronounced rise in LMW-Clq. Analysis of LMW-Clq concentrations in the 71 patients with SLE, from whom blood samples were obtained within six weeks after the appearance of clinical symptoms, did not show significantly lower LMW-Clq concentrations in patients with renal disease than in those without renal symptoms. Apparently, more serial measurements of LMW-Clq concentrations in patients with active SLE are needed in combination with detailed Clq turnover studies to show whether there are differences in Clq synthesis between patients with various manifestations of SLE. Also, the possibility that the less pronounced rise of LMW-Clq in patients with nephritis results from loss through proteinuria will have to be excluded.

The inability to show impaired biosynthesis of Clq by culture derived macrophages from 10 patients with SLE does not exclude an impairment in vivo. Firstly, none of these particular patients had active disease according to clinical and laboratory indices: decreased synthesis of Clq in vivo may be temporary and restricted to a certain phase in the disease course, as has been shown for C3.\textsuperscript{27} Secondly, cultured cells from healthy donors as well as from patients with SLE (table 2) secreted excessive amounts of LMW-Clq in comparison with functional Clq. This suggests that the in vivo situation itself may impede the production of normal Clq, possibly masking any differences between patients and controls. We are currently investigating this phenomenon.