Assessment of complement activation in human disease

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Chapter 5

Studies on the haemolytic activity of circulating C1q-C3/C4 complexes
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Abstract
During classical complement pathway activation, the internal thio-ester of both C3 and C4 becomes exposed which enables C3 and C4 to bind covalently to nearby molecules. Recently, we described that C3 and C4 bind to C1q, the recognition molecule of the classical pathway, upon activation of this pathway. Covalently linked complexes between C1q and activated C4 (C1q-C4 complexes) are specific markers for classical complement pathway activation. In the present study we further investigated the molecular characteristics of complexes between C1q and activated C3 or C4 that occur in vivo. In human serum only complexes of C1q with C3d or C4d fragments were detected but not those with the larger C3b/bi or C4b/bi fragments. We identified that C1q-C4 complexes circulate as part of the intact C1 complex instead of as free C1q. Finally, we investigated whether deposited C3d or C4d affect C1 haemolytic activity. We observed that C1q-C3 and C1q-C4 complexes are less active in a C1q-haemolytic assay than non-complexed C1q. Thus, the dominant types of C1q complexes that circulate in vivo are C1q-C3d and C1q-C4d complexes. These complexes are still able to interact with C1r and C1s to form a C1-complex, but seem to have a reduced activity as compared to C1q not carrying C3- or C4-fragments.
Introduction

The effector pathway of the complement system can be activated via three pathways, the classical, the lectin or the alternative pathway (1, 2). The classical pathway (CP) is triggered by interaction of C1 with immune complexes, containing either IgG or IgM. C1, the first component of the CP, is a calcium-dependent complex of the recognition molecule C1q and a tetramer comprising two copies of each of the C1r and C1s serine proteases (3-5). The interaction of C1 with immune complexes induces a conformational change in the C1q molecule, thereby converting the inactive pro-enzymes C1r and C1s to activated proteases (4, 6). Activated C1s cleaves the C4 molecule, thereby generating C4b, which attaches to a pathogenic surface.

The C4 molecule contains an internal thio-ester. In intact C4 this thio-ester is shielded, and becomes exposed upon cleavage by C1s, which allows it to be hydrolysed or to bind to biological targets. The thio-ester of the majority of activated C4 molecules reacts with surrounding water molecules and will thereby be inactivated. The thio-ester of a substantial part of activated C4, however, will bind covalently to hydroxyl- or amino-groups on nearby surfaces (7, 8). Fixation of the complement protein C3 to biological targets occurs via an identical mechanism i.e. via an internal thio-ester that is exposed upon cleavage of the C3 molecule (9-11).

Recently we described that upon CP activation, C4 and C3 not only bind to the activator, but also to the C1q molecule itself (12). The resulting covalently linked complexes between C1q and activated C4 (C1q-C4 complexes) appear to be specific and highly stable activation markers for the CP in patients suffering from complement mediated disease such as rheumatoid arthritis (13). In the present study we further elucidated the molecular characteristics of circulating complexes between C1q and activated C3 or C4 (C1q-C3 or C1q-C4 complexes, respectively). We studied the composition of the complexes circulating in humans. Furthermore we investigated the functional activity of C1q-C3 and C1q-C4 complexes.
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Materials and Methods

**Plasma and serum samples**
Normal human serum and plasma were obtained from healthy blood bank donors. To obtain recalcified plasma, frozen EDTA plasma was thawed and reconstituted with 12 mM CaCl₂ after which the plasma was allowed to clot at 37°C for 20 min. Thereafter the sample was centrifuged for 10 min at 1,300 g to remove the fibrin clot. All samples were stored at -70°C until tested.

**Proteins and antibodies**
C1q was isolated from pooled human recalcified plasma according to the method of Tenner (14). The protocol was preceded by a precipitation step with polyethylene glycol-6000 (PEG; Merck, Darmstadt, Germany) (4% w/v in 0.1 M boric acid) at 4°C. MAb anti-C1q-2 is directed against the collagen part (“stem”) of C1q and has been described before (12, 15). MAb anti-C1q-85, which is directed against the globular heads of C1q and inhibits activation of C1q by immune complexes, also has been described before (15, 16). MAb anti-C4-4 is directed against the C4d fragment, recognizing both native and activated C4. The use of this antibody in immunoassays has been described previously (12, 17). MAb anti-C3-19 reacts with an epitope on the C3d region of the C3 molecule (18). Both mAbs anti-C4-1 and anti-C3-9 react specifically with neo-epitopes on C4b/bi/c (C4b/c), or C3b/bi/c (C3b/c), respectively, and were also described previously (19).

**Sucrose gradient ultracentrifugation**
Isokinetic sucrose density gradients (5-25%, w/v, sucrose; Merck) were prepared in either veronal buffered saline, pH 7.4 (VB), supplemented with 10 mM CaCl₂ and 2 mM MgCl₂ (VB⁺⁺) or in phosphate buffered saline, pH 7.4 (PBS), containing 10 mM EDTA (PBS/EDTA). Serum and plasma (300 μl, diluted threefold in the appropriate buffer) were layered onto sucrose gradients that were made in VB⁺⁺ or PBS/EDTA, respectively. The gradients were centrifuged in a Beckman swing out rotor type SW 41 at 36,000 rpm for 20 hrs at 4°C. Fractions of 500 μl were collected. Specific protein concentrations were determined by ELISA except for IgM levels which were determined by nephelometry.

**C1q determination**
MAb anti-C1q-2, diluted to a concentration of 2 μg/ml in 0.1 M carbonate buffer, pH 9.6, was coated onto ELISA plates (Nunc Maxisorp; NalgeNunc Int., Roskilde, Denmark) by overnight incubation at room temperature. This and all other incubations were performed with a final
volume of 100 μl. After five washes with PBS containing 0.02%, w/v, Tween-20 (PT), plasma samples, diluted in ELISA buffer (high performance ELISA buffer [HPE; Business Unit Immune reagents, Sanquin, Amsterdam, the Netherlands] supplemented with 0.5 M NaCl to prevent non-specific binding of C1q to the IgG coated on the plates) were incubated for 1 h at room temperature. Subsequently, plates were washed five times in PT and incubated for 1 h at room temperature with biotinylated anti-C1q-85 to detect C1q. After five washes with PT, wells were incubated with 0.1%, v/v, streptavidin-peroxidase (Amersham/Pharmacia, Uppsala, Sweden) for 30 min at room temperature. Finally, peroxidase activity was visualized with 100 μg/ml tetramethylbenzidine in 0.11 M sodium acetate, pH 5.5, containing 0.003%, v/v, H₂O₂. The reaction was stopped with 2 M H₂SO₄ and absorbance was measured at 450 nm with a Titertek multiscan.

**C1q-C3 and C1q-C4 determination**

C1q-C3 and C1q-C4 complexes were measured in an ELISA procedure recently described (12). However, testing of the sucrose gradient fractions was done without separation of C1q-C3 and C1q-C4 complexes from unbound C3 and C4 by ammonium sulphate precipitation, since these compounds were already separated on the gradient because of their different molecular size. In the ELISA mAbs anti-C3-19 and anti-C4-4, recognizing the C3d and the C4d fragment, respectively, were used as catching antibodies. Biotinylated mAb anti-C1q-85 was used for detection. For optimal results, polymerized streptavidin-peroxidase (Business Unit Reagents, Sanquin Research) was used. Results were expressed in arbitrary units (au), 100 au being the amount of complexes in a C1q sample purified from human plasma. To detect complexes between C1q and C3b/bi or C4b/bi fragments, a similar assay was used but with the mAbs anti-C3-9 and anti-C4-1 as catching antibodies.

**Determination of activated C4**

Overall complement activation was measured by assessing the amount of activated C4 using an ELISA for C4b/c as described previously (19). Briefly, mAb anti-C4-1 was used as catching antibody and biotinylated polyclonal rabbit anti-human C4c for detection. Notably, the activation products recognized in the assay are C4b, C4bi and C4c, which are together referred to as C4b/c. Results were expressed as nM, referring to an in house standard containing known levels of activated C4.
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**Isolation of C1q-C3 and C1q-C4 complexes**

C1q-C3 and C1q-C4 complexes were isolated from purified C1q by affinity chromatography. C1q (purified from normal human plasma and containing both C1q-C3 and C1q-C4 complexes) was applied onto either anti-C3-19 or anti-C4-4 coupled Sepharose (CNBr-activated Sepharose 4B; Amersham/Pharmacia), which was first equilibrated with application buffer (PBS/0.5 M NaCl). Bound C1q-C3 and C1q-C4 complexes were eluted with 0.1 M citrate buffer, pH 3.5. Fractions of 500 μl were collected and immediately neutralized with 2 M Tris buffer, pH 8.8. The concentration of C1q-C3 and C1q-C4 complexes in the preparation was determined by ELISA as described above. Positive fractions were pooled and dialysed against PBS. The isolated complexes were stored at -70°C.

**C1q haemolytic assay**

Sheep red blood cells (SRBC) were sensitized with mouse monoclonal IgM antibodies against SRBC by incubation in a shaking water bath for 30 min at 37°C. The sensitized cells were suspended at a concentration of 1.5×10⁸ cells/ml in VB containing 5.8% (w/v) sucrose, 0.5% (w/v) human serum albumin (HSA), 1 mM MgCl₂, 0.2 mM CaCl₂, pH 7.4 (VBS). 70 μl sensitized SRBC suspension was incubated with C1q depleted serum (C1qD; Quidel, San Diego, USA, 30 μl, diluted fourfold in VB supplemented with 0.05% (w/v) gelatine (VBG)). Finally, 100 μl C1q, C1q-C3 or C1q-C4 (appropriately diluted in VBG) was added and assessed for haemolytic activity. After 1 h incubation at 37°C in a shaking water bath, the cells were centrifuged and absorbance was measured at 412 nm on a Titertek multiscan. Lysis was expressed as percentage of total SRBC lysis in water, which was 100%. Background lysis in the absence of C1q sample was 6.8%.

**Statistics**

To analyze differences in complement activation by PEG in recalcified plasma and EDTA plasma, a paired Student t-test was performed. Mann-Whitney test was performed to analyze differences in haemolytic activity between uncomplexed C1q and C1q-C3 and C1q-C4 complexes. Two sided P-values less than 0.05 were considered to be statistically significant.
Results

**Human plasma only contains complexes between C1q and late cleavage products C3d and C4d**

Using mAb anti-C4-4, which recognizes an epitope on the C4d fragment, as catching antibody, C1q-C4 complexes were detected at low levels in plasma obtained from healthy donors. C1q-C4 complexes were also detected in a C1q preparation purified from human plasma and this preparation was used as a standard in the assay (Fig. 1A). Since the anti-C4-4 antibody recognizes activated and non-activated C4, we questioned which fragment of C4 is attached to the C1q-molecule. To examine this, we performed a C1q-C4 complex ELISA with mAb anti-C4-1 as catching antibody, which is specific for a neo-epitope on C4b/bi/c, thereby detecting C1q-C4b/bi complexes (C4c is fluid phase).

As shown in figure 1B, C1q-C4b/bi complexes could not be detected in human plasma, while this plasma tested positive for C1q-C4d complexes. As control, we tested whether anti-C4-1 can be used as catching antibody in another assay with polyclonal anti-C4 as detecting antibody.

![Figure 1](image-url)

**Figure 1.** Human plasma contains C1q-C4d but no C1q-C4b/bi complexes. (A) C1q-C4 complexes were measured using anti-C4-4 (against C4d) as a catching antibody. Samples tested were recalcified plasma, purified C1q or recalcified plasma precipitated with 4% PEG. (B) C1q-C4 complexes could not be detected in recalcified plasma using anti-C4-1 (C4b/bi specific) as catching antibody. Results were compared with anti-C4-4 coat. (C) To test the catching ability of anti-C4-1, recalcified plasma was titrated on both anti-C4-4 and anti-C4-1 with polyclonal anti-C4 as detecting antibody. Results in the figures represent mean and SEM (error bars) of 6 (A) or 3 (B and C) donors.
Figure 1C shows that anti-C4-1 is a good catching antibody, which was expected as we have used this antibody previously in assays for activated C4 (19). Most likely, C4b bound to C1q was completely cleaved to C4d by factor I and its cofactors present in plasma, thereby releasing the epitope that is recognized by the anti-C4-1 antibody. For C1q-C3 complexes similar results were found. C1q-C3b/bi complexes could not be detected in human plasma, while C1q-C3d complexes were found to be present (data not shown).

In the C1q standard (which was purified from human plasma obtained from healthy donors), we observed much higher levels of C1q-C4 complexes as compared to C1q-C4 levels in normal plasma (Fig. 1A). The first step in C1q purification is precipitation of recalcified plasma with 4% w/v PEG. To establish whether the high C1q-C4 levels in the standard may be explained by precipitation with PEG, we performed the C1q-C4 ELISA in a PEG precipitate from recalcified plasma. This precipitate was prepared by incubating one volume of recalcified plasma with one volume of 8%, w/v, PEG-6000 in 0.1 M boric acid. After incubation for one h at 4°C precipitates were centrifuged for 30 min at 1,300 g and dissolved in ELISA buffer. Figure 1A shows that C1q-C4 levels were indeed markedly raised in plasma after incubation with PEG, notwithstanding the fact that C1q purification is carried out at 4°C.

To investigate whether the increase in C1q-C4 levels after incubation with PEG is due to classical pathway activation, we measured levels of activated C4 in PEG treated plasma using the C4b/c ELISA. As depicted in figure 2, C4b/c levels were significantly increased after incubation of recalcified plasma with 4% PEG. Recalcification alone was not sufficient to activate complement and EDTA inhibited the PEG-induced increase in C4b/c levels, indicating that the measured complement activation indeed resulted from incubation with PEG.

![Figure 2. PEG induces complement activation in recalcified plasma.](image)

Complement activation was assessed by C4b/c ELISA in EDTA plasma and recalcified plasma (RP) of healthy donors. Incubation with 4% PEG resulted in significantly elevated C4b/c levels in RP ($P < 0.0001$), as compared to EDTA plasma. C4b/c levels are depicted as nM. Data are presented as means (n=3) with standard deviations.
C4 is deposited on C1q in the C1 complex

Under physiologic conditions, it is assumed that hardly any free C1q is present in the circulation since most, if not all, C1q is associated with 2 C1r and 2 C1s molecules to form the Ca\(^{++}\) dependent C1-complex. Only after activation of the CP, the C1 complex is dissociated: C1-inhibitor (C1inh) binds to activated C1r and C1s to generate two C1inh-C1s-C1r-C1inh complexes which subsequently dissociate from the C1q molecule (20). Thus, theoretically in the human circulation, C1q-C4 complexes could exist either as part of the C1-complex (i.e. associated with the pro-enzymes C1r and C1s) or as free C1q molecules with C4 fragments attached to it. To this end, EDTA-plasma and serum (obtained from a healthy donor with measurable amounts of C1q-C4 complexes) were fractionated on sucrose gradient ultracentrifugation. The position of C1q, measured with ELISA, in the gradient is indicated with solid lines and that of C1q-C4 with dashed lines. The position of IgM and albumin, measured as internal markers, is indicated by arrows. Top of the gradient is to the right of the figure.

**Figure 3.** C1q-C4 complexes circulate as part of the C1-complex. EDTA plasma (A) and serum (B) of a healthy donor with measurable C1q-C4 levels were fractionated on sucrose gradient ultracentrifugation. The position of C1q, measured with ELISA, in the gradient is indicated with solid lines and that of C1q-C4 with dashed lines. The position of IgM and albumin, measured as internal markers, is indicated by arrows. Top of the gradient is to the right of the figure.
In figure 3A, the position of C1q and C1q-C4 in fractionated EDTA plasma is shown, as determined with ELISA. In this sucrose gradient, the C1q peak was found in fraction 12 and the peak of C1q-C4 complexes in fraction 11. The C1 complex will be completely dissociated in C1q and two molecules of each C1r and C1s, because of calcium chelation by EDTA. Therefore, we conclude that the C1q peak indicated in fraction 12 is the position of free, non-associated C1q.

The position of both C1q and C1q-C4 in fractionated serum is indicated in figure 3B. In this sucrose gradient we identified the C1q peak in fraction 10 and that of C1q-C4 complexes in fraction 9. The gradient was run in the presence of calcium, so the C1-complex remained intact. Since we detected C1s in fraction 10 as well (data not shown) and C1q and C1s migrate together under calcium containing conditions (because they are both part of the C1-complex), we conclude that fraction 10 contained intact C1.

When serum was fractionated on a sucrose density gradient under physiologic conditions, C1q-C4 complexes migrated like intact C1, indicating that in human serum, C1q-C4 complexes circulate as part of the intact C1-complex. In both serum and plasma C1q-C4 complexes sediment slightly faster than C1q; this is in agreement with the increased molecular weight as result of C4 deposition.

**Purification of C1q-C3 and C1q-C4 complexes**

C1q-C3 and C1q-C4 complexes were isolated from a purified C1q preparation by affinity chromatography. The C1q-C3 and C1q-C4 complexes were purified using anti-C3-19 and anti-C4-4 coupled to Sepharose, respectively. Figure 4 shows the enrichment for C1q-C4 complexes after purification on anti-C4-4 coupled to Sepharose. Relative to the C1q concentration, the isolated C1q-C4 sample contained 36 times more C1q-C4 complexes as compared to the starting material. Assuming that the isolated sample completely consisted of C1q-C4 complexes, 2.8% of the C1q molecules in the starting material would be in complex with activated C4. However, we cannot exclude the possibility that the purified C1q-C4 sample contains some uncomplexed C1q molecules.

In a previous study, we have immunoprecipitated C1q-complement complexes from a purified C1q preparation using either anti-C3-19 or anti-C4-4 coupled Sepharose. Precipitates were electrophoresed and subsequently immunostained with anti-C4-4 on Western blot (12). It appeared that precipitated C1q-C3 complexes could not be made visible on blot with anti-C4-4, indicating that C3d- and C4d fragments are attached to separate C1q molecules.
C1q-C3 and C1q-C4 complexes have a lower haemolytic activity than C1q

To investigate whether deposition of C3 and C4 affects the haemolytic activity of the C1q molecule, we performed a C1q haemolytic assay (21) in which C1qD was supplemented with either C1q, isolated C1q-C3 or isolated C1q-C4 complexes. Equimolar amounts of purified proteins were added, which was based on the C1q concentration in the samples. As shown in figure 5A, purified C1q restored the haemolytic activity of C1qD. To reach 50% lysis of SRBC under the experimental conditions, C1q at a mean final concentration of 5 ng/ml had to be added to C1qD (Fig. 5B).

It cannot be excluded that the elution procedure of C1q-C3 and C1q-C4 might affect the haemolytic activity of C1q. Therefore we eluted C1q from anti-C1q-2 coupled Sepharose with 0.1 M Citrate buffer pH 3.5, similar to the isolation of the complexes. This low pH treatment resulted in a minimal change in haemolytic activity of C1q (Fig. 5), indicating that any observed reduction in haemolytic activity of isolated C1q-C3 or C1q-C4 complexes likely resulted from the deposition of C3 or C4 on the C1q molecule.

C1q-C4 complexes purified from the C1q preparation were significantly \( (P < 0.05) \) less active in the haemolytic assay, i.e. to achieve 50% haemolysis a mean concentration of 10.5 ng/ml of C1q complexed to C4 was needed. However, C1q-C3 complexes were much less active, requiring a mean of 25.3 ng/ml to reach 50% lysis \( (P < 0.01) \) (Fig. 5B).
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Figure 5. Haemolytic activity of C1q, isolated C1q-C3 and isolated C1q-C4 complexes
(A) SRBC lysis by C1qD supplemented with purified C1q, purified C1q treated with 0.1 M citrate pH 3.5, isolated C1q-C4 complexes and isolated C1q-C3 complexes. Percentage of lysis was compared to SRBC lysis by water, which was set at 100%. Background lysis by buffer alone was 6.8%. This figure is representative for three experiments which yielded similar results. (B) For the various C1q preparations the concentration that yielded 50% lysis of SRBCs is depicted. Results represent mean and SEM (error bars) of 3 experiments.

Discussion
C1q-C4 complexes are specific activation markers of the classical pathway of complement. In the current paper, we further analysed the molecular characteristics of these covalently linked complexes as occurring in the human circulation. Using various monoclonal antibodies against different forms of C3 and C4, we found that human serum only contained complexes between C1q and the late cleavage fragments C3d or C4d. We did not detect complexes between C1q and the larger fragments C3b/bi or C4b/bi in serum. This may be explained by the presence of factor I and its cofactors C4-binding protein and factor H in human serum, which cleave the activated C3 and C4 molecules leaving only the thio-ester containing fragments (C3d and C4d) on C1q. The amount of C1q-C4 in a purified C1q preparation was set at 100 au/ml and C1q-C4 levels in human plasma were compared to this standard. In normal plasma, C1q-C4 levels never exceeded 1 au/ml, which is hundred times lower than the amount of complexes present in the standard. In patients suffering from complement-mediated disease such as rheumatoid arthritis, C1q-C4 levels are higher than in healthy controls (22) but never that high as in purified C1q. This discrepancy apparently was due to the effect of PEG, which is used in the purification procedure of C1q. After incubation of recalcified plasma with PEG, levels of C1q-C3 and C1q-C4
complexes were markedly elevated. We found increased overall complement activation following incubation with PEG, as assessed by measuring C4b/c levels. This phenomenon only occurred in recalcified plasma and was completely abolished in the presence of EDTA. The above mentioned results indicate that incubation of human recalcified plasma with PEG, eventually leads to activation of the classical pathway, even at 4°C (PEG incubation occurs at 4°C). PEG has been described before to enhance the binding of C1q to circulating immune complexes but also to monomeric IgG (23). Presumably, PEG also induces in vitro aggregation of immunoglobulins, thus forming immune complex-like aggregates, which are capable to activate the classical pathway of complement.

As discussed in the previous paragraph, the high levels of C1q-complexes measured in the standard preparation were artificially induced by incubation with PEG. In contrast, ammonium sulphate, which is used in the C1q-C4 ELISA to separate C1q-C4 complexes from unbound C4, does not activate the classical pathway presumably due to its high ionic strength which prevents fruitful interaction of C1q with IgG aggregates. Moreover, C1q-C4 complexes are usually determined in EDTA plasma, which does not allow complement activation via the classical pathway, as was also shown for PEG precipitation.

It should be noted that with the C1q-C4 ELISA, we may have underestimated the amount of C1q molecules that are complexed with activated C4. The C4 molecule is a highly polymorphic protein that consists of two main isotypes, C4A and C4B. The monoclonal antibody that is used in the C1q-C4 ELISA (anti-C4-4) is specific for the C4A isotype. Therefore, we only measure complexes between C1q and C4A and complexes between C1q and C4B are neglected. Currently, we are investigating whether these latter complexes are also formed upon classical pathway activation.

On sucrose gradients, C1q-C4 complexes in serum migrated at the position of the intact C1 complex, indicating that C4 fragments deposited on C1q do not abrogate the interaction with the other constituents of the C1 complex. In agreement herewith, significant haemolytic activity of C1q complexes was found. We have no information on the number of C3d or C4d molecules on one molecule of C1q. Hence, it remains to be elucidated whether additional C3 or C4 deposited on a C1q molecule, as for example may occur in inflammatory conditions, may further hamper or even abrogate the haemolytic function of C1q.

To investigate the haemolytic activity of C1q-C3 and C1q-C4 complexes, we isolated the complexes from a purified C1q preparation by affinity chromatography on anti-C3-19 or anti-C4-4, respectively, coupled to Sepharose beads. C1q-C3 and C1q-C4 ELISAs were performed in the flow through as well as in the eluted fractions. It appeared that in general C3 and C4
fragments are attached to separate C1q molecules, since the majority of C1q-C3 complexes were recovered in the flow through from the anti-C4-4 Sepharose column, and vice versa. This is in accordance with earlier observations in which immunoprecipitated C1q-C3 complexes could not be detected on Western blot with mAb anti-C4-4 (12). However, trace amounts of complexes containing both C3 and C4 fragments could be detected by ELISA in the eluate of both anti-C3-19 and anti-C4-4 columns.

We observed significantly reduced haemolytic activity of isolated C1q-C3 and C1q-C4 complexes as compared to uncomplexed C1q. Since we found no effect of the isolation procedure, we conclude that the reduction in haemolytic activity is likely caused by the deposition of C3 and C4 activation fragments on the C1q molecule. However, we cannot exclude that the isolated complexes contain some contaminating non-complexed C1q. Since part of the observed haemolysis by the isolated complexes might be caused by contaminating non-complexed C1q, we may even have underestimated the inhibitory effect of C3 and C4 deposition on C1q.

We observed no difference in binding to IgG between C1q and isolated C1q-C4 complexes (data not shown), indicating that the observed reduced haemolytic activity of the isolated complexes was not caused by hindering C1q binding to its ligands. Furthermore, we have shown that C1q-C4 complexes circulate as part of the intact C1-complex, indicating that deposition of C4 to C1q does not prevent the interaction of C1q with the proteases C1r and C1s. One possible explanation for the reduced haemolytic activity of the isolated complexes is that deposition of C3 and C4 fragments might influence the ability of C1q to change its conformation which is necessary for activation of C1r after ligand binding, thereby limiting classical pathway activation. An alternative explanation is that deposition of C4 and C3 fragments to C1q within the C1 complex reduces the access of C1s to its substrates C4 and C2. This would lead to reduced haemolytic activity of the isolated complexes.

In 1986 Ziccardi and colleagues described a mechanism of feedback inhibition whereby C1 turnover is inhibited by nascent C3b and C4b (24). This would act preventively against excessive complement activation in vivo at low concentrations of immune complexes. A possible mechanism behind this inhibition was suggested by the same group in 1988 (25). Nascent C3b binds to the antigen moiety of an immune complex, thereby displacing the complement activating antibody from the complex. Only C1 turnover is influenced by this mechanism and not C1 binding to IgG itself. This effect was mainly described for C3. In addition to these findings, we propose an extra mechanism by which activated C3 and C4 may help preventing excessive complement activation in vivo. This mechanism postulates that activated C3 and C4 may cause
reduced activity of the C1 molecule by forming covalent complexes with C1q. Future studies should reveal to what extent this mechanism is relevant for human inflammatory disease. Moreover, it cannot be excluded that because of C3 or C4 deposition, the C1q molecule gains in function.

References


