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INTERACTION OF Clq SUBCOMPONENT WITH IMMUNOGLOBULIN M

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The affinity of human Clq subcomponent for IgM of normal human serum and Waldenström macroglobulins of patients Sew and Zuk were investigated by the polyethylene glycol 6000 immune complexes precipitation test. This test was calibrated with heat-aggregated γ-globulin (HAGG); maximum fixation of Clq ranged from 60 to 80% (measured as percentage of radioactivity of the immune complexes precipitate) and was observed when the Clq:HAGG concentration ratio was about 1:250. At the ratio of 1:20 the radioactivity of the precipitate was about 43%, of the total. The capacity of polyclonal IgM and Waldenström macroglobulins for Clq fixation is low and variable. The percentage of Clq fixed at the Clq:IgM ratio of 1:20 for polyclonal IgM and Zuk macroglobulin was about 9%, whereas for Sew it was only about 1%.

Fixation of Clq subcomponent to immune complexes is the first step of the classical pathway of complement system activation [1]. It is generally agreed that the Fc fragments of IgG or IgM, and the globular head of the Clq molecule are involved in these interactions [2, 3]. The affinity of immunoglobulin for Clq seems to be dependent on conformational or allosteric changes of the Fc region resulting from antigen binding, aggregation of immunoglobulins or physico-chemical modification of immunoglobulin molecule [4].

There is no general agreement as far as interaction of Clq with intact immunoglobulin molecules is concerned. Some authors believe that native IgM does not interact with activated Clq subcomponent. On the other hand, there is ample evidence that the pentameric structure of IgM enables significant fixation of Clq, although it does not seem to activate further steps of the complement pathway [5].
In the present study we tried to evaluate the interaction of human C1q with IgM isolated from normal human serum and from serum of patients with Waldenström macroglobulinaemia.

MATERIAL AND METHODS

Human IgM and Waldenström macroglobulins were isolated from normal serum and from sera of patients Sew and Zuk, respectively, as described previously [6].

C1q subcomponent was purified from human serum by the repeated precipitation method [7].

Purity of all proteins was assessed by polyacrylamide gel electrophoresis [8] and immunoelectrophoresis [9] against anti-whole, anti-μ and anti human C1q antisera (Behring, Frankfurt, F.R.G.).

Concentration of IgM was measured by radial immunodiffusion on ready-made Immunoplates (Hyland Lab., Costa Mesa, CA U.S.A.). For C1q determination single radial immunodiffusion [10] was performed using 1% agarose containing appropriately diluted rabbit antisera against human C1q in 50 mM Tris buffer, pH 8. The lowest limit of sensitivity was approximately 0.2 mg/ml.

Iodination of C1q was performed by the chloramine T method [11]. Both chloramine T and pyrosulphite solutions were prepared ex tempore. Na$^{125}$I (0.5 mCi/ml, 150 Ci/mol) was from OPIDI (Świerk, Poland). Iodinated C1q was separated from free Na$^{125}$I by gel filtration on Sephadex G-25 column and stored frozen at −20°C until use.

The interaction of IgM with C1q was estimated by the immune complexes precipitation test with polyethylene glycol$^1$ 6 000 [12], adapted to our conditions. Protein solutions (50 μl) were incubated for 30 min at 37°C with 100 μl of 200 mM EDTA, pH 7.5, containing 2.5% of human serum albumin. After cooling in the ice-bath, 50 μl of iodinated C1q and 1 ml of 3% PEG 6 000 in 100 mM borate buffer, pH 8.3, were added. The mixture was incubated for 60 min at 4°C and centrifuged at 1 500 g for 20 min at 4°C. The supernatant was discarded and the radioactivity of the precipitate was measured in a gamma counter ZM-701 (Polon, Warszawa, Poland).

The calibration curve was prepared using increasing concentrations of HAGG. The solution of human γ-globulin, 25 mg/ml (Serum and Vaccine Factory, Lublin, Poland) was incubated for 12 min at 63°C and then for 30 min at 4°C. After centrifugation, appropriate dilutions were prepared in 200 mM EDTA, pH 7.5, containing 2.5% of human albumin. The blank sample contained 50 μl of EDTA buffer instead of protein solution. In controls

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$^1$ Abbreviations used: PEG 6 000, polyethylene glycol 6 000; HAGG, heat-aggregated γ-globulin
for radioactivity determination, 1 ml of 10% trichloroacetic acid was used instead of PEG solution. The interaction of C1q with the isolated IgM was estimated in triplicate using five different dilutions of each sample.

RESULTS

The IgM preparations isolated from normal human serum and sera of patients Sew and Zuk with Waldenström macroglobulinaemia showed on polyacrylamide gel electrophoresis only one slowly migrating band. Their homogeneity was confirmed by immunoelectrophoresis in which a single precipitation arc against whole anti-human rabbit antiserum was observed in the slow γ region. Final concentrations of polyclonal, Sew and Zuk IgM solutions were 2.5, 3.0 and 2.8 mg/ml, respectively. No contamination by IgG was found on immunodiffusion against antiserum to human IgG (sensitivity of this method is lower than 2 μg/ml).

The purified C1q was also homogeneous in polyacrylamide gel electrophoresis and on immunoelectrophoretic examination. On immunoelectrophoresis against whole anti-human rabbit antiserum a single precipitation arc near the application place was observed. Final concentration of C1q was 0.57 mg/ml.

This C1q solution was used for 125I iodination as described in Methods. Results of separation of the radioiodinated C1q from free Na125I are presented in Fig. 1.

![Fig. 1. Sephadex G-25 gel filtration of 125I-C1q. Three 1 ml fractions showing high radioactivity and protein content were pooled](image)

The fractions corresponding to the first peak of radioactivity, eluted at the void volume of the Sephadex G-25 column, were collected and concentrated by dialysis against dry Dextran. Final concentration of C1q was 100 μg/ml and specific radioactivity 0.42 μCi/μg of protein.

The ability of iodinated C1q to form complexes was checked by addition of HAGG in the PEG immune complexes precipitation test (Fig. 2).
The addition of increasing amounts of HAGG resulted in an increase of radioactivity in the precipitate formed in the PEG 6000 precipitation test. However, even when HAGG concentration was raised to 1.25 mg/sample (C1q:HAGG ratio of 1:1250) only about 80% of the control radioactivity was recovered.

The effect of immunoglobulin concentration on C1q fixation by polyclonal IgM and macroglobulins of patients Sew and Zuk is summarized in Table 1; the radioactivity of these proteins, expressed as percentage of control radioactivity, is presented in Fig. 3.

![Graph](image1)

**Fig. 2.** C1q (5 μg/sample) fixation by increasing amounts of heat-aggregated γ-globulin, expressed as percentage of control radioactivity in the PEG 6000 immune complexes precipitation test.

![Graph](image2)

**Fig. 3.** C1q (5 μg/sample) fixation by increasing amounts of IgM isolated from normal human serum (○), and from Waldenström macroglobulins from patients Sew (●) and Zuk (▲), expressed as percentage of control radioactivity in the PEG 6000 immune complexes precipitation test.

The affinity of C1q for IgM is rather low and there are significant differences in C1q fixation by polyclonal IgM and one of the Waldenström macroglobulins. The maximum C1q fixation did not exceed 12%, even at the C1q:IgM ratio of about 1:25. The percentage of 125I-C1q remaining in the immune complexes precipitate at the ratio of proteins 1:20 was about 8.4% for polyclonal IgM, 10.3% for Zuk macroglobulin and only 0.6% for IgM isolated from serum of Sew patient.
Table 1

Amount of polyclonal IgM and Waldenström macroglobulins used for fixation of 5 µg Clq in the PEG 6000 immune complexes precipitation test

The results are expressed as percentage of the control sample radioactivity found in the PEG 6000 immune complexes precipitate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Polyclonal IgM</th>
<th>Macroglobulin Sew</th>
<th>Macroglobulin Zuk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td>% of fixation</td>
<td>µg</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>1.4</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
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<td>3</td>
<td>75</td>
<td>6.0</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>125</td>
<td>12.4</td>
<td>150</td>
</tr>
</tbody>
</table>

DISCUSSION

It is now generally accepted that the Clq binding site is localized within a region corresponding to the Cµ4 domain of the IgM molecule [13]. The Clq binding capacity may be related to the amino acid sequence but the conformation of the µ chain in this region seems to be more important [14]. The variable affinity for Clq of intact IgM molecules and their (Fc), µ fragments [15], soluble immune complexes [16] and cell-bound IgM [5] supports this supposition.

Our experiments suggest that the interaction of Clq with polyclonal IgM is weak when both proteins are solubilized, and it probably does not play a significant role in vivo unless IgM conformation is changed by immunological reactions. Although some modification of Clq and IgM molecules during the isolation procedure, and its effect on bonding properties should be considered, this hypothesis is in agreement with previous results of other authors. For example, Füst et al. [17] demonstrated low affinity of Cl for various IgM preparations using measurement of Cl consumption by the haemolytic method. As far as we know, polyclonal IgM from normal human serum has not been used before in this kind of experiments.

The opinions on the affinity of various monoclonal IgM for Clq are divergent. Augener et al. [18] did not observe significant differences between 12 monoclonal IgM preparations. On the other hand, Füst et al. [17] found even 12-fold differences in the Clq fixation capacity of various monoclonal immunoglobulins. Also in our experiments significant differences between monoclonal IgM from patients Sew and Zuk were observed, whereas the
amount of the Clq-IgM precipitates formed with Zuk and polyclonal IgM were nearly the same.

In the previous study [19] the differences in amino acid composition of the IgM μ chains from normal human serum and some monoclonal IgM have been described. It seems possible that in some cases these differences may concern the amino acid sequence of the domain responsible for Clq fixation and thus affect the capacity to form complexes with Clq molecules, as well as some other immunoglobulin properties.

REFERENCES


