Relative contribution of contact and complement activation to inflammatory reactions in arthritic joints


Abstract

Although both the complement and contact system are thought to contribute to the inflammatory reaction in arthritic joints, only activation of complement has so far been well established, whereas contact activation and its contribution to arthritis has not been systematically explored. Complement and contact activation were assessed in 71 patients with inflammatory arthropathies and 11 with osteoarthritis using sensitive assays for C3a, and Cl-inhibitor (CIINH)-kallikrein and CIINH-factor XIIa complexes respectively.

Increased plasma concentrations of kallikrein-and factor XIIa-CIINH complexes were found in two and seven of the 71 patients with inflammatory arthropathies, respectively, and in none of the patients with osteoarthritis. Increased synovial fluid concentrations of kallikrein and factor XIIa complexes occurred in 13 and 15 patients with inflammatory joint diseases respectively, and in two patients with osteoarthritis. Contact system parameters did not correlate with clinical symptoms, local activity, or neutrophil activation.

In contrast, synovial fluid concentrations of C3a and CIINH-C1 complexes were increased in all patients and in 20 patients with inflammatory arthropathies respectively, and were higher in patients with a higher local activity score. Synovial fluid C3a correlated with parameters of neutrophil activation such as lactoferrin. Increased plasma concentrations of C3a and CIINH-C1 complexes occurred in 13 and 11 patients with inflammatory joint diseases, and in one and two patients with osteoarthritis respectively. Plasma concentrations of C3a correlated with the number of painful joints.

Thus contact activation occurs only sporadically in patients with arthritis and contributes little if anything to the local inflammatory reaction and neutrophil activation. These latter events are significantly related to the extent of complement activation.


Patients and methods

PATIENTS AND SAMPLES

The study was approved by the medical ethical committee of the Daniel Den Hoed Clinic. Eighty two patients (37 women, 45 men) who visited the outpatient clinic of the hospital were studied. Their median age was 49 years (range 16–94). Fifty two patients had RA, seven gout, 12 seronegative spondyloarthropathies, and 11 osteoarthritis. Patients with RA fulfilled the respective American Rheumatism Association criteria.

Gout was diagnosed based on the
presence of at least five of the criteria described by Wallace et al.26 All seven patients who fulfilled these criteria had chronic gout. Patients with seronegative spondyloarthopathies included two with ankylosing spondylarthritis, defined according to the New York criteria,27 four with psoriatic arthritis as diagnosed by the presence of characteristic skin lesions or nail pits, one with Reiter’s syndrome (seronegative peripheral arthritis with a non-specific conjunctivitis), and five patients had ‘unclassifiable’ seronegative peripheral arthritis as described by Prakash et al.28 Patients with osteoarthritis had knee radiographs showing loss of cartilage, subchondral sclerosis or osteophytes, or both. The activity of the disease was estimated in 49 patients (35 patients with RA, three with gout, and eight with seronegative spondylarthritis) by assessing the Ritchie index. In 48 patients the aspirated joint was examined and assessed for parameters of inflammation: increased warmth (0–2 points), effusion (0–2 points), tenderness (0–2 points), and swelling (0–2 points). From these parameters the local activity was calculated (0–8 points).

Synovial fluid was aspirated from the joint (from the knee in 79 patients and from the shoulder in three) when therapeutically indicated (clinically inflamed or an effusion persistent for at least three months) with a plastic syringe and placed immediately in a siliconised vacuum tube that contained 10 mM EDTA and 0.05% (w/v) Polybrene (hexadimethrine bromide from Janssen, Beersse, Belgium) to prevent in vitro activation of the complement and contact systems.23 Blood from patients was collected at the time of arthrocentesis in similar tubes to those used for synovial fluid. Cells were removed by centrifugation and synovial fluid and plasma samples were stored in aliquots at −70°C until tested. Plasma samples from 28 healthy volunteers were collected in a similar manner as from the patients and stored at −70°C in individual aliquots and in a pool prepared by mixing equal volumes of plasma from each subject (pooled normal plasma).

METHODS

Complexes between CIINH and factor XIIa, kalikrein, or activated C1 were measured as described previously.23 (In this paper, CIINH-C1 complexes designate the complex that consists of activated C1s, activated C1r, and CIINH, in a molar ratio of 1:1:2.) In brief, a monoclonal antibody which specifically reacts with complexed CIINH,23 mcAb-KOK12, was coupled to Sepharose (cyanogen bromide activated Sepharose 4B from Pharmacia Fine Chemicals (Uppsala, Sweden)), and incubated with samples. Bound CIINH-factor XIIa, CIINH-kalikrein, and CIINH-C1 complexes were subsequently detected by incubation with purified 125I-labelled polyclonal antibodies to factor XIIa, kalikrein, and C1s respectively. Concentrations of complexes were expressed in nmol/l by reference to dextran sulphate activated plasma25 (containing 375 nM CIINH-factor XIIa and 341 nM CIINH-kalikrein complexes) or to serum to which aggregated IgG was added29 (containing 360 nM CIINH-C1 complexes). Plasma and synovial fluid concentrations of complexes in patients were considered to be increased when they exceeded the mean +2SD of the plasma concentrations in 28 healthy volunteers, i.e. > 0.19 nM for CIINH-factor XIIa, > 0.85 nM of CIINH-kalikrein, and > 18 nM for CIINH-C1 complexes.

Concentrations of C3a, prekallikrein, factor XII, functional CIINH, and total CIINH in plasma and synovial fluid were determined by radioimmunoassay as described previously.23 24 (In plasma, and presumably also in synovial fluid, C3a is rapidly converted to C3a-desarg.) In this paper we use C3a to designate both C3a and C3a-desarg.) C5a concentrations were determined with a commercially available kit obtained from Behringwerke (Marburg, Germany).

Concentrations of inactivated cleaved CIINH, i.e. CIINH cleaved at or near its reactive site, were measured as described previously with a monoclonal antibody that specifically reacts with this form of CIINH.30

RESULTS

CONTACT ACTIVATION IN SYNOVIAL FLUID

Concentrations of CIINH-kalikrein and CIINH-factor XIIa complexes in synovial fluid from 71 patients with inflammatory arthropathies and 11 patients with osteoarthritis are shown in fig 1. Concentrations of the two complexes were comparable in all patient groups. Increased concentrations of CIINH-kalikrein complexes were found in seven of 52 patients with RA, four of seven patients with gout, three of 12 patients with seronegative spondyloarthopathies, and two of 11 patients with osteoarthritis. Increased concentrations of CIINH-factor XIIa complexes were found in 10 patients with RA, two with gout, three with seronegative spondyloarthopathies, and two with osteoarthritis. CIINH-kalikrein and CIINH-factor XIIa complexes correlated strongly with each other (r=0.78; p<10−9).

Total synovial fluid concentrations of factor XII
Complement and contact activation in arthritic joints

Figure 1 Synovial fluid concentrations of CIINH complexes in patients with inflammatory arthropathies. The concentrations of CIINH-kallikrein complexes (A) and CIINH-factor XIIa complexes (B) in synovial fluid from 52 patients with rheumatoid arthritis (●), seven patients with gout (□), 12 patients with seronegative spondyloarthropathies (△), and 11 with osteoarthritis (○) are shown. The broken lines indicate the highest level of CIINH-kallikrein (A) and CIINH-factor XIIa complexes (B) respectively, detected in plasma from 28 healthy volunteers; the solid lines indicate the median of the patient group presented.

Table 1 Contact system parameters in various arthropathies. Results are mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Inflammatory arthropathies (n=71)</th>
<th>Osteoarthritis (n=11)</th>
<th>Healthy volunteers (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma† (nM)</td>
<td>Plasma‡ (nM)</td>
<td>Plasma† (nM)</td>
</tr>
<tr>
<td>Prekallikrein (%)</td>
<td>Plasma† 90 (21)</td>
<td>111 (31)</td>
<td>100 (22)</td>
</tr>
<tr>
<td></td>
<td>Synovial fluid†</td>
<td>46 (16)</td>
<td>35 (11)</td>
</tr>
<tr>
<td>Factor XII (%)</td>
<td>Plasma 97 (36)</td>
<td>108 (44)</td>
<td>95 (29)</td>
</tr>
<tr>
<td></td>
<td>Synovial fluid†</td>
<td>56 (25)</td>
<td>47 (16)</td>
</tr>
</tbody>
</table>

*The inflammatory group was different from the group with osteoarthritis (p=0.008) and from the healthy volunteers (p=0.045).
†The inflammatory group was different from the group with osteoarthritis (p=0.046).

Table 2 CIINH species in patients with various arthropathies. Results are mean (SD) except for CIINH which is median (range)

<table>
<thead>
<tr>
<th></th>
<th>Total CIINH (µM)</th>
<th>Functional CIINH (µM)</th>
<th>Functional index (functional/antigenic)</th>
<th>Inactivated CIINH (nM)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Plasma†</td>
<td>Plasma‡</td>
<td>Plasma†</td>
<td>Plasma†</td>
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<tr>
<td></td>
<td>Synovial fluid‡</td>
<td>Synovial fluid‡</td>
<td>Synovial fluid‡</td>
<td>Synovial fluid‡</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=52)</td>
<td>3.7 (0.8)</td>
<td>1.7 (0.8)</td>
<td>3.8 (1.1)</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>1.03 (0.19)</td>
<td>0.94 (0.18)</td>
<td></td>
<td>103 (53-262)</td>
</tr>
<tr>
<td>Gout (n=7)</td>
<td>4.2 (0.5)</td>
<td>1.8 (0.6)</td>
<td>3.9 (1.1)</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>0.94 (0.12)</td>
<td>0.92 (0.18)</td>
<td></td>
<td>105 (63-262)</td>
</tr>
<tr>
<td>Seronegative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spondyloarthropathies</td>
<td>3.6 (0.9)</td>
<td>1.5 (0.5)</td>
<td>3.5 (0.6)</td>
<td>1.4 (0.5)</td>
</tr>
<tr>
<td>(n=12)</td>
<td>0.98 (0.14)</td>
<td>0.96 (0.09)</td>
<td></td>
<td>102 (53-187)</td>
</tr>
<tr>
<td>Osteoarthritis (n=11)</td>
<td>3.0 (0.7)</td>
<td>1.0 (0.4)</td>
<td>3.1 (0.6)</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td></td>
<td>1.04 (0.08)</td>
<td>0.94 (0.25)</td>
<td></td>
<td>92 (68-136)</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>2.9 (0.7)</td>
<td>2.9 (0.6)</td>
<td>2.9 (0.6)</td>
<td>0.99 (0.09)</td>
</tr>
<tr>
<td>(n=28)</td>
<td></td>
<td></td>
<td></td>
<td>73 (34-138)</td>
</tr>
</tbody>
</table>

*The inflammatory group was different from both the group with osteoarthritis (p<0.017) and from healthy volunteers (p<0.0001).
†The inflammatory group was different from the group with osteoarthritis (p<0.001).
‡The inflammatory group was different from the healthy volunteers (p<0.0001).
§The inflammatory group was different from the group with osteoarthritis (p<0.0001).

in plasma, and 0.21 and 0.09% in synovial fluid, and was significantly higher in synovial fluid than in plasma (p<0.0001 for CIINH-kallikrein and CIINH-factor XIIa complexes).

Synovial fluid concentrations of total and inactivated CIINH were higher in patients with inflammatory joint diseases compared with osteoarthritis (p<0.001 for both, table 2). When the amount of inactivated CIINH was expressed as a fraction of total CIINH, however, no significant difference was found between these patient groups (p=0.370). Also the functional index of CIINH, i.e. the ratio of functional/antigenic CIINH, did not differ significantly between patient groups. None of the contact system parameters correlated with local activity of the joints.

CONTACT ACTIVATION IN PLASMA

In plasma from 71 patients with arthritis, we occasionally found increased concentrations of CIINH complexes (table 2). Six patients (five with RA and one with gout) had increased CIINH-factor XIIa complexes (i.e. >0.19 nM) and three patients (all with RA) had increased CIINH-kallikrein complexes (i.e. >0.85 nM).

In two of 28 volunteers we also found increased concentrations of CIINH-kallikrein and CIINH-factor XIIa complexes. Concentrations of total, functional, and inactivated CIINH in plasma from patients with inflammatory joint diseases were significantly higher than in plasma from patients with osteoarthritis and healthy volunteers. The fraction of inactivated CIINH did not differ between patient groups, whereas total concentrations of prekallikrein were higher in synovial fluid from patients with inflammatory joint diseases compared with osteoarthritis (p<0.05, table 1). The percentage of complexed kallikrein and factor XII, i.e. the concentration of complexes compared with the total concentration of prekallikrein/kallikrein and factor XII respectively, was 0.08 and 0.04%
(inactivated C1INH/antigenic C1INH) was higher in patients with gout compared with healthy volunteers (p=0.038), patients with RA (p=0.023), and patients with osteoarthritis (p=0.03).

Plasma concentrations of prekallikrein were lower in patients with inflammatory joint diseases compared with healthy volunteers (p=0.045) and patients with osteoarthritis (p=0.008) (table 1). In the group of patients with inflammatory arthropathies, patients with RA had lower plasma concentrations of prekallikrein compared with patients with gout (p=0.009). Plasma concentrations of factor XII did not differ significantly between patient groups or between patient groups and healthy volunteers. No correlation between contact activation parameters in plasma and clinical symptoms was found.

COMPLEMENT ACTIVATION IN SYNOVIAL FLUID

In synovial fluid from patients with inflammatory joint diseases concentrations of C3a and C1INH-C1 complexes were higher than in patients with osteoarthritis (p=0.006 and p=0.0001 respectively, table 3). The highest concentration of C3a found in patients with osteoarthritis was 81 nM, whereas 42 of the 71 patients with inflammatory arthropathies had C3a concentrations greater than 80 nM.

In 42 patients we also measured C5a concentrations. In 25 patients with RA mean (SD) plasma concentrations were 51 (3-4) ng/ml whereas synovial fluid concentrations were 14 (114) ng/ml. In three patients with gout these concentrations were 43 (3-2) and 43 (1-5), in nine patients with seronegative spondyloarthropathies 59 (2-4) and 66 (4-5), and in five patients with osteoarthritis 39 (2-1) and 43 (3-2) respectively. C3a and C5a concentrations correlated with each other (r=0.74; p<0.001), with C1INH-C1 complexes (r=0.78 and r=0.71 with p<0.001 and p<0.005 respectively) and with levels of activated C1INH (r=0.67 and r=0.62 with p<0.001 for both). C3a and C5a concentrations also correlated with parameters of neutrophil activation such as lactoferrin (r=0.58 and r=0.67 with p<0.001 and p<0.008 respectively) and with levels of C1INH (r=0.36 and r=0.002 for both) (table 4). In 48 patients (37 with RA, three with gout, and eight with seronegative spondyloarthritides) the local activity of the aspirated joint was clinically assessed. When patients were divided into two approximately equal groups according to their score for local activity, we found that patients with a higher local activity score (median score in these patients was 6, range 5–8) had higher concentrations of C3a and C1INH-C1 complexes (p<0.005) than patients with a lower score (median 3, range 2–4) (fig 2).

Table 3 Concentrations of C3a and C1INH-C1 complexes in various arthropathies. Results given as median (range)

<table>
<thead>
<tr>
<th>Arthropathy</th>
<th>Plasma</th>
<th>Synovial fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory arthropathies</td>
<td>5 (2–26)</td>
<td>93 (10–1098)</td>
</tr>
<tr>
<td>(n=52)</td>
<td></td>
<td>12 (1–35)</td>
</tr>
<tr>
<td>Gout (n=7)</td>
<td>5 (2–68)</td>
<td>106 (16–1098)</td>
</tr>
<tr>
<td>Seronegative spondyloarthropathies (n=12)</td>
<td>4 (2–12)</td>
<td>33 (10–132)</td>
</tr>
<tr>
<td>Osteoarthritis (n=11)</td>
<td>4 (2–9)</td>
<td>24 (12–81)</td>
</tr>
<tr>
<td>Healthy volunteers (n=28)</td>
<td>3 (2–22)</td>
<td>8 (4–18)</td>
</tr>
</tbody>
</table>

*The inflammatory group was different from the healthy volunteers (p=0.006 for C3a, and p=0.0001 for C1INH-C1). The inflammatory group was different from the group with osteoarthritis (p=0.001 for C3a and p=0.007 for C1INH-C1). The group with rheumatoid arthritis was different from the group with osteoarthritis (p=0.0002 for C3a and p=0.001 for C1INH-C1), and from the seronegative spondyloarthritides (p=0.007 for C3a and p=0.001 for C1INH-C1).

Figure 2 Relation between complement activation and local activity of joints. Sympovial fluid concentrations of C1INH-C1 complexes (A) and C3a (B) in patients with rheumatoid arthritis (37 patients for C3a, 34 patients for C1INH-C1), gout (three patients) (C), and seronegative spondyloarthritides (eight patients) (D) are shown.

Patients were divided into two groups based on the local activity (see under Methods) of the aspirated joints: (a) local activity score <3 (22 patients), and (b) local activity ≥3 (26 patients). The difference in C3a and C1INH-C1 complexes between groups (a) and (b) was significant (p<0.005 for both). The straight line indicates the median of the patient group.
COMPLEMENT ACTIVATION IN PLASMA

In 71 patients with inflammatory arthropathies, plasma concentrations of C3a and CI-C1INH were higher than in 28 healthy controls (p=0.006 and p<0.0001, respectively) (table 3). Increased concentrations of C3a (i.e. >7 nM) and of CIINH-C1 complexes (i.e. >18 nM) were found in 18 and 15% of patients with inflammatory arthropathies respectively. Plasma concentrations of C3a correlated with concentrations of CIINH-C1 complexes (r=0.35; p=0.004) and C3a and CIINH-C1 complexes correlated with concentrations of ICIIINH (r=0.28; p=0.019 and r=0.47; p<0.10). Plasma C3a correlated with the Ritchie index in 49 patients (r=0.40; p=0.004), and higher plasma concentrations of C3a were found in the 24 patients with more than four painful joints (p=0.0077).

Discussion

The complement system and the contact system have been implicated as major contributors to the inflammatory reaction in arthritis. Whereas activation of the complement system has been well established in inflammatory joints, however,7-11 studies of the contact system in arthritis are rare. In this study we analysed for the first time the extent of complement and contact activation in the same patients and assessed the contribution of the two systems to neutrophil activation and clinical symptoms.

In vitro observations that the contact system is activated by exposure to connective tissue elements12 and urate crystals13 14 suggested that activation of the contact system is a major source of kinins in inflammatory arthritis such as RA and gout. With assays for factor XIIa- and kallikrein-C1INH complexes which can detect 0.05% of activation of factor XII and prekallikrein,2 we found that in arthritic joints the contact system is activated only in approximately 20% of patients with inflammatory arthropathies. However, concentration of complexes did not differ between patients with a higher or a lower local activity, nor between the different patient groups. These observations do not support the hypothesis that the contact system is important in the inflammatory reactions in arthritis.

In plasma from patients with arthritis we rarely found increased concentrations of CIINH-factor XIIa or CIINH-kallikrein complexes. Plasma concentrations of prekallikrein were, however, lower in patients with RA compared with patients with gout and healthy controls. An explanation for this observation could be that the prekallikrein concentration was lowered due to consumption, most likely caused by contact activation. The observed normal plasma concentration of factor XIIa can be explained by the fact that during contact activation one mole of factor XIIa can activate three moles of kallikrein, thus three times more CIINH-kallikrein than CIINH-factor XIIa complexes are formed.23 The absence of CIINH-kallikrein and CIINH-factor XIIa complexes in plasma was presumably due to the low grade of activation and to the rapid clearance of the complexes from the circulation. Consequently, these data may point to a systemic low grade activation of the contact system in some patients with inflammatory joint diseases. As it did not correlate with clinical symptoms, however, the relevance of this activation is not clear. In contrast with contact activation, complement activation, as reflected by C3a (and C5a) concentrations, was found in most synovial fluid samples from arthritic joints. Concentrations appeared to be in the same range as those reported by Moxley and Ruddy,9 but substantially higher than those reported by Wagner and Hugli.35 Also, in synovial fluid from patients with gout and seronegative spondyloarthropathies increased C3a concentrations were found, the amount of C3a being lower in synovial fluid from patients with seronegative spondyloarthropathies than in patients with other inflammatory joint diseases. In addition, CIINH-C1 complexes were often increased (38%) in synovial fluid from patients with RA and not in synovial fluid from other inflammatory joint diseases and osteoarthritis, in agreement with one previous study.34 Concentrations of these complexes correlated with C3a, suggesting that most of the C3a in synovial fluid from patients with RA was generated via the classical pathway. The complement activation observed in the other inflammatory joint diseases is presumably due to the presence of activators other than the immune complexes, such as urate crystals in gout.36-38

Few clinical studies have compared the degree of local clinical inflammation with the levels of intra-articular complement activation. As a measure of local activity we used a summed score of four clinically assessed parameters: increased warmth, effusion, swelling, and tenderness. We found that local activity was higher in patients who had more pronounced intra-articular complement activation. This is in keeping with the study of Doherty et al.,19 though these workers reported a larger increase in synovial fluid concentrations of local activity. This can in part be attributed to the fact that they excluded patients with an intermediate activity from their analysis. Also concentrations of CIINH-C1 complexes were higher in patients with a high local activity score. This is not surprising as 37 of the patients in whom local activity was assessed had RA, and activation of the classical pathway of the complement system (by immune complexes) is probably the main route for complement activation in RA.7 8 Mølles and Paus13 did not find a correlation between complement activation and clinical activity of the joint expressed as a functional score (the knee score). This is presumably explained by the fact that this knee score results from ongoing and previous processes, whereas the amount of complement activation in synovial fluid is the result of processes occurring at the time of the arthrocentesis.

CIINH is the most important inhibitor of activated C1s and C1r, and of kallikrein and factor XIIa.22 The plasma concentration of CIINH was higher in patients with inflammatory arthropathies than in healthy volunteers, in agreement with its acute phase behaviour.39
The functional index of C1INH and the fraction of inactivated C1INH in plasma did not differ significantly between patient groups, though occasional patients showed a fraction of inactivated C1INH two to three times the normal level. This is in keeping with the higher fractional catabolic rate of C1INH observed in some patients with RA. The correlation of inactivated C1INH with C3a and C1INH-C1 suggests that inactivated C1INH was the result of complement and not of contact activation. Analysis of C1INH in synovial fluid of patients with arthritis showed no major abnormalities. Increased synovial fluid concentrations of inactivated C1INH that were occasionally observed correlated with complement activation (table 4) and thus presumably also resulted from complement activation.

Neutrophils are the predominant cells present in arthritic joints.1 2 41 The observed correlation between complement activation and neutrophil counts or neutrophil activation products, or both, by several workers11-13 and in this study suggests that split products of complement components are the predominant chemoattractants or agonists, or both, for these cells in arthritis. Kalilikrein and factor XIIa have been shown in vivo to possess chemotactic or agnostic activity, or both, for neutrophils.14-17 42 The absence of any relation between C1INH-kalliikrein complexes or other contact system parameters and neutrophil parameters suggests that this phenomenon does not have a pathogenetic role in arthritis.

In conclusion, in arthritic joints the contact system was activated only in occasional patients. In contrast, activation of the complement system was a general finding in arthritic joints, and appears to be responsible for the local inflammatory reaction and the influx and activation of neutrophils in arthritis.

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34 Molins E T, Pais A. Complement activation in synovial fluid and tissue from patients with juvenile rheumatoid arthritis. Arthritis Rheum 1986; 29: 1359-64.
Relative contribution of contact and complement activation to inflammatory reactions in arthritic joints.


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