Survey of synovial fluid cryoprecipitates

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SUMMARY Synovial fluid cryoproteins from various inflammatory and noninflammatory arthritides were examined for the presence of immunoglobulin, fibrinogen, antiglobulin activity, and third component of complement and correlated with the synovial fluid leucocyte count. The majority of rheumatoid synovial fluid cryoproteins contained either IgG-IgM complexes or IgG alone. Contrary to previous reports, many synovial fluid cryoproteins from psoriasis, Reiter's syndrome, nonspecific acute polyarthritis, gout, and gonococcal arthritis also contained IgG and occasionally IgG-IgM complexes. Noninflammatory synovial fluids were less likely to contain any immunoglobulin. The highest concentration of cryoprotein was found in Reiter's syndrome. There was a significant ($P < 0.05$) correlation between the presence of immunoglobulin and the concentration of the synovial fluid cryoprotein with the synovial fluid leucocyte count. Since synovial fluid cryoproteins containing immunoglobulin are present in the synovial fluids of many diverse rheumatic diseases not postulated to be immune complex mediated, they may be a nonspecific phenomenon related to the degree of inflammation.

The occurrence of synovial fluid cryoproteins was first described by Marcus and Townes (1971) and subsequently by Cracchiolo *et al.* (1971). The synovial fluid cryoproteins of rheumatoid arthritis were well characterised and were shown to consist of mixed immunoglobulins, rheumatoid factor, antinuclear antibody, fibrinogen, and small amounts of DNA. Rheumatoid synovial fluid cryoproteins were found to have significant complement fixing activity which resided in the 19S region, whereas nonrheumatoid synovial fluid cryoproteins were devoid of such activity (Marcus and Townes, 1971).

The significance of cold insoluble proteins in the rheumatoid synovial fluid is unclear. There is speculation that the cryoproteins represent soluble antigen-antibody complexes, as described in the serum of patients with lupus nephritis (Winfield *et al.*, 1975) and that they play a role in synovial inflammation. Although IgM with antiglobulin activity is found complexed with IgG in rheumatoid joint fluid, it is not clear whether that reaction is the initial event in the synovitis or merely an epiphenomenon.

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Little attention has been paid to the cryoproteins of other joint disorders, such as gout, osteoarthritis, Reiter's syndrome, and psoriatic arthritis. Immune complexes have not been implicated in the arthritis of the so-called rheumatoid variants (Bunch *et al.*, 1974), but there has been no other suitable explanation for the intense inflammation often seen with those diseases.

The purpose of this study was further to expand the investigation of cryoproteins in various rheumatic conditions, including seronegative arthritides and acute nonspecific polyarthritis, and to compare those findings to that of rheumatoid arthritis. The cold insoluble proteins were characterised as to their immunoglobulin content and presence of complement and rheumatoid factor activity and to evaluate their occurrence in relation to the degree of synovial inflammation.

Materials and methods

Seventy-four synovial fluids were obtained from 65 patients with diagnoses of definite or classical rheumatoid arthritis (18), acute gout with sodium urate crystals (7), psoriatic arthritis (4), Reiter's syndrome (6), gonococcal arthritis (4), osteoarthritis (17), systemic lupus erythematosus (1), and acute nonspecific inflammatory polyarthritis (8).

Any patient who developed arthritis of less than 6 weeks' duration and did not fall in any definite diagnostic category was termed acute nonspecific polyarthritis. Serial synovial fluids were occasionally obtained from the same patient.

After aspiration the synovial fluid was immediately placed in warmed plain glass tubes and incubated for at least 1 hour at 37°C. Hyaluronidase (Wyeth) 0.1 unit per ml of synovial fluid was added to the synovial fluid and mixed. The tube was centrifuged at room temperature and the supernatant was placed in 5 ml aliquots at 4°C for 5 days for maximum cryoprecipitation. In addition synovial fluid was placed in Wintrobe tubes at 4°C for 5 days, spun at 2500 rpm for 10 minutes at 4°C and read to determine the cryocrit.

The synovial cryoprecipitate was solubilised by the technique of Weisman and Zvaifler (1975) as follows: 5 ml of the synovial fluid was spun at 2500 rpm for 10 minutes at 4°C. The supernatant was removed and stored at 4°C. A volume of cold normal saline equal to the volume of the original fluid was added to the cryoprotein button, mixed thoroughly, and respun at 2500 rpm for 10 minutes at 4°C. The procedure was repeated 3 times. To resolubilise the cryoprotein, 0.5 ml of 0.3 M NaCl was added to the washed cryoprotein button and incubated for one hour at 37°C. Then 0.5 ml of distilled water was added and the solution was reincubated for 1 hour at 37°C. The final 1 ml volume in normal saline (0.15 M) was centrifuged at room temperature, and any insoluble material was discarded. Further immunochemical tests were performed at room temperature.

The resolubilised cryoprecipitate was subjected to immunoelectrophoresis (1% agarose, pH 8.6) with

antisera monospecific for human immunoglobulin IgG, IgA, IgM, fibrinogen, C'3, and for whole human serum (Miles Laboratory). Antiglobulin activity was measured at room temperature by latex agglutination and positive samples were titrated by the method of Singer and Plotz (1956). Total protein concentration of the solubilised cryoprecipitates was measured by the method of Lowry *et al.* (1951), except that sodium citrate was used in place of sodium tartrate to provide a more stable reagent. The protein concentration of the final solution, expressed as mg/dl, represented a concentration of 5 times with respect to the initial sample volume.

Synovial fluid leucocyte count was performed by standard techniques on fresh synovial fluid collected in heparinised tubes.

Results

Visible protein-containing cryoprecipitates were detected in 73 of 74 synovial fluids examined. Characterisation of the cryoproteins revealed that most patients (40/57) with inflammatory arthritis, that is, rheumatoid arthritis, gout, Reiter's syndrome, systemic lupus erythematosus, and gonococcal arthritis, had qualitatively detectable IgG and occasionally IgM (Table 1). Patients with non-inflammatory effusions only occasionally (4/17) had IgG detected.

Cryocrit determinations on the synovial fluid cryoprecipitates revealed that all values were less than 2.0%. There did not appear to be any significant differences between the inflammatory and noninflammatory groups.

The immunoglobulin content of synovial fluid cryoproteins varied within the inflammatory arthritis

Table 1 *Characterisation of synovial fluid cryoprecipitates*

Patient		Immunoglobulin content	Fibrinogen	C'3	Protein conc. (mg/dl)	Latex titre	Leucocyte count (mm ³)
RA	R1	0	0	0	0.397	40	5500
	R2	0	0	0	ND	ND	ND
	R3	IgG	+	0	1.905	0	8900
	R4	IgG	+	0	0.254	0	ND
	R5	IgG-IgM	0	0	0.885	640	ND
	R6	IgG-IgM-IgA	+	0	1.415	160	54 000
	R7	IgG-IgM	0	0	0.694	0	ND
	R8	IgG-IgM	0	0	ND	ND	11 600
	R9	IgG	0	0	3.789	0	36 800
	R10	IgG	0	0	1.157	0	ND
	R11	IgG-IgM	0	0	8.64	80	32 000
	R12	IgG	0	0	3.281	0	ND
	R13	IgG	+	0	2.872	0	9100
	R14	IgG-IgM	0	0	1.566	0	11 200
	R15	IgG-IgM	0	0	1.901	0	ND
	R16	0	+	0	1.583	0	ND
	R17	IgG-IgM	+	0	2.383	0	5800
	R18	IgG	0	0	2.040	0	ND
	R19	IgG	0	0	4.246	0	13 700
	R20	IgG	0	0	2.247	0	ND

Table 1—continued

Patient		Immunoglobulin content	Fibrinogen	C'3	Protein conc. (mg/dl)	Latex titre	Leucocyte count (mm ³)
Acute non-specific polyarthritis	A1	IgG	0	0	2.096	0	27 300
	A2	IgG	0	0	1.930	0	2100
	A3	0	0	0	0.735	0	5850
	A4	0	+	0	0.810	0	10 000
	A5	0	0	0	0	0	3200
	A6	IgG	+	0	1.515	0	450
	A7	IgG	+	0	1.703	0	25 500
	A8	IgG	+	0	5.948	0	28 000
Reiter's syndrome	RT1	IgG	0	0	ND	0	15 200
	RT2	IgG	+	0	3.519	0	ND
	RT3	IgG-IgM	+	0	7.45	0	8400
	RT4	0	0	0	2.072	0	26 500
	RT5	IgG	0	0	4.10	0	9200
	RT6	0	+	0	0.624	0	12 600
	RT7	IgG-IgM	+	0	4.190	0	ND
	RT8	IgG	+	0	3.651	0	30 000
Psoriasis	PS1	0	0	0	1.106	0	ND
	PS2	0	0	0	3.194	0	ND
	PS3	IgG	0	0	1.839	0	9000
	PS4	IgG	0	0	1.219	0	36 200
	PS5	IgG	+	0	1.904	0	ND
	PS6	0	+	0	0.549	0	5035
	PS7	IgG	0	0	2.567	0	ND
Gout	G1	IgG	+	0	1.212	0	ND
	G2	IgG	+	0	6.742	0	18 100
	G3	IgG	0	0	1.446	0	2400
	G4	IgG	0	0	1.044	0	34 000
	G5	0	0	0	1.651	0	3800
	G6	0	0	0	0.228	0	27 000
	G7	0	0	0	0.665	0	2150
Gonococcal infection	G1	0	0	0	0.166	0	ND
	G2	IgG	+	0	1.459	0	17 500
	G3	0	0	0	0.183	0	2850
	G4	IgG-IgM	0	0	1.410	0	30 500
	G5	IgG-IgM	+	0	1.929	0	44 100
DJD	D1	0	0	0	1.202	0	350
	D2	0	0	0	0	0	400
	D3	IgG	+	0	3.386	0	ND
	D4	0	0	0	0.617	0	350
	D5	0	0	0	1.039	0	200
	D6	0	0	0	1.041	0	300
	D7	0	0	0	0.802	0	ND
	D8	IgG	+	0	1.783	0	ND
	D9	0	0	0	0.905	0	200
	D10	0	0	0	0.362	0	300
	D11	IgG	+	0	0.863	0	ND
	D12	0	0	0	1.490	0	50
	D13	0	+	0	0.438	0	600
	D14	0	0	0	1.025	0	100
	D15	0	0	0	0.713	0	100
	D16	0	0	0	0.665	0	200
	D17	IgG	0	0	1.254	0	450
Misc	SLE	IgG-IgM	+	0	3.82	0	3500
	JRA	IgG	+	0	5.182	0	14 000

ND=not done.

group. In rheumatoid arthritis IgG was present in 85%, IgM in 40%, and IgA in 5% of the synovial fluid cryoproteins. By comparison, acute nonspecific polyarthritis synovial fluids yielded only IgG in 5 of 8 samples. The acute Reiter's syndrome and psoriatic arthritis synovial fluids had IgG detected in 10 of 15 cryoproteins with IgM, being present

twice serially in a single patient with Reiter's syndrome. The cryoproteins from 7 patients with gouty arthritis contained IgG only in 4 of 7 fluids. Finally, disseminated gonococcal arthritis synovial fluids contained IgG in 3 of 5 samples, while IgM was present in 2 of those 3.

Study of the noninflammatory osteoarthritis

fluids revealed that only 4 of 13 samples had detectable IgG, while none had IgM. Two patients had a concomitant history of pseudogout, but both fluids were noninflammatory and 1 was positive for a small amount of IgG.

The synovial fluids cryoproteins were examined for other components. In all samples, C/3 was not detected. Fibrinogen was intermittently present but was detected in somewhat higher frequency in inflammatory than noninflammatory synovial fluid cryoproteins. Rheumatoid factor activity in the rheumatoid arthritis synovial cryoprecipitates was present in 4 samples. Serum rheumatoid factor comparisons were generally not available because the Singer-Plotz method was not used. In the non-rheumatoid inflammatory diseases the latex fixation tests were uniformly negative despite that IgM was occasionally detected.

Measurement of the protein concentration of the synovial fluid cryoproteins (Table 2) revealed that the highest values occurred in Reiter's syndrome followed by rheumatoid arthritis, gout, nonspecific polyarthritis, and psoriatic arthritis. The lowest values tended to occur with the osteoarthritis group, consistent with the low incidence of any immunoglobulin detection. It was felt that immunoglobulins made up the greatest portion of the cryoprotein measured although serum proteins, such as fibrinogen, were occasionally detected.

In examining the relationship between the leucocyte count of the synovial fluid and the IgM, IgG, and IgA content of the cryoglobulin (Fig. 1), significant differences were noted between the 3 groups. ($P < 0.001$ by the F test). The mean synovial fluid leucocyte count for the cryoproteins with IgG and IgM was $22\,320/\text{mm}^3$ ($22 \times 10^9/\text{l}$) compared to $16\,310/\text{mm}^3$ ($16 \times 10^9/\text{l}$) for IgG cryoproteins and $4\,680/\text{mm}^3$ ($4.7 \times 10^9/\text{l}$) for cryoproteins without detectable immunoglobulin. Two-way comparison tests showed that immunoglobulin complexes of IgG-IgM or IgG alone were significantly associated with higher synovial fluid leucocyte counts ($P < 0.01$)

Fig. 1 Relationship of synovial fluid leucocyte count to presence of cryoprotein immunoglobulin. Detection of immunoglobulin IgM-IgG or IgG alone is associated with significantly higher mean synovial fluid leucocyte counts.

compared to the absent immunoglobulin group. There was no significant difference between the means of the synovial fluid leucocyte count of the immunoglobulin complexes containing IgG-IgM versus IgG alone.

Fig. 2 illustrates the correlation between synovial fluid leucocyte count and the protein concentration of the cryoproteins. The osteoarthritis fluids were obviously grouped in the low protein levels and low leucocyte counts. The inflammatory synovial fluids tended to range from relatively low protein concentration to as high as 8.74 mg/dl . There was a significant relationship for the entire group between the degree of inflammation, that is, the synovial fluid leucocyte count and the concentration of detectable synovial fluid cryoprotein ($P < 0.05$). However, examination of the individual subgroups of inflammatory arthritides did not reveal a significant relationship except for gonococcal arthritis, $P < 0.05$.

Electron microscopic investigation was carried out on 6 selected synovial fluid cryoproteins, including those from patients with rheumatoid arthritis, Reiter's syndrome, and psoriasis. No viral particles or 'immune complexes' could be identified.

Table 2 Synovial fluid cryoprotein measurements in rheumatic diseases

Rheumatic condition evaluated	No. of synovial fluid samples	Mean protein conc. (mg/dl)
Reiter's syndrome	7	3.66
Rheumatoid arthritis	18	2.29
Gout	7	1.85
Acute nonspecific polyarthritis	8	1.84
Psoriatic arthritis	7	1.81
Gonococcal arthritis	5	1.029
Osteoarthritis/noninflammatory arthritis	17	1.034

Fig. 2 Relationship of synovial fluid leucocyte count to cryoprotein concentration. Raised synovial fluid leucocyte counts in several inflammatory diseases were found to be associated with higher cryoprotein concentration. Osteoarthritis synovial fluids had low synovial fluid leucocyte count and concentration of cryoprotein. RA—rheumatoid arthritis. Reiter's—Reiter's syndrome. PS—psoriatic arthritis. GC—gonococcal arthritis. NS—Polyarthrititis—Nonspecific polyarthrititis. OA—Osteoarthritis. Gout—Gouty arthritis.

Discussion

The purpose of these studies was to survey synovial fluids in a variety of rheumatic conditions for the presence and character of cryoproteins. With the methods outlined virtually all synovial fluids examined yielded some cryoprecipitable material. As demonstrated by Cracchiolo *et al.* (1971) and Marcus and Towers (1971), rheumatoid arthritis fluid cryoproteins contain immunoglobulin IgG and/or IgM. However, careful examination in our study of psoriatic, Reiter's syndrome, gout, and acute nonspecific polyarthrititis revealed similar immunoglobulin findings. The major differences were the less frequent detection of IgM and the absence of any antigammaglobulin activity in the latter diseases. Osteoarthritis fluids were occasionally found to yield immunoglobulin in small amounts despite the noninflammatory nature of the diseases.

In examining the magnitude of inflammation and

cryoprotein concentration we found that psoriatic and Reiter's syndrome fluids showed inflammatory values equal to those of rheumatoid arthritis. In fact, the mean cryoprotein concentration for Reiter's syndrome was higher than rheumatoid arthritis. Pekin and Zvaifler (1964) showed that Reiter's syndrome synovial fluids were high in total synovial fluid protein values, leucocyte counts, and total haemolytic complement values.

An interesting aspect of this study was the presence of synovial fluid cryoglobulins in rheumatic diseases not generally considered to involve immunologic mechanisms. As described, Reiter's syndrome, psoriatic, gout, and gonococcal arthritis fluids contained immunoglobulin IgG or occasionally IgM. Hunder *et al.* (1977) showed that the same rheumatic conditions were associated with in-vitro activation of C3 and/or factor B in their joint fluids. In addition, there was a positive correlation between the amount of C3 conversion and synovial

fluid polymorphonuclear leucocyte response. Our study suggests that the synovial fluid leucocyte count is correlated with the type of cryoprotein immunoglobulin present; in particular the detection of IgG-IgM or IgG complexes is associated with the highest counts. These findings in both rheumatoid and non-rheumatoid inflammatory synovial fluids may indicate that immunoglobulin is important in perpetuating the inflammatory response through further complement activation and release of chemotactic factors (Ruddy *et al.*, 1972). Evidence supporting the role of immune complexes in psoriatic and Reiter's syndrome synovial fluids is still lacking.

Since the magnitude of the inflammatory process of the nonrheumatoid conditions, apart from osteoarthritis, appeared to be proportional to the concentration of immunoglobulin, the synovial fluid cryoprotein may simply be another index of inflammation along with the leucocyte count, volume of exudative fluid, total protein concentration (Ropes and Bauer, 1953), and total haemolytic complement (Pekin and Zvaifler, 1964). In generalised joint inflammation large protein molecules are found in joint fluid in greater amounts than when the synovial membranes are not inflamed. As a result of this phenomenon cryoprecipitable protein is more likely to be detected in inflammatory fluids. Although total protein concentration of the synovial fluid was not measured, our synovial fluid cryoprotein measurements seem to parallel the protein measurements of the studies by Pekin and Zvaifler (1964). In our study the cryoprecipitable protein concentration was higher by 5 to 10 fold than Marcus and Townes (1971) and Cracchiolo *et al.* (1971) found. This may be attributed to differences in techniques of harvesting cryoproteins and a higher concentration ratio of final solution to initial volume rather than to the collection of more inflamed joint fluids found in earlier reports.

The significance of these cryoproteins in various synovial fluids remains to be explained. Earlier studies have postulated that those complexes represent cold insoluble antigen-antibody complexes by virtue of the presence of DNA antigen and defined antibody activities. Cracchiolo *et al.* (1971) found a large percentage of rheumatoid fluid cryoproteins containing DNA antigen after heat denaturation, a process that was felt to expose antigenic sites. One cannot rule out absolutely the presence of viral antigens in the cryoglobulin complexes, since in arthritis associated with hepatitis B surface antigen (McIntosh *et al.*, 1976) and adenovirus related arthritis (Utsinger, 1977) viral antigens were detected by various techniques in the serum cryoglobulins.

Nonimmunological material has been found at

times in synovial fluid cryoproteins. Alpha 2-macroglobulin was detected occasionally by Marcus and Townes (1971). Zvaifler (1973) has suggested that extremely cold insoluble fibrin monomers, fibrinogen, and fibrin degradation products, found commonly in rheumatoid effusions, might make up a large component of the cryoprotein. Certainly a significant portion of the cryoprotein never solubilises in saline and might contain other protein important to the inflammatory response.

In summary, synovial fluid cryoproteins are found in the majority of joint fluids if they are examined for them. It remains to be determined whether these cryoproteins contain unique antigens or antibodies playing a critical role in all synovial inflammation or are just nonspecific cold insoluble proteins that appear in the synovial fluid as a consequence of inflammation.

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