

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

J J Abbink, A M Kamp, J H Nuijens, A J M Eerenberg, A J G Swaak, C E Hack

## 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 C1-inhibitor (C1INH)-kallikrein and C1INH-factor XIIa complexes respectively.

Increased plasma concentrations of kallikrein- and factor XIIa-C1INH 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 C1INH-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 C1INH-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.

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Neutrophils are the predominant cells present in the synovial cavity of patients with rheumatoid arthritis (RA) and gout.<sup>1,2</sup> The presence of increased concentrations of elastase, lactoferrin, and other neutrophil granule constituents in arthritic joints suggests that these cells are activated.<sup>3,4</sup> Activation of the complement and the contact system results in the production of factors that are chemotactic or agonistic, or

both, for neutrophils in vitro (for review, see Vogt<sup>5</sup> and Kaplan<sup>6</sup>).

Evidence for complement activation in arthritic joints is abundant. For example, increased concentrations of split products of the third component of the complement system, C3, in joints of patients with RA and gout have often been reported.<sup>7-11</sup> Intra-articular complement activation has also been shown to correlate with leucocyte levels.<sup>11-13</sup>

The contact system of coagulation is also thought to contribute to the inflammatory response observed in arthritis (for reviews, see Kaplan<sup>6</sup> and Cochrane and Griffin<sup>14</sup>). Activation of this pathway generates active factor XII (factor XIIa) and kallikrein.<sup>6,14</sup> Kallikrein cleaves bradykinin, a potent vasodilator, from its precursor high molecular weight kininogen. Kallikrein and factor XIIa can aggregate neutrophils and cause elastase secretion.<sup>15-17</sup> The presence of kinins in arthritic joints has long been recognised.<sup>18,19</sup> The role of contact activation in the generation of these, however, and the contribution of this system to the arthritic reaction in relation to complement activation has not yet been explored.

Factor XIIa and kallikrein are inhibited by the serine proteinase inhibitor, C1INH,<sup>20,21</sup> which is also the only known inhibitor of activated C1s and C1r from the classical pathway of complement.<sup>22</sup> As factor XIIa and kallikrein readily form complexes with C1INH, complexes between C1INH and kallikrein and factor XIIa are currently measured to assess contact activation in biological states.<sup>23</sup>

We have developed highly sensitive assays for C3a<sup>24</sup> and C1INH-kallikrein and C1INH-factor XIIa complexes.<sup>23</sup> In the work reported here we used these assays to study the occurrence of contact activation in 71 patients with inflammatory arthropathies and 11 patients with osteoarthritis. We also assessed the relative contribution of complement and contact activation to clinical symptoms in these patients.

## 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.<sup>25</sup> Gout was diagnosed based on the

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands  
J J Abbink  
A M Kamp  
J H Nuijens  
A J M Eerenberg  
C E Hack

Department of Rheumatology, Dr Daniel den Hoed Clinic, Rotterdam, The Netherlands  
A J G Swaak

Correspondence to: Dr C E Hack, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, PO Box 9406, 1006 AK Amsterdam, The Netherlands.

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presence of at least five of the criteria described by Wallace *et al.*<sup>26</sup> All seven patients who fulfilled these criteria had chronic gout. Patients with seronegative spondyloarthropathies included two with ankylosing spondylarthritis defined according to the New York criteria,<sup>27</sup> 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.*<sup>28</sup> 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 (38 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.<sup>23</sup> 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  $-70^{\circ}\text{C}$  until tested. Plasma samples from 28 healthy volunteers were collected in a similar manner as from the patients and stored at  $-70^{\circ}\text{C}$  in individual aliquots and in a pool prepared by mixing equal volumes of plasma from each subject (pooled normal plasma).

#### METHODS

Complexes between C1INH and factor XIIa, kallikrein, or activated C1 were measured as described previously.<sup>23</sup> (In this paper, C1INH-C1 complexes designate the complex that consists of activated C1s, activated C1r, and C1INH, in a molar ratio of 1:1:2.) In brief, a monoclonal antibody which specifically reacts with complexed C1INH,<sup>23</sup> mcAb-KOK12, was coupled to Sepharose (cyanogen bromide activated Sepharose 4B from Pharmacia Fine Chemicals (Uppsala, Sweden)), and incubated with samples. Bound C1INH-factor XIIa, C1INH-kallikrein, and C1INH-C1 complexes were subsequently detected by incubation with purified  $^{125}\text{I}$ -labelled polyclonal antibodies to factor XIIa, kallikrein, and C1s respectively. Concentrations of complexes were expressed in nmol/l by reference to dextran sulphate activated plasma<sup>23</sup> (containing 375 nM C1INH-factor XIIa and 341 nM C1INH-kallikrein complexes)

or to serum to which aggregated IgG was added<sup>29</sup> (containing 360 nM C1INH-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 C1INH-factor XIIa,  $> 0.85$  nM of C1INH-kallikrein, and  $> 18$  nM for C1INH-C1 complexes.

Concentrations of C3a, prekallikrein, factor XII, functional C1INH, and total C1INH in plasma and synovial fluid were determined by radioimmunoassay as described previously.<sup>23, 24</sup> (In plasma, and presumably also in synovial fluid, C3a is rapidly converted to C3a<sub>desarg</sub>. In this paper we use C3a to designate both C3a and C3a<sub>desarg</sub>.) C5a concentrations were determined with a commercially available kit obtained from Behringwerke (Marburg, Germany).

Concentrations of inactivated cleaved C1INH, i.e. C1INH cleaved at or near its reactive site, were measured as described previously with a monoclonal antibody that specifically reacts with this form of C1INH.<sup>30</sup>

#### STATISTICAL ANALYSIS

Data were analysed with a standard statistical computer program (SPSS-PC). The normal distribution of the data was checked at a 0.05% level of significance by the Kolmogorov-Smirnov test.

Differences in plasma and synovial fluid concentrations of various parameters between patient groups were evaluated with Student's *t* test when parameters were distributed normally and with the Wilcoxon-Mann-Whitney test otherwise. A *p* value of less than 0.05 was considered to indicate statistical significance.

The relation between parameters was studied with linear regression (Pearson product moment correlation when parameters were distributed normally, and Spearman rank correlation otherwise). To correct for multiple correlations, only a *p* value less than 0.005 was considered to indicate a significant correlation.

#### Results

##### CONTACT ACTIVATION IN SYNOVIAL FLUID

Concentrations of C1INH-kallikrein and C1INH-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 C1INH-kallikrein complexes were found in seven of 52 patients with RA, four of seven patients with gout, three of 12 patients with seronegative spondyloarthropathies, and two of 11 patients with osteoarthritis. Increased concentrations of C1INH-factor XIIa complexes were found in 10 patients with RA, two with gout, three with seronegative spondyloarthropathies, and two with osteoarthritis. C1INH-kallikrein and C1INH-factor XIIa complexes correlated strongly with each other ( $r=0.78$ ;  $p<10^{-9}$ ). Total synovial fluid concentrations of factor XII

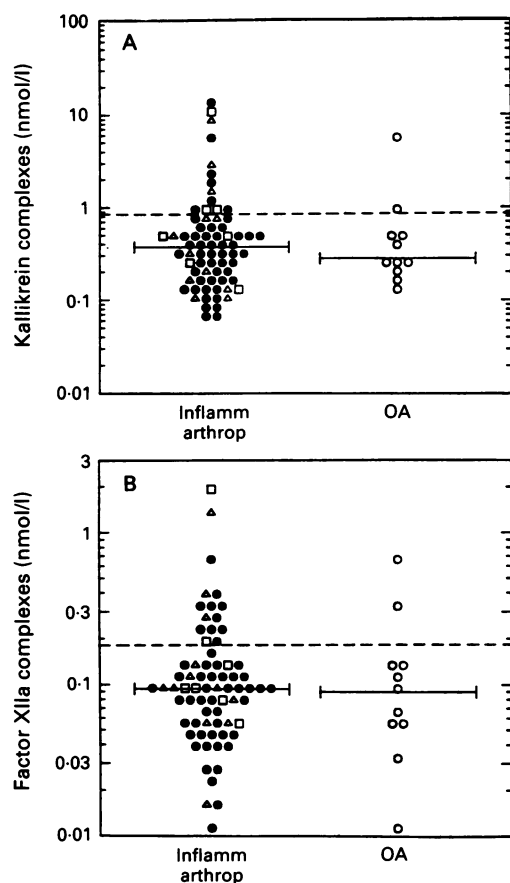


Figure 1 Synovial fluid concentrations of CIINH complexes in patients with inflammatory arthropathies. The concentrations of CIINH-kallikrein complexes (A) and CIINH-factor XIIa complexes (B) in synovial fluid from 52 patients with rheumatoid arthritis (●), seven patients with gout (□), 12 patients with 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.

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%

in plasma, and 0.21 and 0.09% in synovial fluid, and was significantly higher in synovial fluid than in plasma ( $p < 0.00001$  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

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

	Inflammatory arthropathies (n=71)	Osteoarthritis (n=11)	Healthy volunteers (n=28)
Prekallikrein (% normal)			
Plasma*	90 (21)	111 (31)	100 (22)
Synovial fluid†	46 (16)	35 (11)	—
Factor XII (% normal)			
Plasma	97 (36)	108 (44)	95 (29)
Synovial fluid	56 (25)	47 (16)	—

\*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)

	Total CIINH ( $\mu$ M)		Functional CIINH ( $\mu$ M)		Functional index (functional/antigenic)		Inactivated CIINH (nM)	
	Plasma*	Synovial fluid†	Plasma‡	Synovial fluid‡	Plasma	Synovial fluid	Plasma‡	Synovial fluid§
Inflammatory arthropathies	3.7 (0.8)	1.7 (0.8)	3.8 (1.1)	1.5 (0.5)	1.03 (0.19)	0.94 (0.18)	103 (53–262)	68 (17–172)
Rheumatoid arthritis (n=52)	3.6 (0.8)	1.8 (0.7)	3.9 (1.1)	1.5 (0.5)	1.07 (0.19)	0.92 (0.18)	105 (63–262)	75 (20–172)
Gout (n=7)	4.2 (0.5)	1.8 (0.6)	3.9 (0.5)	1.8 (0.3)	0.94 (0.12)	1.05 (0.28)	91 (71–152)	62 (34–126)
Seronegative spondyloarthropathies (n=12)	3.6 (0.9)	1.5 (0.5)	3.5 (0.6)	1.4 (0.5)	0.98 (0.14)	0.96 (0.09)	102 (53–187)	60 (17–107)
Osteoarthritis (n=11)	3.0 (0.7)	1.0 (0.4)	3.1 (0.6)	0.9 (0.3)	1.04 (0.08)	0.94 (0.25)	92 (68–136)	31 (18–63)
Healthy volunteers (n=28)	2.9 (0.7)		2.9 (0.6)		0.99 (0.09)		73 (34–138)	

\*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 ( $p < 0.02$ ).

‡The inflammatory group was different from the healthy volunteers ( $p < 0.0001$ ).

§The inflammatory group was different from the group with osteoarthritis ( $p < 0.0001$ ).

(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 nor 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 5.1 (3.4) ng/ml whereas synovial fluid concentrations were 14 (11.4) ng/ml. In three patients with gout these concentrations were 4.3 (3.2) and 4.3 (1.5), in nine patients with seronegative spondyloarthropathies 5.9 (2.4) and 6.6 (4.5), and in five patients with osteoarthritis 3.9 (2.1) and 4.3 (3.2) respectively. C3a and C5a concentrations correlated with each other ( $r=0.74$ ;  $p<10^{-7}$ ), with C1INH-C1 complexes ( $r=0.78$  and  $r=0.71$  with  $p<10^{-6}$  for both), and with levels of inactivated C1INH ( $r=0.67$  and  $r=0.62$  with  $p<10^{-4}$  for both). C3a and C5a concentrations also correlated with parameters of neutrophil activation such as lactoferrin ( $r=0.58$  and  $r=0.67$  with  $p<10^{-8}$  for both) (table 4). In 48 patients (37 with RA, three with gout, and eight with seronegative spondylarthritis) 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 ( $p<0.05$ ) than patients with a lower score (median 3, range 2–4) (fig 2).

Table 4 Relation between parameters of complement activation, C1INH species, and neutrophil parameters in synovial fluid using Spearman rank correlation analysis

	$r^*$	$p$	$n^\dagger$
C1INH-C1 complexes			
Leucocytes	0.18	0.27	38
C3a	0.78	10 <sup>15</sup>	67
iC1INH	0.68	10 <sup>10</sup>	67
Functional index C1INH	-0.32	0.007	67
Lactoferrin	0.29	0.016	66
C3a			
Leucocytes	0.30	0.06	40
Lactoferrin	0.58	10 <sup>7</sup>	70
iC1INH	0.67	10 <sup>10</sup>	70
Functional index C1INH	-0.36	0.002	70

\*Correlation coefficient.

†Number of patients with the two parameters recorded.

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

	C3a		C1INH-C1 complexes	
	Plasma*	Synovial fluid†	Plasma*	Synovial fluid†
Inflammatory arthropathies				
Rheumatoid arthritis (n=52)	5 (2-68)	93 (10-1098)	12 (1-35)	10 (1-67)
Gout (n=7)	5 (2-68)	106 (16-1098)	13 (4-29)	16 (1-67)
Seronegative spondyloarthropathies (n=12)	5 (4-35)	72 (20-365)	8 (5-20)	9 (2-14)
Osteoarthritis (n=11)	4 (2-12)	33 (10-132)	10 (1-35)	5 (1-14)
Healthy volunteers (n=28)	4 (2-9)	24 (12-81)	11 (4-24)	6 (1-12)
	3 (2-22)		8 (4-18)	

\*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 spondylarthritis group ( $p=0.0007$  for C3a and  $p=0.001$  for C1INH-C1).

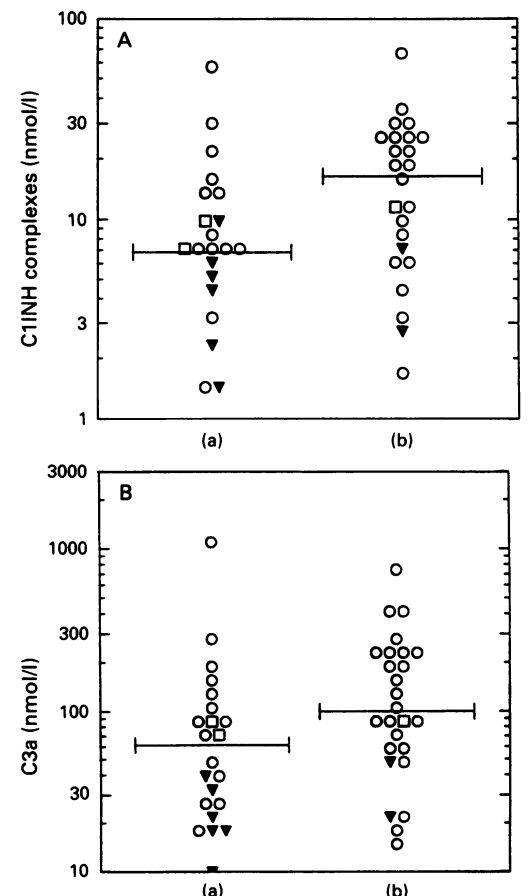


Figure 2 Relation between complement activation and local activity of joints. Synovial fluid concentrations of C1INH-C1 complexes (A) and C3a (B) in patients with rheumatoid arthritis (37 patients for C3a, 34 patients for C1INH-C1) (○), gout (three patients) (□), and seronegative spondyloarthropathies (eight patients) (▼) are shown. Patients were divided into two groups based on the local activity (see under Methods) of the aspirated joint: (a) local activity score  $<5$  (22 patients), and (b) local activity  $\geq 5$  (26 patients). The difference in C3a and C1INH-C1 complexes between groups (a) and (b) was significant ( $p<0.05$  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 C1-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 C1INH-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 C1INH-C1 complexes ( $r=0.35$ ;  $p=0.004$ ) and C3a and C1INH-C1 complexes correlated with concentrations of iC1INH ( $r=0.28$ ;  $p=0.019$  and  $r=0.47$ ;  $p<10^{-5}$ ). 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,<sup>7-11 31</sup> 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 elements<sup>32</sup> and urate crystals<sup>33 34</sup> suggested that activation of the contact system is a major source of kinins in inflammatory arthritides 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,<sup>23</sup> we found that in arthritic joints the contact system is activated only in approximately 20% of patients with inflammatory arthropathies. Synovial fluid concentrations 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 C1INH-factor XIIa or C1INH-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 XII 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 C1INH-kallikrein than C1INH-factor XIIa complexes are formed.<sup>23</sup> The absence of C1INH-kallikrein and C1INH-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. Con-

clusively, 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,<sup>9</sup> but substantially higher than those reported by Wagner and Hugli.<sup>35</sup> 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, C1INH-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.<sup>31</sup> 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 immune complexes, such as urate crystals in gout.<sup>36-38</sup>

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 summated 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.*,<sup>10</sup> though these workers reported a larger difference between higher and lower 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 C1INH-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.<sup>7 8</sup> Mollnes and Paus<sup>11</sup> 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.

C1INH is the most important inhibitor of activated C1s and C1r, and of kallikrein and factor XIIa.<sup>20-22</sup> The plasma concentration of C1INH was higher in patients with inflammatory arthropathies than in healthy volunteers, in agreement with its acute phase behaviour.<sup>39</sup>

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.<sup>40</sup> The correlation of inactivated C1INH with C3a and C1INH-C1 suggest 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.<sup>1,2,41</sup> The observed correlation between complement activation and neutrophil counts or neutrophil activation products, or both, by several workers<sup>11-13</sup> and in this study suggests that split products of complement components are the predominant chemoattractants or agonists, or both, for these cells in arthritis. Kallikrein and factor XIIa have been shown in vitro to possess chemotactic or agonistic activity, or both, for neutrophils.<sup>15-17,42</sup> The absence of any relation between C1INH-kallikrein 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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- 1 Brown K A. The polymorphonuclear cell in rheumatoid arthritis. *Br J Rheumatol* 1988; 27: 150-5.
- 2 Weissmann G. Activation of neutrophils and the lesions of rheumatoid arthritis. *J Lab Clin Med* 1982; 100: 322-33.
- 3 Hadler N M, Spitznagel J K, Quinet R J. Lysosomal enzymes in inflammatory synovial effusions. *J Immunol* 1979; 123: 572-6.
- 4 Kleesiek K, Reinards R, Brackertz D, Neumann S, Lang H, Greiling H. Granulocyte elastase as a new biochemical marker in the diagnosis of chronic joint diseases. *Rheumatol Int* 1986; 6: 161-9.
- 5 Vogt W. Anaphylatoxins: possible roles in disease. *Complement* 1986; 3: 177-88.
- 6 Kaplan A P. The intrinsic coagulation, fibrinolytic and kinin-forming pathways of man. In: Kelley W N, Harris E D, Ruddy S, Sledge C B, eds. *Textbook of rheumatology*. Philadelphia: Saunders, 1985: 95-114.
- 7 Nydegger U E, Zubler R H, Joliat G G, Karagevrekis Ch, Lambert P H, Miescher P A. Circulating complement breakdown products in patients with rheumatoid arthritis. *J Clin Invest* 1977; 59: 862-8.
- 8 Perrin L H, Nydegger U E, Zubler R H, Lambert P H, Miescher P A. Correlation between levels of breakdown products of C3, C4, and properdin factor B in synovial fluids from patients with rheumatoid arthritis. *Arthritis Rheum* 1977; 20: 647-52.
- 9 Moxley G, Ruddy S. Elevated C3 anaphylatoxin levels in synovial fluids from patients with rheumatoid arthritis. *Arthritis Rheum* 1985; 28: 1089-95.
- 10 Doherty M, Richards N, Hornby J, Powell R. Relation between synovial fluid C3 degradation products and local inflammation in rheumatoid arthritis, and crystal induced arthropathy. *Ann Rheum Dis* 1988; 47: 190-7.
- 11 Mollnes T E, Paus A. Complement activation in synovial fluid and tissue from patients with juvenile rheumatoid arthritis. *Arthritis Rheum* 1986; 29: 1359-64.
- 12 Hunder G G, McDuffie F C, Mullen B J. Activation of complement component C3 and factor B in synovial fluids. *J Lab Clin Med* 1977; 89: 160-71.
- 13 Berkowicz A, Kappelgaard E, Petersen J, et al. Complement C3c and C3d in plasma and synovial fluid in rheumatoid arthritis. *Acta Pathol Microbiol Immunol Scand Sect C* 1983; 91: 397-402.
- 14 Cochrane C G, Griffin J H. The biochemistry and pathophysiology of the contact system of plasma. *Adv Immunol* 1982; 33: 241-304.
- 15 Kaplan A P, Kay A B, Austen K F. A prealbumin activator of prekallikrein. II. Appearance of chemotactic activity for neutrophils by the conversion of human prekallikrein to kallikrein. *J Exp Med* 1972; 135: 81-97.
- 16 Schapira M, Despland E, Scott C F, Boxer L A, Colman R W. Purified human plasma kallikrein aggregates human blood neutrophils. *J Clin Invest* 1982; 69: 1199-202.
- 17 Schapira M, Henry J, Wachtfogel Y T, et al. A role for plasma kallikrein in rheumatoid arthritis. *Clin Res* 1983; 31: 454A.
- 18 Melmon K L, Webster M E, Goldfinger S E, Seegmiller J E. The presence of a kinin in inflammatory synovial effusion from arthritides of varying etiologies. *Arthritis Rheum* 1967; 10: 13-20.
- 19 Keele C A, Eisen V. Plasma kinin formation in rheumatoid arthritis. *Adv Exp Med Biol* 1970; 8: 471-5.
- 20 Pixley R A, Schapira M, Colman R W. The regulation of human factor XIIa by plasma proteinase inhibitors. *J Biol Chem* 1985; 260: 1723-9.
- 21 van der Graaf F, Koedam J A, Bouma B N. Inactivation of kallikrein in human plasma. *J Clin Invest* 1983; 71: 149-58.
- 22 Sim R B, Reboul A, Arlaud G J, Villiers C L, Colomb M G. Interaction of <sup>125</sup>I-labelled complement subcomponents C1r and C1s with protease inhibitors in plasma. *FEBS Lett* 1979; 97: 111-5.
- 23 Nuijens J H, Huijbregts C C M, Eerenberg A J M, et al. Quantification of plasma factor XIIa-C1-inhibitor and kallikrein-C1-inhibitor complexes in sepsis. *Blood* 1988; 72: 1841-8.
- 24 Hack C E, Paardekooper J, Eerenberg A J M, et al. A modified competitive inhibition radioimmunoassay for the detection of C3a. Use of 125I-C3 instead of 125I-C3a. *J Immunol Methods* 1988; 108: 77-84.
- 25 Arnett F C, Edworthy S M, Bloch D A, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
- 26 Wallace S L, Robinson H, Masi A T, Decker J L, McCarty D J, Yu T. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum* 1977; 20: 895-900.
- 27 Moll J M H, Wright V. New York clinical criteria for ankylosing spondylitis. *Ann Rheum Dis* 1973; 32: 354-63.
- 28 Prakash S, Mehra N K, Bhargava S, Malaviya A N. HLA B27 related 'unclassifiable' seronegative spondyloarthropathies. *Ann Rheum Dis* 1983; 42: 640-3.
- 29 Hack C E, Hannema A J, Eerenberg A J M, Out T A, Aalberse R C. A C1-inhibitor-complex assay (INCA): a method to detect C1 activation in vitro and in vivo. *J Immunol* 1981; 127: 1450-3.
- 30 Nuijens J H, Eerenberg-Belmer A J M, Huijbregts C C M, et al. Proteolytic inactivation of plasma C1-inhibitor in sepsis. *J Clin Invest* 1989; 84: 443-50.
- 31 Inman R D, Harpel P C. C1 inactivator-C1s complexes in inflammatory joint disease. *Clin Exp Immunol* 1983; 53: 521-8.
- 32 Moskowitz R, Schwartz R J, Michel B, Ratnoff O D, Astrup T. Generation of kinin-like agents by chondroitin sulfate, and human articular cartilage: possible pathophysiological implications. *J Lab Clin Med* 1970; 776: 790-8.
- 33 Kellermeyer R W, Breckenridge R T. The inflammatory process in acute gouty arthritis. Activation of Hageman factor by sodium urate crystals. *J Lab Clin Med* 1965; 65: 307-14.
- 34 Ginsberg M H, Jaques B, Cochrane C G, Griffin J H. Urate crystal-dependent cleavage of Hageman factor in human plasma and synovial fluid. *J Lab Clin Med* 1980; 95: 497-506.
- 35 Wagner J L, Hugli T E. Radioimmunoassay for anaphylatoxins: a sensitive method for determining complement activation products in biological fluids. *Anal Biochem* 1984; 136: 75-88.
- 36 Byers P H, Ward P A, Kellermeyer R W, Naff G B. Complement as a mediator of inflammation in acute gouty arthritis. II. Biological activities generated from complement by the interaction of serum complement and sodium urate crystals. *J Lab Clin Med* 1973; 81: 761-9.
- 37 Hasselbacher P. C3 activation by monosodium urate monohydrate and other crystalline material. *Arthritis Rheum* 1979; 22: 571-8.
- 38 Doherty M, Whicher J T, Dieppe P A. Activation of the alternative pathway of complement by monosodium urate monohydrate crystals and other inflammatory particles. *Ann Rheum Dis* 1983; 42: 285-91.
- 39 Kalter E S, Daha M R, Ten Cate J W, Verhoef J, Bouma B N. Activation and inhibition of Hageman factor-dependent pathways and the complement system in uncomplicated bacteremia or bacterial shock. *J Infect Dis* 1985; 151: 1019-27.
- 40 Woo P, Lachmann P J L, Harrison R A, Amos N. Simultaneous turnover of normal and dysfunctional C1 inhibitor as a probe of in vivo activation of C1 and contact activatable proteases. *Clin Exp Immunol* 1985; 61: 1-8.
- 41 Schumacher H R. Synovial fluid analysis. In: Kelley W N, Harris E D, Ruddy S, Sledge C B, eds. *Textbook of rheumatology*. Philadelphia: Saunders, 1985: 561-7.
- 42 Colman R W, Wachtfogel Y T, Kucich U, et al. Effect of cleavage of the heavy chain of human plasma kallikrein and its functional properties. *Blood* 1985; 65: 311-8.