Metabolism of Human Clq

STUDIES IN HYPOGAMMAGLOBULINEMIA, MYELOMA, AND SYSTEMIC LUPUS ERYTHEMATOSUS

PETER F. KOHLER and HANS J. MÜLLER-EBERHARD

From the Division of Clinical Immunology, Department of Medicine, University of Colorado Medical Center, Denver, Colorado 80220, and the Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California 92037

ABSTRACT The in vivo metabolism of radioiodine-labeled Clq was determined in patients with hypogammaglobulinemia, multiple myeloma, systemic lupus erythematosus (SLE), and in healthy controls. Marked differences in metabolic behavior were observed with a much more rapid disappearance of plasma radioactivity in patients as compared with controls. Estimated plasma volumes at 10 min after injection (time 0) were normal in controls and the SLE patient, mean 40 ml/kg, whereas they were grossly elevated, 57-82 ml/kg, in the hypogammaglobulinemic and myeloma patients, indicating a significant loss of Clq-¹²⁵I during the initial mixing period. Absence of a distinct initial equilibration phase of radioactivity loss from the plasma suggested a significant reversible interaction of the labeled Clq with plasma proteins and density gradient studies provided evidence for in vivo uptake into the circulating trimolecular first component complex (Clq, r, s). In controls and the SLE patient 0.51-0.75 of the Clq was retained in the plasma space while only 0.28 or less was in the others. The daily plasma pool fractional Clq catabolism was 0.65-0.67 in controls compared with 0.95-4.80 in the patients. Clq synthetic rates in controls were 4.64 and 4.34 mg/kg per day while higher rates, 4.94-37.40 occurred in the patients.

These experiments clearly indicate that the metabolism of Clq is markedly influenced by serum IgG concentrations, probably related to the reversible inter-

INTRODUCTION

Serum Clq is the subunit of the first component of complement (C1) which is endowed with the capacity to recognize immunoglobulins and to initiate the complement reaction. It is a basic glycoprotein of molecular weight 400,000 with an electrophoretic mobility of a slow gamma globulin and has been isolated in a highly purified and functionally active form (1). Originally defined by its ability to induce precipitation of soluble gamma globulin complexes (2, 3) Clq has receptors directed to sites on the heavy polypeptide chains of immunoglobulins (Ig) * G and M but not A, D, or E (4).* The binding affinity to IgG molecules varies in dependence on their heavy chain subclass; it is greater for G3 than for G1 and G2 and apparently zero for G4.

Decreased serum Clq has been observed in a limited number of diseases. In active systemic lupus erythematosus (SLE) it may be low and correlated with the total serum complement (5-7). Precipitation of the serum cryoglobulins which are frequently present in SLE was

1 Abbreviations used in this paper: Ig, immunoglobulin(s); SLE, systemic lupus erythematosus; TCA, trichloroacetic acid.

first shown by Christian, Hatfield, and Chase to be dependent on the presence of Clq (8). In addition, some patients with active chronic "hypocomplementemic" nephritis have reduced serum Clq (9). Complement-dependent immunopathogenic processes are thought to be operative in both of these diseases and the decreased serum Clq has been attributed to increased utilization by the underlying pathogenic process (5–7, 9).

In addition to the above diseases, even more consistent reductions of Clq occur in patients with certain immunoglobulin deficiencies in whom hemolytic total complement and other component levels are normal. Müller-Eberhard and Kunkel found low Clq concentrations in serum from three individuals with Brutton's agammaglobulinemia (2). A profound decrease in Clq was subsequently reported in a 3 wk female with Swiss agammaglobulinemia (10, 11). These observations have been recently extended by studies employing the quantitative radial diffusion technique (12–14).

Our previous study revealed a direct relationship between the serum concentration of Clq and IgG, but not IgA or IgM (12). The lowest Clq concentrations were found in patients with deficient IgG synthesis. In addition, close to one-half of multiple myeloma and Waldenström's macroglobulinemia sera had low Clq while markedly elevated concentrations were observed in heavy chain disease sera of the IgGs subclass (12). Gewurz and associates (13) found a reduction of Clq to 27% in three infants with Swiss-type agammaglobulinemia and 75% of normal in other agammaglobulinemic syndromes. In their report, the Clq reduction was felt to be related to the type of agammaglobulinemia rather than to the extent of IgG deficiency (13).

The present study was undertaken to define the basis for the reduced Clq concentrations in patients with immunoglobulin abnormalities and SLE by determining the in vivo metabolism of radioiodine-labeled protein. In addition, attention was directed to the possible in vivo uptake of the injected Clq into the native trimolecular Cl complex.

**METHODS**

**Study group.** A total of seven adults were studied. Two male physicians served as controls. The experimental group consisted of two patients with acquired hypogammaglobulinemia, two with multiple myeloma, and one with active SLE. A brief summary of the pertinent clinical and laboratory information concerning these subjects is presented in Table I. All were in a stable clinical condition during the studies save for the patient with IgG myeloma (case 7) who required transfusions of packed red cells because of gastrointestinal bleeding.

**Preparation of isolated, radioactive-labeled Clq for intravenous injection.** Clq was isolated from the fresh serum of healthy adults by a recently described procedure (1). All buffers were autoclaved and contained 5 × 10^{-4} M kanamycin.

The purified Clq was traced labeled with ^{3}I by the chloramine T method as described by McConahey and Dixon (15). After extensive dialysis to remove nonprotein-bound

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age</th>
<th>Clq</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>Study initiated</th>
<th>Treatment</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>M</td>
<td>34</td>
<td>0.182</td>
<td>8.50</td>
<td>2.55</td>
<td>0.59</td>
<td>6/20/69</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Acquired hypogamma</td>
<td>M</td>
<td>53</td>
<td>0.132</td>
<td>2.61</td>
<td>0.117</td>
<td>0.078</td>
<td>6/23/69</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IgA myeloma</td>
<td>F</td>
<td>52</td>
<td>0.091</td>
<td>1.13</td>
<td>0.216</td>
<td>0.096</td>
<td>6/24/69</td>
<td>Prednisone (30 mg) and cyclophosphamide (100 mg/day)</td>
<td>Multiple osteolytic lesions, no proteinuria</td>
</tr>
<tr>
<td>4</td>
<td>Acquired hypogamma</td>
<td>M</td>
<td>19</td>
<td>0.094</td>
<td>0.76</td>
<td>0.0</td>
<td>0.116</td>
<td>6/23/69</td>
<td>Prednisone (40 mg), azathioprine (100 mg), and hydroxychloroquine (600 mg/day)</td>
<td>Recurrent respiratory infections since age 1, brother's immunoglobulins normal</td>
</tr>
<tr>
<td>5</td>
<td>SLE</td>
<td>M</td>
<td>25</td>
<td>0.134</td>
<td>18.0</td>
<td>5.83</td>
<td>0.85</td>
<td>6/27/69</td>
<td>Prednisone (40 mg), azathioprine (100 mg), and hydroxychloroquine (600 mg/day)</td>
<td>Proteinuria, 1–1.5 g/24 hr, low total C, C3 and C4, cryoglobulins</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>M</td>
<td>37</td>
<td>0.151</td>
<td>11.88</td>
<td>2.55</td>
<td>2.26</td>
<td>6/30/69</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>IgG myeloma</td>
<td>M</td>
<td>58</td>
<td>0.240</td>
<td>24.30</td>
<td>1.25</td>
<td>0.56</td>
<td>7/5/69</td>
<td>Transfusions of packed red blood cells</td>
<td>Proteinuria 1 g/24 hr, G.I. bleeding and fever 38°C during study</td>
</tr>
</tbody>
</table>

**Normal range, mg/ml** 0.134–0.246 6.20–14.00 0.68–3.14 0.93–3.20

**Metabolism of Human Clq**

869
isotope, the Clq-<sup>125I</sup> was centrifuged at 49,000 rpm for 60 min in a Spincos model L-2 ultracentrifuge (Spincos Div., Beckman Instruments, Inc., Palo Alto, Calif.) to remove aggregated protein. This step was also necessary as a precaution to insure that any bacterial contaminants were removed since it had been previously observed that Millipore filtration (0.45 μm pore size [Millipore Corporation, Bedford, Mass.]) caused retention of a large proportion of Clq and, therefore, could not be used for sterilization. Subsequent to ultracentrifugation the supernatant fluid was removed sterilely and diluted with sterile pyrogen-free saline to a protein concentration of 80 μg/ml. Sterile human albumin was then added as a carrier at a final concentration of 1 mg/ml and the material placed in sterile rubber-stoppered vacuum vials which were kept thereafter between 0 and 4°C. After anaerobic and aerobic cultures and rabbit pyrogen testing (16) of the labeled Clq were negative, it was judged to be suitable for in vivo use.

The specific activity of the labeled protein was 77.4 μCi/μg. Over 97% of the radioactivity was protein bound as determined by duplicate precipitation in 10% trichloroacetic acid (TCA). To determine the specificity of labeling excess anti-Clq was incubated with the labeled Clq for 16 hr at 4°C. The resulting precipitate was isolated by centrifugation at 2500 rpm for 30 min and washed three times in saline. The rabbit anti-Clq-Clq-<sup>125I</sup> precipitate contained 92% of the total radioactivity. As an additional check, direct radioimmunoelectrophoresis of the Clq-<sup>125I</sup> was performed using as antigen (a) the same preparation of unlabeled Clq (1 mg/ml), (b) heavy chain disease serum Mat (12) which has an elevated Clq (0.410 mg/ml), and (c) normal human serum (case 1). The immunoelectrophoresis conditions were those described by Morse and Christian (17) except for the use of 1% agarose instead of 0.75% agar. Specific rabbit anti-Clq and goat anti-whole human serum (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) were used. The precipitin lines developed over a 24-hr period and the slides were then washed with multiple changes of normal saline followed by distilled water, photographed, dried, and stained with amido black. The slides were incubated for 1 wk with Kodak K. X-ray film (Eastman Kodak Co., Rochester, N. Y.) which was then developed. Only the Clq precipitin line was present on the radioautograph further indicating the labeling specificity and purity of the Clq preparation (Fig. 1).

Immunochemical quantitation. A highly potent rabbit antiserum to human Clq was prepared as previously described (12) and used in the single radial diffusion quantitation of serum Clq in the study subjects and for specificity testing of the labeled Clq. The serum concentration of C3, C4, and C5 were also measured by single radial diffusion (18). Measurement of IgG, A, and M was done by a modified Oudin tube method as described by Ciaman and Merrill (19).

Total complement. Titers of total hemolytic complement were measured in 50% hemolytic U (CH50) by the method of Mayer and Kabat (20).

Administration of labeled Clq and sample collections. Lugol's solution, 30 drops/day, was taken from 24 hr before to 48 hr after completion of the study by all subjects. From 29 to 37 μCi Clq-<sup>125I</sup> was administered i.v. to the controls and patients from disposable plastic syringes. The amount of material injected was determined by the difference in syringe weight before and after injection. Heparinized and clotted blood samples were obtained at 10 min after the Clq injection, at intervals up to 8 hr and daily thereafter for 7-9 days. Fresh serum and plasma were stored at −70°C in an electric freezer. In addition, 2-ml portions of plasma were kept in sterile 12 × 74 mm plastic tubes at 4°C before counting.

During the initial 72 hr of the study, fecal loss of <sup>125I</sup> was determined in four patients (cases 2, 3, 4, and 6) and found to be negligible in every instance. Urines were collected for each 24 hr period, the volumes recorded, and portions stored at 4°C for isotope analysis.

These studies were carried out over a 23 day period from 6/20/69 to 7/12/69. The first study was done in a control (case 1) and the other control (case 6) received the labeled Clq on 6/30/69. In this way the effect of storage at 4°C on the subsequent in vivo retention of labeled Clq could be evaluated. The starting dates of the individual studies are indicated in Table I.

Measurement of radioactivity in 2-ml vol of plasma, urine, and homogenized stool was done in a Nuclear-Chicago automatic gamma scintillation counter (Nuclear-Chicago, Des Plaines, Ill.) equipped with a 1 inch NaI scintillation crystal. Both the total and TCA precipitable radioactivity were determined in all plasma samples. Proteinuria was determined by approximately 1 g/day was present in cases 5 and 7 and their urine TCA precipitable radioactivity was also measured and found to be less than 0.5% in each patient. The patient with SLE (case 5) had a cryoprecipitate consisting of IgG and Clq. After determination of isotope in 2 ml of plasma, the cryoprecipitate which developed after 72 hr at 4°C was isolated by precipitation by centrifugation, washed three times in ice-cold saline, and analyzed for radioactivity in parallel with the supernatant plasma which lacked cryoprecipitable protein.

Data calculation. The urine/plasma radioactivity<sup>3</sup> clearance method (21, 22) as modified by Waldmann, Wochner, Drews, and Strober was used for determination of plasma volume, daily fractional catabolism of Clq and the Clq turnover rate (23, 24). A summary of labeled calculation is outlined as follows: (a) Plasma volume (ml/kg) = radioactivity administered/radioactivity per milliliter plasma at 10 min after injection (time 0) × body wt. in kilograms. (b) Total body radioactivity = radioactivity administered − cumulative radioactivity excreted. (c) Fraction of total body Clq intravascular = plasma volume × plasma radioactivity/ radioactivity retained in body. This was determined after equilibration of labeled protein among body compartments was complete, e.g., 24 hr. (d) Total circulating Clq = plasma volume × plasma concentration of protein. (e) Total exchangeable pool of Clq = total circulating protein/fraction of protein that is intravascular. (f) Fraction of circulating Clq catabolized per day = radioactivity excreted in each 24 hr period/mean circulating radioactivity during the same period. (g) Turnover rate = total circulating Clq × fraction of circulating Clq catabolized per day. The latter fraction was determined for each day and the mean values for the steady-state days was used in determining the turnover rate.

The concentration of serum Clq in each subject was measured on days 1, 3, and 6 and the mean value determined. The variation from the mean was less than 10% in every instance, hence, the assumption of a steady state was considered valid and the daily Clq synthetic rate was therefore equal to the turnover rate (mg/kg per day).

Density gradient studies. Density gradient ultracentrifugation was utilized to evaluate the possible in vivo incorporation of the injected Clq-<sup>125I</sup> into the circulating trimolecular Cl complex. Two different buffers were used: (a) a stan-

---

<sup>3</sup> Plasma protein-bound (TCA precipitable) radioactivity was used for the calculations.
dard complement-veronal buffer containing 1.5 x 10^{-4} \text{ M Ca}^{++} and 5 \times 10^{-3} \text{ M Mg}^{++}, and (b) an EDTA-veronal buffer in which Ca^{++} and Mg^{++} were omitted and 0.05 \text{ M Na}_{2}EDTA was added. Samples of serum and plasma obtained at 10 min (time 0)-8 hr after injection of the labeled Clq were diluted 1:1 in buffer and 0.1 ml portions were applied to 4.5-ml gradients of 7-31% sucrose. The individual samples were run simultaneously in both buffers at 50,000 rpm for 6 hr at 4°C in a Spinco model L ultracentrifuge (Spinco Div., Beckman Instruments, Inc.) with a SW50 rotor.

Fractions were collected with a Buchler gradient fractionation device and analyzed for radioactivity and for protein by the Folin method. As a reference for the rate estimation, purified Clq-^{35}I was added to fresh heparinized human plasma in vitro and run in gradients containing EDTA-veronal and standard complement buffer.

**RESULTS**

Serum Clq and immunoglobulin concentrations in the control and experimental subjects are listed in Table I. A decreased Clq was present in every patient except case 7 (IgG myeloma). Three of the four patients with low Clq also had decreased serum IgG ranging from 0.76 to 2.61 mg/ml while an elevated IgG of 18.00 mg/ml was present in the patient with SLE.

Significant differences in the in vivo behavior of the labeled Clq were observed between controls and experimental subjects and also within the experimental group. A major variation occurred in the estimated plasma volumes determined by dividing the total radioactivity administered by the radioactivity per milliliter of plasma at 10 min after injection (Table II). The controls and patient with SLE had volumes in the normal range of 38-44 ml/kg while the remaining four patients had moderately to grossly elevated values of 57-82 ml/kg. The highest volume was present in the patient with IgG myeloma (case 7) who had an initial hematocrit of 30\%. This reduction in red cell volume could only partially explain his estimated plasma volume of 82 ml/kg. Hematocrits were normal and stable in all other subjects. Since accurate plasma volumes are essential for determining total plasma pool Clq and in turn the protein's distribution, fractional catabolism, turnover, and synthetic rates, it was necessary to correct the plasma volumes to 40 ml/kg in cases 2, 3, and 4 and to 47 ml/kg in case 7 for these calculations.

The plasma disappearance curves of the labeled Clq are illustrated in Figs. 1-3. In patients the Clq survival was clearly shorter as compared with controls.

Another distinctive feature of the plasma disappearance curves was the presence of a single phase of radioactivity in controlling plasma, with an initial rapid phase followed by a gradual linear phase. As a further check of the estimated plasma volumes, the initial Clq-^{35}I disappearance in the baby SLE plasma was associated with the cryoprecipitate throughout the study.

As the plasma volumes in the controls and SLE patient averaged 40 ml/kg body wt. (Table II) this value was taken as the "normal." A value of 47 ml/kg was assigned to case 7 on the basis of his reduced red cell mass (hematocrit = 30\%).

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Plasma pool Clq mg/kg</th>
<th>Plasma volume ml/kg</th>
<th>Total &quot;exchangeable&quot; Clq mg/kg</th>
<th>Fraction in plasma pool mg/kg</th>
<th>Daily fractional pool catabolism mg/kg</th>
<th>Daily plasma pool turnover rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>38</td>
<td>6.92</td>
<td>13.54</td>
<td>0.51</td>
<td>0.67</td>
<td>4.64</td>
</tr>
<tr>
<td>2</td>
<td>Acquired hypogamma</td>
<td>57</td>
<td>5.28</td>
<td>19.04</td>
<td>0.28</td>
<td>1.90</td>
<td>10.04</td>
</tr>
<tr>
<td>3</td>
<td>IgA myeloma</td>
<td>72</td>
<td>3.64</td>
<td>19.15</td>
<td>0.19</td>
<td>2.65</td>
<td>9.65</td>
</tr>
<tr>
<td>4</td>
<td>Acquired hypogamma</td>
<td>65</td>
<td>3.76</td>
<td>28.18</td>
<td>0.13</td>
<td>4.80</td>
<td>18.05</td>
</tr>
<tr>
<td>5</td>
<td>SLE</td>
<td>39</td>
<td>5.21</td>
<td>8.52</td>
<td>0.61</td>
<td>0.95</td>
<td>4.94</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>44</td>
<td>6.64</td>
<td>8.78</td>
<td>0.75</td>
<td>0.65</td>
<td>4.34</td>
</tr>
<tr>
<td>7</td>
<td>IgG myeloma</td>
<td>80</td>
<td>10.70</td>
<td>66.45</td>
<td>0.16</td>
<td>1.86</td>
<td>37.40</td>
</tr>
</tbody>
</table>

* Corrected to 40 ml/kg for calculation of plasma pool protein.
† Corrected to 47 ml/kg for calculation of plasma pool protein.
activity loss rather than a distinct two-phase loss as previously observed in the metabolism of other complement proteins. This indicates that an initial rapid intra- to extravascular equilibration of the labeled Clq did not occur and is best illustrated by the activity curves in the controls and patient with SLE (Fig. 1).

The plasma and total body Clq activity curves were essentially parallel in the controls and SLE patient indicating a prompt excretion of the $^{125}$I released from the catabolized labeled Clq in these subjects. In contrast, considerable divergence between the plasma and total body radioactivity curves occurred in the hypogammaglobulinemic and myeloma patients indicating retention of label as either free iodine or more likely Clq-$^{125}$I, in an extravascular compartment (Fig. 4).

Another difference was found in the distribution of Clq, i.e., plasma/total exchangeable protein (Table II). In the controls and patient with SLE from 0.51 to 0.75 of the protein remained intravascularly compared with from 0.13 to 0.28 in the other patients.

Values for the total plasma and exchangeable Clq are shown in Table II. While the plasma concentration, compared to controls, was reduced in every patient except case 7, the total exchangeable Clq was higher in the hypogammaglobulinemia and myeloma patients and lower in the patient with SLE.

When the daily catabolism of the labeled Clq was calculated from the urine activity loss for each 24 hr period, a constant value was evident only when expressed as a fraction of the mean plasma pool and not of the total body or extravascular radioactivity as illustrated by the patient with IgA myeloma (Fig. 5). This indicates that Clq catabolism was occurring primarily within or at least in close association with the plasma pool. In all patients, the daily fractional plasma pool catabolism was increased, ranging from 0.95 in the patient with SLE to 4.80 in the 19 yr old male with hypogammaglobulinemia, compared with 0.67 and 0.65 in the controls (Table II). The standard deviation from the mean was from 2 to 13% in every subject except for the patient with IgG myeloma who was not stable clinically as indicated previously.

![Figure 2](http://www.jci.org)  Plasma radioactivity curves of the two patients with hypogammaglobulinemia plotted as in Fig. 1.

![Figure 3](http://www.jci.org)  Plasma radioactivity curves of the two patients with multiple myeloma plotted as in Figs. 1 and 2.

![Figure 4](http://www.jci.org)  Total body and plasma radioactivity curves in the patient with SLE compared with the patient with IgA myeloma. The curves for both the total body and plasma activity are parallel in the patient with SLE indicating prompt excretion of released isotope. In contrast, a marked divergence of the curves is apparent in the patient with myeloma compatible with a large extravascular pool of labeled Clq which is not in rapid equilibrium with the plasma space.

P. F. Kohler and H. J. Müller-Eberhard
The concentration of serum Clq remained constant throughout the individual studies and the assumption of a steady state was considered valid in all subjects with the possible exception of case 7. Under these circumstances the amount of circulating Clq catabolized per day was equal to the synthetic rate expressed as milligrams per kilogram. The rates in the controls were 4.64 and 4.34 mg/kg per day and ranged from 4.94 to 37.40 in the patients (Table II).

The density gradient studies provided evidence for the in vivo incorporation of injected Clq\textsuperscript{131I} into a higher molecular weight complex (Fig. 6). In the presence of 0.005 M EDTA the radioactivity peak occurred in the upper or slow (11S) region of the gradients. In contrast a faster sedimenting radioactivity peak of approximately 18S was consistently present when the standard buffer containing Ca\textsuperscript{++} and Mg\textsuperscript{**} was employed.

**DISCUSSION**

The association between decreased serum Clq and hypogammaglobulinemia was described in one of the initial studies (2) of this complement protein and subsequently in an infant with Swiss-type agammaglobulinemia (10, 11). This observation has been recently confirmed and extended by studies from different laboratories in larger numbers of patients with hypogammaglobulinemic syndromes and a variety of other immunoglobulin disorders including myeloma, Waldenström's macroglobulinemia, and heavy chain disease (12-14). The correlation between the concentration of serum Clq and IgG, but not IgA or M, the presence of lower Clq in patients with deficiencies of IgG synthesis and the presence of a normal Clq concentration in a patient with intestinal lymphangiectasia who had a serum IgG of 0.90 mg/ml but normal IgG synthesis, led us to postulate that synthesis of these proteins could be linked (12). Gewurz and associates also suggested that the lowered Clq present in their agammaglobulinemic patients was secondary to a deficiency in Clq synthesis (13).

The present metabolic studies clearly indicate that the decreased serum Clq associated with IgG deficiency is not related to impaired Clq synthesis. Two distinctly different mechanisms leading to hypercatabolism of Clq were evident and in turn were responsible for the lowered serum concentrations.

In the patients with acquired hypogammaglobulinemia and myeloma, the Clq\textsuperscript{131I} disappeared very promptly from the plasma space. This was apparent from the over-estimated plasma volumes in these four subjects, the shortened plasma disappearance times and the

![Figure 5](http://www.jci.org)  Figure 5 The ratios of urine radioactivity during each 24 hr period over the mean radioactivity during the same period in the plasma, extravascular, and total body compartment are plotted in the IgA myeloma patient against time. The constancy of plasma Clq catabolism indicates that protein breakdown occurred either within or in close association with the plasma space. Similar results were also present in the other subjects.

![Figure 6](http://www.jci.org)  Figure 6 Density gradient ultracentrifugation of plasma samples obtained 10 min (time 0) after injection in the patient with IgA myeloma. In buffer containing Ca\textsuperscript{++} and Mg\textsuperscript{**} the peak Clq radioactivity localizes to the 19S region of the gradient indicating in vivo uptake into the Clq, r, s complex. The radioactivity peak is in the 11S region in the presence of EDTA buffer. When labeled Clq was added in vitro to fresh serum, plasma, or whole blood (with optimal Ca\textsuperscript{++} and Mg\textsuperscript{**}) there was no evidence for its uptake into the native Clq, r, s complex in contrast to consistent uptake in vivo. An explanation for this difference in behavior is not available. The minor radioactivity peak at fraction 4 may represent binding with IgM and the slower peak subunits of Clq.
altered distribution of the labeled Clq with only 28% or less remaining intravascularly (Table II).

The lack of a parallel decline in plasma and total body radioactivity indicates that in these subjects a large proportion of the Clq was in an extravascular compartment which was not in rapid equilibrium with the plasma pool. The constant fractional catabolism of the plasma pool Clq (but not the extravascular or total body) indicated that breakdown of protein occurred primarily within the space even though its distribution was altered.

The reversible protein-protein interactions between Clq and IgG have been defined by in vitro studies employing analytical ultracentrifugation techniques (4). In conditions of IgG excess, a single Clq molecule may reversibly combine with five to six IgG molecules. The basis for the prompt plasma disappearance of labeled Clq in the two individuals with hypogammaglobulinemia and the patient with IgA myeloma presumably relates to their decreased serum IgG concentrations which limited the normal Clq-IgG interactions thereby allowing the injected labeled protein to be rapidly cleared from the intravascular space.

This explanation cannot account for the very similar in vivo behavior of the labeled Clq in the patient with IgG myeloma and over 24 mg/ml IgG. His estimated plasma volume was 82 ml/kg compared with an expected 47 ml/kg. Thus in the initial 10 min mixing period approximately 40% of the injected Clq left the plasma space. This was not related to any physico-chemical alteration of the preparation during storage since the initial and the sixth studies in the controls provided the expected estimated plasma volumes of 38 and 44 ml/kg. His myeloma protein was in the IgG subclass.

The mean concentrations of Clq and IgG in normal adults are 0.185 mg/ml and 10.0, respectively, as determined by the immunodiffusion methods used in this laboratory (7, 19). Applying the estimated molecular weights of 400,000 for Clq and 160,000 for IgG and Avogadro’s number, approximately 25 × 10⁴ molecules Clq and 25 × 10⁴ IgG/ml are normally present with a molar Clq IgG ratio of approximately 1:1000. It would therefore appear that even with marked reductions of serum IgG below 1 mg/ml as in cases 3 and 4, a surplus of IgG molecules would be present capable of interacting with Clq. However, the application of in vitro data concerning Clq-IgG interactions to the actual in vivo events is hazardous at best. It has been shown that Clq preferentially binds with the IgG heavy chains of subclass 3 and 1, to a lesser extent with IgGs and little, if at all, with IgM molecules (4). It is likely that

* Determined by Dr. Howard Grey.

the quantitative distribution of IgG subgroups is of importance in the metabolism of Clq.

A second mechanism for the decreased Clq was suggested in the patient with SLE. In this instance, the increased turnover could be related to the underlying active disease process which, in addition to a decreased Clq, was manifested by a low total complement, decreased C4 and C3, the presence of serum cryoproteins, an abnormal urine sediment, and mild arthralgias. By immunoelectrophoresis of the washed concentrated cryoprecipitate, which contained a high fraction of the plasma Clq-{³¹P} (mean 0.61 ± 0.05) throughout the study, only IgG and Clq were detected. Hanauer and Christian found both IgG and IgM in addition to Clq in their studies of SLE cryoproteins which were analyzed with antisera to individual cryoproteins from six patients (25). We did not attempt to raise specific antisera to our patient’s cryoprecipitate.

Morse, Müller-Eberhard, and Kunkel (5) first reported low serum Clq in SLE as have Hanauer and Christian, using radial immunodiffusion (6). In both studies this decrease was associated with disease activity and low total complement titers. Kohler and ten Bensel found that the most consistent complement abnormality in SLE was a decreased fourth component which often occurred with normal total complement activity and normal concentrations of Clq, C3, and C5 and in the absence of overt disease (7). Subsequent metabolic studies have demonstrated an increased turnover of C4 in patients with SLE even when their disease is inactive and their levels of C4 are normal (26). It is probable that the increased Clq turnover present in the patient with SLE was due to utilization by the disease process, a mechanism distinctly different from that present in the hypogammaglobulinemic and myeloma patients.

More profound decreases of serum Clq have been documented in individuals with Swiss-type lymphopenic agammaglobulinemia compared with the two hypogammaglobulinemic patients in the present study (11, 13, 14). Of great interest is the fact that normal Clq concentrations are present in Swiss-type patients by both hemolytic and radial diffusion quantitation suggesting that the synthesis and/or catabolism of these subunits of C1 are independent (11, 14). In all likelihood the synthesis of Clq and C1s is independent. However, the fact that density gradient studies indicated uptake of injected Clq-{³¹P} into the circulating trimolecular C1 complex suggests that the catabolism of all three subunits of C1 is interrelated. The possibility also remains that a Clq synthesis defect may be present in Swiss-type lymphopenic agammaglobulinemia whereas it is clearly not in the immunoglobulin disorders included in this study.
ACKNOWLEDGMENTS

The excellent technical assistance of Mr. Marlin Noziger and Miss Mary Ann Calcott is gratefully acknowledged. We thank Dr. E. B. Reeve for his critical review of the experimental data.

This work was supported by grant (FR-51) from the General Research Centers Program of the Division of Research Resources, National Institutes of Health and U. S. Public Health Services grants HD-A1 03381 and A1 00013.

REFERENCES


Metabolism of Human C1q 875