Complement metabolism in the seronegative arthritides

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The complement system, through the action of its nine components and various cleavage products, plays a major role in the inflammatory response. In rheumatoid arthritis a depression of total haemolytic complement (Hedberg, 1963, 1964, 1967; Pekin and Zvaifler, 1964; Fostiropoulos, Austen, and Bloch, 1965; Barnett, Bienenstock, and Bloch, 1966; Peltier, Coste, and Delbarre, 1966; Vaughan, Barnett, Sobel, and Jacox, 1968; Ruddy and Austen, 1970; Gligore, Bolosiu, Dutu, and Podut, 1971) and the concentrations of individual components (Fostiropoulos and others, 1965; Ruddy and Austen, 1970; Ruddy, Everson, Schur, and Austen, 1971; Britton and Schur, 1971) in synovial fluid compared with serum has been accepted as evidence of intra-articular complement activation, and has been confirmed by the finding of conversion products of C3 (Zvaifler, 1969; Hedberg, Lundh, and Laurell, 1970), cleavage products of C5, and the trimolecular chemotactic factor C567 (Ward and Zvaifler, 1971). Increased catabolic rates for C3 have also been found in synovial fluid (Ruddy, Müller-Eberhard, and Austen, 1971). In most of the above-mentioned studies, the serum complement system appeared to be normal. Activation of the complement system in the joint cavity is thought to be due to fixation by immune complexes consisting of IgG and rheumatoid factor. Although the presence of IgG antiglobulin factors in various seronegative arthritides has been documented (Torrighiani and Roitt, 1967; Torrighiani, Ansell, Chown, and Roitt, 1969; Torrighiani, Roitt, Lloyd, and Corbett, 1970; Panush, Bianco, and Schur, 1971), the status of the complement system in these diseases has not been described.

In this paper we report the results of measurements of the Clq, C3, and C7 components of complement, glycine-rich- β -glycoprotein (GBG or C3 proactivator) concentrations in the plasma of patients with seronegative forms of arthritis. In addition, we have used crossed antibody electrophoresis for measuring C3 conversion and immunoelectrophoresis for GBG

conversion, an important index of alternate pathway activation (Götze and Müller-Eberhard, 1971).

Materials and methods

PATIENTS STUDIED

Thirty patients with ankylosing spondylitis (Gofton, 1968), nine with Reiter's disease (Bauer and Engelman, 1942), nineteen with Still's disease (Ansell and Bywaters, 1959), eighteen with psoriatic arthritis (Moll and Wright 1973), and ten with seronegative rheumatoid arthritis (Ropes, Bennett, Cobb, Jacox, and Jessar, 1958) were studied. In addition, sixteen patients with the sicca syndrome (Whaley, Williamson, Chisholm, Webb, Mason, and Buchanan, 1972) were studied as examples of localized chronic inflammatory disease controls and fourteen osteoarthritic patients acted as chronic non-inflammatory joint disease controls. The mean age, duration of disease, and articular indices for these patients are shown in Table I. Of the patients with ankylosing spondylitis, seven were in stage I (disease localized to the vertebral column), eight stage II (hip joints involved), and fifteen stage III (generalized polyarthritis).

SEROLOGICAL TESTS

All patients were screened for rheumatoid factor by the Rheumaton test (Warner Laboratories), sera being tested neat and at a 1/10 dilution. Positive sera were then titrated in the R3 latex test (Denver Laboratories) starting at a dilution of 1/16. Patients were said to be seropositive for rheumatoid factor when their sera produced agglutination at a titre of at least 1/16. Antinuclear factor was detected by immunofluorescence using rat kidney as substrate and screening sera at a dilution of 1/16. Positive sera were titrated by quadrupling dilution until an end point of nuclear staining was achieved (Beck, 1961).

COMPLEMENT STUDIES

Serum and EDTA plasma samples were stored at -70°C within 2 hrs of venepuncture, and thawed once only. All except the anticomplementary assays and the reactive lysis test for C56 assays were performed on plasma samples.

Plasma concentrations of Clq, C3, and GBG were measured by radial immunodiffusion using monospecific antisera prepared as follows.

Table I *Clinical features of patients studied*

<i>Clinical group</i>	<i>No.</i>	<i>Sex</i>		<i>Age (yrs)</i> <i>Mean ± SD</i>	<i>Duration (yrs)</i> <i>Mean ± SD</i>
		<i>M</i>	<i>F</i>		
Ankylosing spondylitis	28	17	11	39.1 ± 10.6	13.5 ± 9.3
Reiter's disease	10	10	—	36.1 ± 5.3	6.9 ± 7.1
Still's disease	9	5	4	20.7 ± 9.8	11.0 ± 11.4
Psoriatic arthritis	18	7	11	47.1 ± 15.6	8.7 ± 5.5
Seronegative RA	10	4	6	32.3 ± 7.8	6.4 ± 5.8
Sicca syndrome	16	1	15	57.7 ± 17.9	12.9 ± 6.7
Osteoarthritis	14	4	10	63.4 ± 9.2	12.4 ± 10.4

Clq Rabbits were immunized with latex particles agglutinated in fresh normal human serum (Thunold, Abeyounis, and Milgrom, 1970) or with purified Clq (Agnello, Winchester, and Kunkel, 1970; Yonemasu and Stroud, 1971). Sera were absorbed with lyophilized heat-inactivated human serum if required.

C3 Rabbits were immunized with zymosan previously incubated in human serum (Mardiney and Müller-Eberhard, 1965) or with alexinated bacteria (Thompson, 1972). Absorption with low C3-containing sera was sometimes required.

GBG Rabbits were immunized with immune precipitates prepared by immunoelectrophoresis (Goudie, Horne, and Wilkinson, 1966).

C7 Concentrations were measured in agarose plates using the reactive lysis procedure (Thompson and Lachmann, 1970). The plates contained unsensitized sheep erythrocytes, activated C56, and 0.01mol/l. EDTA. Generation of C56 in agarose by certain sera was shown by reactive lysis (Thompson and Rowe, 1968).

C3 conversion was measured by crossed antibody electrophoresis (Laurell, 1965) and GBG conversion to glycine-rich γ -glycoprotein (GGG) was detected by immunoelectrophoresis. Serum anticomplementary activity was measured by incubating 0.1 ml heat inactivated

serum with 3 units of complement, and then titrating the residual complement activity.

Results

The plasma concentrations of Clq, C3, C7, and GBG are shown in Table II. High values of different components were seen in individuals in all but the disease control groups (the sicca syndrome and osteoarthritic control patients), and the mean levels of Clq, C3, and C7 were significantly higher than at least one of the control groups in the patients with ankylosing spondylitis, Still's disease, and psoriatic arthritis, whereas with the exception of the levels of C7 in Reiter's disease and C3 in seronegative rheumatoid arthritis no other significant differences were observed (Table II). The high levels of the individual complement components were found in patients with more widespread disease, but were not related to clinical or laboratory parameters of disease activity. Conversion of C3 (Table III) was not present in any of the osteoarthritis or sicca syndrome plasma samples, whereas it was detected

Table II *Plasma complement component concentrations in seronegative arthritides (mean ± SEM)*

<i>Component</i>	<i>Normal range (% of pool)</i>	<i>Ankylosing spondylitis (no. = 30)</i>	<i>Reiter's disease (no. = 9)</i>	<i>Still's disease (no. = 8)</i>	<i>Psoriatic arthritis (no. = 18)</i>	<i>Sero-negative rheumatoid arthritis (no. = 10)</i>	<i>Sicca syndrome (no. = 10)</i>	<i>Osteo-arthritis (no. = 14)</i>
Clq	70-140	132.7 ± 7.6**†	128.2 ± 7.8	51.7 ± 14.6*†	128.5 ± 4.0***†	123.5 ± 5.6	113.0 ± 6.8	115.2 ± 2.7
C3	70-155	148.9 ± 8.6***†††	131.7 ± 8.6	180.1 ± 10.9***†††	160.9 ± 12.9***†††	136.6 ± 8.9†	120.9 ± 7.2	116.1 ± 7.2
C7	75-155	144.3 ± 8.1***†	135.3 ± 10.0*	142.4 ± 15.0*†	136.9 ± 9.2*	127.7 ± 9.8	115.3 ± 6.0	119.7 ± 6.5
GBG	65-120	105.8 ± 5.1	95.6 ± 4.9	106.5 ± 5.2	102.3 ± 4.7	99.9 ± 4.2	97.0 ± 4.3	95.7 ± 4.0

Symbols indicate significance values
(* = *v.* sicca syndrome † = *v.* osteoarthritis).
* or † = *P* < 0.05.
** or †† = *P* < 0.01.
*** or ††† = *P* < 0.001.

Table III Complement metabolism in the seronegative arthritides

	Ankylosing spondylitis	Reiter's disease	Still's disease	Psoriatic arthritis	Sero- negative RA	Sicca syndrome	Osteo- arthritis
C3 conversion	7/30	7/9	4/9	1/18	3/10	0/16	0/14
Production of C56 in agarose	6/30	5/9	0/9	6/18	2/10	0/16	0/14
Anticomplementary activity	6/30	3/9	ND	ND	4/10	0/16	0/14

GBG conversion not detected in any samples.

in a variable number of samples from the other disease groups (from 1 of 19 patients with psoriatic arthritis to 7 of 9 patients with Reiter's disease). GBG conversion was not detected in any of the plasma samples studied. Reactive lysis indicating generation of C56 (Table III) was detected in certain sera from all disease groups except Still's disease, the sicca syndrome, and osteoarthritic groups. Serum anticomplementary activity (Table III) was looked for in the sicca syndrome, osteoarthritis, ankylosing spondylitis, Reiter's disease, and seronegative rheumatoid arthritis groups. In the first two groups none was detected, whereas it was found in 6 of 30 patients with ankylosing spondylitis, 3 of 9 with Reiter's disease, and 4 of 10 patients with seronegative rheumatoid arthritis.

Closer examination of the ankylosing spondylitis group of patients (Table IV) revealed that 8 of 30 patients had rheumatoid factor, detected by the Rheumatoid test, present in their sera. The presence of C3 conversion and anticomplementary activity, and the generation of C56 in agarose were significantly higher in this group of patients ($P < 0.01$; $P < 0.005$, respectively). These patients all fell into disease stage III, i.e. ankylosing spondylitis with pronounced peripheral joint involvement. In the other groups of patients with seronegative spondyloarthropathies the presence of abnormalities of the

complement system was also associated with widespread disease. No correlations were found between these abnormalities and ESR, serum albumin and globulin concentrations, or the presence of antinuclear factor.

Discussion

In this study we have shown that the mean circulating complement component concentrations, Clq, C3, C7, but not GBG, are frequently raised in the seronegative arthritides. Increased serum total haemolytic complement concentration components have previously been found in patients with a variety of chronic inflammatory diseases, including seronegative arthritis (Vaughan, Bayles, and Favour, 1951; Laurell and Grubb, 1958; Ellis and Felix-Davies, 1959). The presence of C3 conversion in a significant proportion of patients with these diseases, which was not found in either of the control groups of patients with osteoarthritis or the sicca syndrome, but which has been shown to occur in the plasma of seropositive rheumatoid arthritis (Versey, Hobbs, and Holt, 1973), shows that the complement system is activated. The raised concentrations of the various components are thus probably due to increased synthesis; as has been previously shown by turnover studies of C3, in patients with rheumatoid arthritis (Weinstein, Peters, Brown, and Bluestone, 1971). That activation of the complement system is occurring by the classical rather than the alternate pathway is shown by the normal plasma concentrations of GBG and the absence of its conversion to GGG (Götze and Müller-Eberhard, 1971), and activation therefore is probably due to the presence of circulating immune complexes. In the ankylosing spondylitis group of patients the association of complement abnormalities with the presence of positive rheumatoid factor tests suggests that in these patients γ -globulin complexes may be responsible for activation of the system, and may play an important role in the perpetuation of the characteristic chronic inflammatory changes present in this group of arthritides.

Table IV C3 conversion, generation of C56, and serum anticomplementary activity in ankylosing spondylitis

	Rheumatoid positive (no. = 8)	Rheumatoid negative (no. = 22)	Significance
C3 conversion	5	2	$\chi^2 = 6.6075$ $P < 0.01$
C56 generation	5	1	$\chi^2 = 8.7547$ $P < 0.005$
Anticomplementary activity	5	1	$\chi^2 = 8.7547$ $P < 0.005$

Summary

The plasma concentrations of Clq, C3, C7, and GGB (C3PA) have been measured in groups of patients with various seronegative spondyloarthritides. The mean levels of Clq, C3, and C7 were raised in patients with ankylosing spondylitis, Still's disease, and

psoriatic arthritis, but not Reiter's disease. Evidence of complement activation was obtained by the finding of C3 conversion on crossed antibody electrophoresis. The absence of GGB conversion to GGG suggests that activation is by the classical pathway, possibly as a result of the presence of circulating immune complexes.

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