Assessment of complement activation in human disease
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Citation for published version (APA):
Chapter 4

Evaluation of classical complement pathway activation in Rheumatoid Arthritis

Measurement of C1q-C4 complexes as novel activation products

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*Arthritis and Rheumatism*

Abstract

Objective. Novel activation products that are stable and minimally susceptible to in vitro artefacts have recently been described in the classical complement pathway. The present study assessed circulating levels of these products, i.e. covalent complexes between the recognition molecule of the classical pathway (C1q) and activated C4, in plasma samples from patients with rheumatoid arthritis (RA) to establish the relationship between these levels and the clinical and immunological parameters in these patients.

Methods. C1q-C4 levels were measured in the plasma of 41 patients with active RA and 43 patients with inactive RA. These levels were related to other complement activation products and to disease activity according to the Disease Activity Score in 28 joints (DAS28), using Spearman’s rank correlations.

Results. C1q-C4 plasma levels were significantly higher in patients with active RA as compared with patients with RA in clinical remission (median 3.3 arbitrary units [AU], range 0.4-13.4 versus 1.7 AU, range 0.2-5.5; \( P = 0.0001 \)), suggesting that activation of the classical pathway reflects disease activity. This is supported by a significant correlation between C1q-C4 levels and the DAS28 (\( r = 0.398, P = 0.0002 \)). Levels of other complement activation products, such as activated C4 (C4b/c), were also significantly elevated in patients with active disease compared with patients with inactive disease (\( P = 0.03 \)), and were correlated with C1q-C4 levels (\( r = 0.329, P = 0.002 \)). Levels of C1q-C4 complexes were higher in synovial fluid samples than in plasma from the 4 patients tested.

Conclusion. Systemic complement activation via the classical pathway in patients with RA correlates with disease activity. These results indicate that C1q-C4 complexes may be used as a biomarker for RA.
Introduction

Rheumatoid arthritis (RA) is an inflammatory autoimmune disease characterized by chronic inflammation of the joints, eventually leading to bone and cartilage destruction. Although the etiology of RA is unknown, complement activation has been implicated in the pathogenesis of the disease. Various studies identify complement activation as a main event in the inflammatory cascade in RA (1-3). Evidence of complement activation in synovial fluid is abundant. For example, levels of complement proteins are depressed in the synovial fluid of patients with RA, reflecting consumption of complement. Moreover, elevated levels of complement cleavage products, such as sC5b-9, C3a, Bb and C1inh-C1s complexes, have been observed in synovial fluid (4-9).

Involvement of complement in the pathogenesis of RA was also confirmed in experimental studies. Collagen-induced arthritis (CIA), an experimental animal model for human RA, was induced in C3- and factor B deficient mice (10). In complement-deficient mice, arthritis was reduced or completely absent, whereas normal mice were susceptible for CIA, indicating an important role of complement in the induction of disease. Consistent with this observation, systemic administration of a monoclonal anti-C5 antibody prevented CIA in susceptible mice (11).

RA patients have increased levels of circulating immune complexes (12, 13). Part of these complexes contains rheumatoid factors (RFs), which are autoantibodies against human IgG. RF-containing immune complexes are capable of activating complement via the classical pathway (14-17), with IgM-RF being considerably more effective in complement activation than IgG-RF (18). Thus, immune complexes, particularly those in the inflamed joints, are often assumed to be the main trigger for complement activation in RA. It is not clear to what extent circulating immune complexes contribute to complement activation in RA (19). However, other activators of complement may also contribute to complement activation in RA. Levels of the acute-phase protein, C-reactive protein (CRP), are elevated in the majority of patients with RA, and are associated with disease activity. CRP bound to a ligand, can activate complement and there is evidence that CRP-mediated activation of complement occurs in RA (20). Both immune complexes and ligand-bound CRP activate complement via the classical pathway (18, 21-23).

A parameter that reliably reflects complement activation in vivo might constitute a biomarker in RA. However, most, if not all, activation markers of the complement system are susceptible to in vitro artefacts, resulting in artificially high levels of activation products in plasma samples. We recently described novel activation products of complement that not only are specific for activation of the classical pathway of complement, but also are remarkably stable in plasma (24).
The present study was performed to investigate plasma levels of this new complement parameter, which consists of covalent complexes between the recognition molecule of the classical pathway, C1q, and activated C4, in RA. We also sought to establish the relationship between classical pathway activation and other immunological parameters in patients with active or inactive RA, and we assessed the association of these parameters with disease activity.

**Patients and Methods**

**Patients**

We selected patients with active RA (n = 41), and patients with inactive RA (n = 43) from a cohort of 187 patients investigated previously (20). All patients with RA fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatology Association) 1987 criteria for RA (25). Inactive RA was defined by the ACR criteria for clinical remission (26). Disease activity was assessed by calculating the modified Disease Activity Score in 28 joints (DAS28) (27). In addition, 10 patients treated with anti-tumor necrosis factor (anti-TNF) antibody (infliximab) were studied. All patients received 3 mg/kg infliximab at weeks 0, 2, 6 and 14 and every 8 weeks thereafter. Evaluation of disease activity and collection of plasma samples were carried out in these patients at baseline and at weeks 22 and 52 after the start of therapy. The protocol was approved by the local institutional ethics review committee. For control experiments, human EDTA plasma was obtained from 21 healthy volunteers.

**Collection of blood samples**

Blood was collected in 10 mM EDTA (final concentration) and centrifuged for 10 minutes at 1,300g to remove the blood cells. Plasma was stored in aliquots at -70°C until use.

**Proteins and antibodies**

Monoclonal antibody (mAb) anti-C1q-85, directed against the globular heads of C1q, has been described previously (28). The mAb anti-C4-4 was obtained from a fusion of spleen cells from a mouse immunized with C4 and is directed against the C4d fragment, recognizing both native and activated C4. The use of this antibody in immunoassays has been described previously (29). The mAb anti-C4-1, directed against an activation-dependent neoepitope, has also been described previously (30). C1q was isolated from human plasma according to the method of Tenner and colleagues (31).
**Enzyme-linked immunosorbent assay (ELISA) for activated C4**

Overall complement activation was measured by assessing the amount of C4b/bi/c (abbreviated further as C4b/c). For the quantification of these activation products, an ELISA was used that has been described previously (29). Briefly, a mAb (anti-C4-1) recognizing a neoepitope on activated C4 was used as catching antibody. Biotinylated polyclonal rabbit anti-human anti-C4 antibody was used for detection. Since C4 can be hydrolysed by surrounding water molecules, C4b/c levels in plasma or serum may be artificially high due to temperature changes. Aged human serum, containing a known amount of activated C4, was used for the calibration curve.

**ELISA for C1q-C4 complexes**

To measure C1q-C4 complexes, an ELISA that was recently described by our group (24) was used. The catching antibody in this ELISA, anti-C4-4, recognizes both native and activated C4. To prevent saturation of the coating antibody C1q-C4 complexes were separated from unbound C4 by ammonium sulphate precipitation. To this end, 1 volume of plasma was incubated with 1 volume of 66% saturated (final concentration 33% or 1.29M) ammonium sulphate (Merck, Darmstadt, Germany), dissolved in phosphate buffered saline (PBS), pH 7.4 containing 10 mM EDTA. Mixtures were placed on ice for 1 hour, and then centrifuged for 30 minutes at 1,300g at 4°C. Precipitates were dissolved in ELISA-buffer (high-performance ELISA buffer [HPE] [Business Unit Immune Reagents; Sanquin, Amsterdam, the Netherlands] to which 0.5 M NaCl and 10 mM EDTA were added to prevent aspecific binding of C1q and in vitro complement activation, respectively).

The ELISA was then performed with plates coated with anti-C4-4 mAb at 5 μg/ml overnight at room temperature (RT), in 0.11M sodium acetate buffer, pH 5.5. Plates were washed 5 times with PBS-0.02% (weight/volume) Tween prior to 1 hour of incubation with dissolved precipitates at RT. After washing 5 times with PBS-0.02% Tween, plates were incubated for 1 hour at RT with biotinylated mAb against C1q (anti-C1q-85) diluted in HPE to detect C1q-C4 complexes. After 5 washes with PBS-0.02% Tween, plates were incubated with polymerized horse radish peroxidase (Business Unit Reagents, Sanquin), diluted 1:10,000 in PBS-2% (v/v) cow milk for 30 minutes at RT. After 5 washes, the ELISA was developed with 100 μg/ml tetramethylbenzidine in 0.11M sodium acetate, pH 5.5, containing 0.003% (v/v) H2O2. Substrate conversion was stopped by addition of 100 μl H2SO4. Absorbance was measured at 450 nm with a Titertek multiscan. Purified C1q, containing C1q-C4 complexes, was used as the calibration curve for this assay. Levels of complexes in plasma were expressed as arbitrary units (AU), based on the amount of complexes in the purified C1q sample.
**Absorption of RFs from plasma**

One volume of plasma was incubated for 30 minutes at RT with 4 volumes of RF neutralization agent (human gamma globulin-coated microparticles; Abbott Laboratories, Abbott Park, IL) and subsequently centrifuged for 10 minutes at 2,000 revolutions per minute. Supernatant was used for testing.

**Determination of IgM-RFs**

IgM-RFs were measured in a regular ELISA using human IgG (25 μg/ml) as antigen. A positive plasma sample containing 200 international units (IU) of IgM-RF was used for the calibration curve. IgM-RF levels above 12.5 IU/ml were considered to be positive.

**Statistical analysis**

Levels of complement activation products above the upper limit of normal values were considered to be increased. Comparisons between patients with active disease and those with inactive disease were made using the Mann-Whitney, the unpaired t-test or the chi-square test, depending on whether the values were normally distributed. Comparisons between samples before and after freezing and thawing were made using the paired t-test. Correlations between the various complement activation products and disease activity were analyzed using Spearman’s rank correlation coefficients. P values (2-tailed) less than 0.05 were considered statistically significant.

<table>
<thead>
<tr>
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<th>Active RA (n = 41)</th>
<th>Inactive RA (n = 43)</th>
<th>P</th>
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<tr>
<td>Age, years</td>
<td>61 (29-84)</td>
<td>59 (24-86)</td>
<td>0.06</td>
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<tr>
<td>Female, no. (%)</td>
<td>35 (85)</td>
<td>30 (70)</td>
<td>0.09</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>7 (0-50)</td>
<td>8 (2-32)</td>
<td>0.79</td>
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<tr>
<td>DAS28</td>
<td>5.4 (0.9-8.1)</td>
<td>1.7 (0.1-3.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DMARD, no. (%)</td>
<td>40 (98)</td>
<td>25 (58)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CRP, mg/liter</td>
<td>14 (0.6-112)</td>
<td>3 (0.1-20)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C4b/c, nM†</td>
<td>36 (9.6-113)</td>
<td>21 (8.7-88)</td>
<td>0.03</td>
</tr>
<tr>
<td>C1q-C4, AU‡</td>
<td>3.3 (0.4-13.4)</td>
<td>1.7 (0.2-5.5)</td>
<td>0.0001</td>
</tr>
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</table>

**Table 1.** Characteristics of the patients with active and inactive rheumatoid arthritis (RA)

Except were indicated otherwise, values are the median (range).

DAS28 = Disease Activity Score in 28 joints; DMARD = disease-modifying antirheumatic drug;
CRP = C-reactive protein; AU = arbitrary units.

† Normal value = 8 nM; ‡ Normal value = 0.9 AU.
Results

Patient characteristics

The characteristics of the 2 groups of patients (active versus inactive RA) are depicted in Table I. There was no significant difference in age, sex or disease duration between the 2 groups of patients. Clinical features such as the DAS28 and the frequency of patients treated with disease-modifying antirheumatic drugs were, as expected, significantly higher among the patients with active disease. Correspondingly, CRP plasma levels and levels of IgM-RF were also higher in the patients with active disease.

Increase of C1q-C4 levels in RA

The median C1q-C4 plasma level in the whole group of 84 RA patients was 2.15 AU (range 0.2-13.4), which was significantly (P < 0.0001) higher than in the healthy control group (median 0.83 AU, range 0.45-1.84) (Figure 1). Increased C1q-C4 plasma levels were found in 80% of the 41 patients with active RA and in 58% of the 43 patients with inactive RA. The median level of C1q-C4 in patients with active RA (3.3 AU, range 0.4-13.4) was significantly higher than that in patients with inactive RA (1.7 AU, range 0.2-5.5) (P = 0.0001, by Mann-Whitney test) (Figure 1). This indicates that the classical pathway of complement is activated to a higher degree in active disease than in inactive disease.

No influence of RF in C1q-C4 ELISA

A large percentage of RA patients have RFs that are mainly IgM autoantibodies against epitopes on the constant region of IgG. Since the antibodies in the C1q-C4 ELISA are of the IgG subclass, RFs may cause false positive signals in the ELISA. Therefore, interference in the assay for C1q-

Figure 1. Plasma levels of C1q-C4 complexes in patients with active rheumatoid arthritis (RA) (n = 41), patients with inactive RA (n = 43) and normal healthy volunteers (n = 21) Bars show the median, boxes show the 25th and 75th percentiles, and bars outside the boxes show the 10th and 90th percentiles (in arbitrary units [au]. * = P = 0.0001; ** = P = 0.0002; *** = P < 0.0001.
C4 needs to be excluded. We did several experiments to address this issue. First, RA samples were tested in an ELISA with irrelevant mAb, which yielded no response (results not shown). Second, we absorbed RFs from RA plasma samples by incubation with latex beads coated with human IgG. Using this method, more than 90% of IgM-RFs were removed from the plasma samples, as assessed in an ELISA for RF (all absorbed samples yielded levels below the normal value of 12.5 IU/ml). However, C1q-C4 levels were unchanged upon absorption of the RF. Thus, both control experiments indicate that RFs have no influence on the C1q-C4 ELISA results.

**Stability of C1q-C4**

To assess the stability of C1q-C4 complexes in RA plasma samples, we tested the effect of repeated freezing and thawing on C1q-C4 levels. We compared this effect with the effect of repeated freezing and thawing on C4b/c levels (30). Fourteen RA plasma samples, randomly chosen from the 84 available samples, were repeatedly frozen at -80°C and subsequently thawed. C4b/c and C1q-C4 levels were measured in the plasma samples after 5 cycles of freezing and thawing, and values were compared to those in fresh samples. The median C4b/c plasma level after 5 cycles of freezing and thawing (426.5 nM, range 237.9-1038.0) was more than 10-fold higher and significantly increased as compared with the median level in fresh samples (41.07 nM, range 13.0-105.0; P < 0.0001, by paired t-test). In contrast, the median C1q-C4 plasma level in the fresh samples (2.8 AU, range 0.4-7.9) did not increase after 5 cycles of freezing and thawing (median 2.6 AU, range 0.3-13.9; P = 0.73, by paired t-test), indicating that C1q-C4 levels are not sensitive to in vitro activation, consistent with observations in previous studies (24).

**Plasma levels of C4b/c in active and inactive RA**

Plasma levels of C4b/c were increased in all 84 RA patients. The median level of C4b/c was significantly higher in patients with active RA than in patients with inactive RA (P = 0.03, by unpaired t-test) (Table 1). Levels of the various complement parameters correlated with each other. Using the Spearman’s rank correlation coefficient, C1q-C4 levels correlated significantly with C4b/c levels (r = 0.329, P = 0.002).
Correlation of C1q-C4 levels to IgM-RF plasma levels

Since IgM-RFs are able to activate the classical pathway of complement (15, 16), we assessed the relationship between IgM-RF levels and C1q-C4 complexes. In all 84 RA patients, levels of IgM-RFs were determined and assessed for correlations with complement activation products. The median level of RF in all RA patients was 51.7 IU/ml, ranging from 1.2 IU/ml to 412.3 IU/ml. Levels of IgM-RF were significantly higher ($P < 0.0001$) in patients with active RA than in patients with inactive RA. Eighty-eight percent of the patients with active RA and 47% of the patients with inactive RA were positive for IgM-RF (i.e., IgM-RF levels were above the normal value of 12.5 IU/ml). No significant correlation was found between positivity for IgM-RF and C4b/c levels ($r = 0.071, P = 0.52$). However, a significant correlation between C1q-C4 levels and the presence of IgM-RF was observed ($r = 0.449, P < 0.0001$) suggesting that the presence of IgM-RF is involved in classical pathway activation (Figure 2A).

Figure 2. Correlation between levels of IgM-rheumatoid factor (RF) and C1q-C4 (A), the Disease Activity Score in 28 joints (DAS) and C1q-C4 levels (B), and the DAS and IgM-RF levels (C) in all rheumatoid arthritis patients ($n = 84$). Associations were determined by Spearman’s rank correlation coefficient. au = arbitrary units; IU = international units.
Chapter 4

Correlation of complement activation to disease activity
C1q-C4 levels were higher in the patients with active RA than in the patients with inactive disease, suggesting that classical pathway activation is related to disease activity. We therefore analyzed the correlation between C1q-C4 complexes and the DAS28, which is an index of disease activity (27). A significant correlation was found between C1q-C4 levels and the DAS28, when using Spearman’s rank correlation coefficient (r = 0.398, \( P = 0.0002 \)) (Figure 2B). Moreover, C4b/c levels correlated significantly with the DAS28 (\( r = 0.361, \ P = 0.0008 \)) (results not shown). As expected, the IgM-RF levels correlated significantly with the DAS28 (\( r = 0.487, \ P < 0.0001 \)) (Figure 2C).

C1q-C4 in synovial fluid of RA patients
We assessed levels of the C1q-C4 complex in the synovial fluid and corresponding EDTA plasma of an additional 4 RA patients. In 3 of the 4 patients, C1q-C4 levels in the synovial fluid were much higher than the levels in the plasma; in 1 patient (patient 4 in Table 2), we found equal C1q-C4 concentrations in both the plasma and the synovial fluid (Table 2). In general, the total protein concentration was somewhat lower in the synovial fluid than in the plasma of these patients. Consistent with these observations,, C1q levels were lower in the synovial fluid of all 4 patients, and especially in patient nr 4, whose C1q concentration in the synovial fluid was very low. Therefore, when corrected for the C1q concentration, all 4 patients had significantly higher C1q-C4 complex levels in the synovial fluid than in the plasma (Table 2 and Figure 3).

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>SF</td>
<td>Plasma</td>
<td>SF</td>
</tr>
<tr>
<td>C1q-C4, AU</td>
<td>4.5</td>
<td>12.6</td>
<td>3.2</td>
<td>13.8</td>
</tr>
<tr>
<td>C1q, μg/ml</td>
<td>163</td>
<td>102</td>
<td>207</td>
<td>125</td>
</tr>
<tr>
<td>Total protein, mg/ml</td>
<td>74</td>
<td>60</td>
<td>82</td>
<td>67</td>
</tr>
<tr>
<td>Adjusted C1q-C4, AU*</td>
<td>2.8</td>
<td>12.3</td>
<td>1.5</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2. Complement parameters in plasma and synovial fluid (SF) samples from 4 patients with rheumatoid arthritis. *Values are the ratio of C1q-C4 to C1q.
C1q-C4 complexes in Rheumatoid Arthritis

Course of C1q-C4 levels during anti-TNF treatment

Ten RA patients receiving anti-TNF therapy were included in this study. Prior to each infusion, the patients were clinically examined and EDTA plasma was collected. At 22 and 52 weeks after the start of treatment, the patients showed clinical improvement compared with baseline values, as reflected by a significantly decreased DAS28 (P < 0.05) (Figure 4A). In parallel with the DAS changes, C1q-C4 plasma levels decreased after anti-TNF treatment (Figure 4B). Although this decrease was not statistically significant, we observed an obvious trend in the decreasing levels of C1q-C4. Furthermore, the difference in C1q-C4 levels at 52 weeks after anti-TNF treatment, as compared with baseline, was borderline significant (P = 0.08).
Discussion

A number of studies (1, 20, 32) including the present one, have demonstrated a relationship between disease activity and complement activation in RA. Nevertheless, among the known biomarkers, plasma levels of complement activation products are rarely used as an inflammatory marker in RA, because analysis of complement activation in patients is hampered by in vitro artefacts. Recently, we described a new marker for complement activation, i.e., C1q-C4 complexes, which are remarkably stable in EDTA plasma. In the present study, we found that C1q-C4 levels were elevated in RA patients as compared with the levels in healthy controls, and that these levels were higher in patients with active RA than in patients who were in clinical remission.

Increased complement activation in RA patients has been described before (1, 2, 20, 32) and is supported in the present study by means of a novel technique to assess classical pathway activation. Since C1q-C4 complexes are a specific parameter of classical pathway activation, increased plasma levels of these complexes in RA indicate involvement of the classical pathway of complement in RA. The molecular size of C1q-C4 complexes depends on whether the attached C4 molecule is completely cleaved into the C4d fragment. Therefore, as long as their precise molecular composition is not clear, calculation of the molar concentration of C1q-C4 complexes will be difficult, if not impossible. For this reason, levels of C1q-C4 complexes were expressed in arbitrary units in the present study, with 100 AU being the amount of complexes in a purified C1q sample. Currently, we are investigating the composition of C1q-C4 complexes in more detail, to improve quantification.

Median plasma levels of the measured complement activation products (C4b/c and C1q-C4) were elevated in patients with active RA compared with those with inactive RA. Although the median values were significantly different, there was overlap between patients with active RA and patients in clinical remission. Some patients in remission had levels of complement activation products that were in the range of those in patients with active disease. This indicates that at least a subgroup of patients who are clinically in remission have ongoing inflammation. At the moment we can only speculate whether the clinical course in these patients differs from that in patients in remission who have low levels of complement activation.

C1q-C4 plasma levels were higher in active RA than in inactive RA, suggesting that classical complement pathway activation is correlated with disease activity. Supporting this assumption, C1q-C4 levels were found to correlate significantly with parameters of disease activity (the DAS28). We also measured C1q-C4 plasma levels in serial samples from RA patients who were receiving infliximab (anti-TNF therapy). In these patients, plasma levels of C1q-C4 decreased
from baseline to week 52, in parallel with the DAS (Figure 4), further supporting the notion that these levels reflect disease activity in RA.

Both CRP and circulating immune complexes are potential activators of the classical pathway (21, 22, 33) and increased levels of both have been described previously in RA. CRP-mediated complement activation may explain at least part of the classical pathway activation in RA (20), but some of the activity is independent of the effects of CRP and may result from interaction of complement with immune complexes. Circulating immune complexes in RA are poor activators of complement (19), but this does not rule out a role for immune complexes in the observed activation of the classical pathway. It has been described that IgM-RF-containing immune complexes are able to activate complement (17, 18, 34-36), particularly in RA patients with vasculitis (37). In the present study, a significant correlation was found between IgM-RF and C1q-C4 complex levels, which supports the role of immune complexes in the observed classical pathway activation.

In general, levels of complement activation products are higher in synovial fluid than in plasma, and it has been suggested that excess production from the joints, or spillover, contributes to plasma levels (8). We measured C1q-C4 complexes in the synovial fluid and corresponding EDTA plasma of 4 RA patients. When corrected for the C1q concentration, we found significantly higher levels of C1q-C4 complexes in the synovial fluid compared with the plasma. Taking into account the molecular size of the C1q-C4 complexes and the ratio between synovial fluid concentration and plasma concentration, it is most likely that C1q-C4 complexes are produced locally in the joint and that the plasma levels measured in RA are the result of spillover. This would explain the higher C1q-C4 plasma levels in patients with active disease, since complement activation in the joint is higher in these patients (5, 6, 38).

Studies on complement activation in human disease are easily blurred because of in vitro activation of the system during processing of samples. Indeed, a major disadvantage of measuring C4b/c is the high sensitivity for in vitro artefacts, due to temperature changes or inaccurate handling of samples. Spontaneous hydrolysis of the intramolecular thio-ester bond may lead to artificially high levels of C4b/c, since the neoepitope recognized by mAb anti-C4-1 is dependent on the integrity of this bond. In the present study, we observed a more than 10-fold increase of C4b/c after 5 cycles of freezing and thawing of RA samples. In contrast, levels of C1q-C4 complexes did not change upon freezing and thawing, demonstrating that C1q-C4 complexes are highly stable in plasma, as has been observed before in plasma samples from normal donors (24). In our opinion, the fact that C1q-C4 complexes are hardly, if at all, generated in vitro in EDTA-containing plasma samples is an important advantage over
conventional methods to assess complement activation. In addition, the assay for C1q-C4 complexes was not influenced by RFs. Because in vitro artefacts play a much lesser role in this new assay, and because C1q-C4 complexes specifically reflect classical pathway activation, we conclude that measurement of these complexes is of extra value in studying complement activation in general, and in RA in particular.

Thus, we have shown that significantly elevated plasma levels of C1q-C4 complexes correlate with disease activity in RA patients. This new complement parameter is remarkably stable in EDTA plasma samples, and is hardly, if at all, influenced by in vitro artefacts. We therefore suggest that C1q-C4 complexes may be helpful for use as a biomarker in assessing inflammatory status and disease activity in RA patients.

References


