Clin. exp. Immunol. (1967) **2**, 501–509.

RHEUMATOID FACTOR IN SYNOVIAL EFFUSIONS: LOCAL PRODUCTION AND CONSUMPTION

K. HANNESTAD AND O. J. MELLBYE

Institute for Experimental Medical Research, University of Oslo, Ullevål Hospital; and Oslo Sanitetsforenings Revmatismesykehus, Oslo

(*Received* 5 *December* 1966; *accepted* 20 *February* 1967)

SUMMARY

The ratio of synovial fluid γM-type rheumatoid factor (RF) to serum RF was compared with the ratio for two other γM-antibodies (trypsin- and periodate agglutinin) and total γM-globulin content in thirteen patients with rheumatoid arthritis.

In one case, the ratio for RF was higher than the ratios for the other γM-globulins and declined after synovectomy, indicating that a significant amount of RF in the synovial fluid was derived from locally produced RF. In three cases, the ratio for RF was lower than the other γM-globulin ratios, indicating local consumption or inhibition of RF in the joint. It was postulated that this phenomenon was due to some form of aggregated γG-globulin. In nine cases, the ratio for RF was equal or almost equal to the ratios for the other γM-globulins, indicating that the synovial fluid RF in these cases was mainly delivered from the circulation.

INTRODUCTION

The proteins of synovial fluid, except polysaccharide proteins, are derived mainly from plasma (Mackiewicz & Fenrych, 1961; Schur & Sandson, 1963). Their rate of diffusion into the synovial space is largely conditioned by their molecular size (Schur & Sandson, 1963). No such discrimination appears to occur during their re-absorption by way of the lymphatics (Rodnan & MacLachlan, 1960). The synovial fluid to serum ratio of proteins may thus be expected to be a function of their size and the permeability of the synovial membrane. This ratio should be equal for proteins of the same size, provided that local synthesis of the proteins or local consumption by immune-reactions do not occur.

Rheumatoid factor (RF) γM-globulin is generally present in the synovial effusions when it is found in serum. The titre in joint fluid is usually equal to or slightly lower than in the corresponding serum (Krehl *et al.*, 1957; Guariglia, Berkowitz & Steinbrocker, 1960; Rodnan, Eisenbeis & Creighton, 1963; Bland & Clark, 1963).

Correspondence: Dr Kristian Hannestad, Institute for Experimental Medical Research, Ullevål Hospital, Oslo, Norway.

Recently, local phenomena in the synovial membrane and fluid have been described with bearing on RF: Synthesis of RF in plasma cells of the rheumatoid synovial membrane has been demonstrated by immunofluorescent techniques (Mellors *et al.*, 1959). Similar techniques have revealed intracytoplasmic localization of γG- and γM-globulins in granulocytes from rheumatoid synovial effusions (Rawson, Abelson & Hollander, 1965; Barnett, Bienenstock & Block, 1966; Zucker-Franklin, 1966), presumed to be complexes between RF and aggregated γG-globulin (Hollander *et al.*, 1965).

The purpose of the present study was to determine if local processes in the synovial membrane and fluid have an influence on the quantity of RF in the synovial fluid as distinct from other γM-globulins.

MATERIALS AND METHODS

Rheumatoid sera and synovial fluids were obtained from twenty-three patients hospitalized at Oslo Sanitetsforening's Rheumatism Hospital, Oslo, and Kronprinsesse Märtha's Institute, Oslo. All fulfilled the American Rheumatism Association's criteria for definite or classical RA (Ropes *et al.*, 1959). The blood and synovial fluid samples were obtained simultaneously in the majority of cases. In one patient (No. 13) the synovial fluid was drawn from a shoulder joint; the other samples were taken from the knee joint. Heparin, 0·4 mg, was added to sterile tubes used for collection of synovial fluids usually giving a concentration of 0·1 mg/ml or less. The joint fluids were centrifuged at approximately 500 ***g*** for 10 min within 1 hr following the tap, stored at 4°C for 1–5 days, then at −20°C for several weeks. Prior to testing, 0·9 ml of synovial fluid was incubated for 4 hr at 37°C with 0·1 ml of bovine testicular hyaluronidase (Penetrase 'Leo') containing 4000 i.u./ml to reduce viscosity, followed by centrifugation at 1000 ***g*** for 1–2 hr to remove cells and debris.

Controls demonstrated that hyaluronidase had no effect on any of the quantitative determinations. This was tested by comparing hyaluronidase-treated and non-treated sera, and in recovery experiments performed by adding non-treated serum or serum proteins to treated serum or synovial fluid. Hyaluronidase did not precipitate with any of the antisera used.

Agglutination tests were performed by testing doubling dilutions in buffered saline of serum, or synovial fluid that was not inactivated, against a 1% suspension of red cells. The tests were performed on glass slides. One drop of diluted serum or synovial fluid was mixed with one drop of the red cell suspension and a third drop consisting of either buffered saline or serum diluted in saline. The slide was incubated at room temperature in a moist chamber and then read macroscopically. The agglutination was graded from + to +++ and the titre was recorded as the highest original dilution giving a + reaction. Each pair of serum and synovial fluid was tested simultaneously, using the same batch of cells and identical incubation time. The specific conditions for each test were as follows:

(a) *Agglutination of human red cells sensitized with rabbit amboceptor (Waaler–Rose test)*

Rabbit amboceptor was obtained commercially from Institut Pasteur, Paris. Cells were sensitized as described by Podliachouk, Eyquem & Jaqueline, (1958). A 5% dilution of normal serum in buffered saline was used as the third drop in the reaction mixture. This serum was negative both in the Waaler–Rose test and in the SHC test. The slide was incubated for 20 min before reading.

(b) *Agglutination of sensitized human O Rh positive cells (SHC test)*

For sensitization of the cells, anti-CD serum Ripley, kindly provided by Dr Marion Waller was used. One volume of packed cells was mixed with nine volumes of anti-CD Ripley diluted 1:8 in buffered saline. After incubation for 90 min at 37°C, the cells were washed four times in saline and made up to a suspension in buffered saline. The other conditions were as for the Waaler–Rose test.

(c) *Agglutination of trypsin-treated human red cells*

Trypsin treatment was performed as described by Morton & Pickles (1947), using bovine trypsin crystallized with $MgSO_4$ (Koch, Light Laboratories, Colnbrook, Buckinghamshire, England). Buffered saline was used as the third drop in the reaction mixture, and the agglutination was read after 10 min incubation.

(d) *Agglutination of periodate-treated human red cells*

Periodate treatment was performed as described by Kipnis & Sacks (1955), except that potassium periodate (Analar grade, Hopkin & Williams Ltd, Chadwell Heath, Essex, England) was used instead of sodium periodate. The other conditions were as for agglutination of trypsin-treated cells.

Reduction and alkylation of disulphide bonds. Synovial fluid was diluted 1:2 in buffered saline and 2-mercaptoethanol (Fluka AG, Buchs, Switzerland) was added to a final concentration of 0·15 M. After incubation for 4 hr at room temperature, the material was dialysed against 0·02 M-iodacetamid (Fluka AG, Buchs, Switzerland) in buffered saline for another 4 hr at room temperature. The reduced and alkylated samples were finally dialysed overnight in the cold against buffered saline.

γM- and γG-globulin concentrations were determined by radial diffusion in gel with specific antibody incorporated in agar, using the technique of Fahey & McKelvey (1964) and an experimental procedure described by Mellbye (1966).

EXPERIMENTS AND RESULTS

The influence of local factors on the content of RF in arthritic joints was evaluated by comparing the ratio between the content of RF in synovial fluid and serum with the corresponding ratios for other γM-antibodies and total γM-globulin.

This experimental principle was based on three assumptions: Firstly, assayed RF in synovial fluid must be γM-globulin only. This was tested in experiment 'b'. Secondly, γM RF must be transported from serum to synovial fluid like other γM-globulins. This assumption is only approximately correct, since RF combines with γG-globulin in a 22S complex (Franklin *et al.*, 1957). Thirdly, the various other γM-antibodies must be catabolized and removed in the same way. This assumption was tested in experiment 'c'.

(a) *The content of RF in serum and synovial fluid from patients with rheumatoid arthritis*

In thirteen patients a titre of 32 or higher was found in serum; these cases were regarded as RF positive. In all these cases some agglutinating activity was found in the synovial

fluid. No atypical agglutination patterns or other diffculties in reading were encountered with synovial fluids. The results for the RF positive patients are given in Table 1.

TABLE 1. Amounts of rheumatoid factor and γG-globulin in synovial fluid (Syn.) and serum of seropositive patients (Ser.)

Patient No.	Fluid tested	Rheumatoid factor (reciprocal of dilutions)		γG-globulin (mg/ml)
		Waaler–Rose	(SHC) Ripley	
1	Syn.	512	1024	
	Ser.	1024	2048	
2	Syn.	512	512	
	Ser.	512	512	
3*	Syn.	1024	2048	22
	Ser.	128	512	23
3†	Syn.	256	256	12
	Ser.	256	256	23
4	Syn.	<2	32	
	Ser.	<2	64	
5	Syn.	256	2048	
	Ser.	256	2048	
6	Syn.	16	16	13
	Ser.	64	2048	23
7	Syn.	2	4	20
	Ser.	64	128	32
8	Syn.	64	16	
	Ser.	64	16	
9	Syn.	64	<2	
	Ser.	256	<2	
10	Syn.	128	<2	12
	Ser.	256	128	15
11	Syn.	16	512	
	Ser.	32	2048	
12	Syn.	512	256	
	Ser.	512	64	
13	Syn.	256	4096	
	Ser.	512	8192	

* Before synovectomy.
† 15 days after synovectomy.

(b) *Immunoglobulin class of RF in synovial fluid*

To exclude the possibility that some of the synovial fluid RF activity in present test systems was due to γG-type RF, the synovial fluids of all RF positive patients were reduced and alkylated. This procedure is known to destroy the agglutinating activity of γM-globulins

but not of γG-globulins (Fudenberg & Kunkel, 1957; Grubb & Swahn, 1958). RF activity of all synovial fluids was abolished by reduction, indicating that no significant amounts of γG-type RF were present.

(c) *Local influence on the content of γM-globulins other than RF*

All the synovial fluids and sera were tested for their content of trypsin agglutinin, periodate agglutinin and total γM-globulin. Trypsin agglutinin and periodate agglutinin are

TABLE 2. Synovial fluid to serum ratios of RF and other γM-globulins

Patient No.	Rheumatoid factor		γM-antibodies		Total γM-globulin
	Waaler	Ripley	Trypsin agglutinin	Periodate agglutinin	
1	1:2*	1:2*	1:4*	1:2*	1:1†
2	1:1	1:1	1:2	1:4	1:2
3‡	**8:1**	**4:1**	1:4	1:4	1:2
3§	1:1	1:1	1:4	1:4	1:2
4	—	1:2	1:1	1:2	1:2
5	1:1	1:1	1:1	1:1	1:1
6	1:4	**1:128**	1:2	1:2	1:2
7	**1:32**	**1:32**	1:2	1:2	1:2
8	1:1	1:1	1:2	1:2	1:2
9	1:4	1:2	1:2	1:1	1:2
10	1:2	**1:64**	1:2	1:2	1:3
11	1:2	1:4	1:2	1:2	1:4
12	1:1	4:1	1:1	1:1	1:1
13	1:2	1:2	1:2	1:1	1:1
14	—	—	1:1	1:1	1:2
15	—	—	1:2	1:2	1:1
16	—	—	1:4	1:4	1:2
17	—	—	1:2	1:1	1:2
18	—	—	1:1	1:1	1:1
19	—	—	1:2	1:4	1:2
20	—	—	1:1	1:2	1:3
21	—	—	1:2	1:2	1:2
22	—	—	1:2	1:2	1:2
23	—	—	1:2	1:1	1:2

* Ratio of the reciprocals of titres.
† Ratio of concentrations, expressed as a proportion to facilitate comparison.
‡ Before synovectomy.
§ 15 days after synovectomy.

γM-globulins (Yachnin & Gardner, 1961; Mellbye, 1965). They are present in normal human sera and react with erythrocytes treated with trypsin and periodate respectively.

The ratios of titres in synovial fluid to titres in serum are shown in Table 2. In twenty-two cases the three ratios were identical, or differed by only one titration step. In one patient a

difference of two steps was observed. These differences can be explained by the errors inherent in all titration techniques. The influence of local factors on the content in synovial fluid of these two randomly chosen γM-antibodies thus did not differ from their influence on total γM-globulin.

(d) *Local influence on the content of RF in synovial fluid*

For all the RF positive patients, the ratio of RF titre in synovial fluid to serum was compared with the corresponding ratios for the other γM-antibodies and for total γM-globulin. The results are shown in Table 2.

In four cases the ratio for RF differed by *more than two* titration steps from those of the other γM-globulins. This was judged to be a significant difference, indicating that the influence of local factors on the content of RF was different from that on the other γM-globulins. In three of these cases the ratio for either both (No. 7) or one (Nos. 6 and 10) of the RF tests was *lower* than for the other γM-globulins. In one case (No. 3), the ratio for both RF tests was *higher* than for the other γM-globulins.

In the other eight patients, the ratio for RF was equal to, or almost equal to the corresponding ratios for the other γM-globulins.

(e) *The content of γG-globulin in serum and synovial fluid*

This was measured in the four cases where deviations for the RF ratio were observed. The results are included in Table 1.

In the three cases where the ratio for RF was lower than for the other γM-globulins, the γG-concentration was lower in synovial fluid than in serum. In the case where the RF was higher than for the other γM-globulins, the concentration of γG-globulin was approximately identical in synovial fluid and serum.

DISCUSSION

The fact that the synovial fluid/serum ratios of the titres for trypsin- and periodate agglutinins were similar to the ratios for total γM-concentrations suggested that the haemagglutination technique was adequate when performed with hyaluronidase-treated synovial fluid. Within the limits of the methods, this correlation indicates that the bulk of γM-antibodies are delivered to and removed or catabolized in the synovial fluid in a similar fashion. γM RF, however, was found to differ from this pattern in several instances.

There is evidence that the RFs are antibodies against human γG-globulin. Interaction between RF and native γG-globulin results in formation of a 22S complex (Franklin *et al.*, 1957). The induction of certain structural changes in the γG-globulin molecules leads to highly active preparations which precipitate with RF. Such changes can be produced experimentally *in vitro* in two different ways: By various treatments of isolated γG-globulin causing aggregation of the molecules (Franklin *et al.*, 1957), or by the preparation of soluble or insoluble immune complexes where γG-globulin functions as a specific antibody (Vaughan 1956; Edelman, Kunkel & Franklin, 1958; Aho, 1961; Harboe, 1961). The latter principle has been utilized in the SHC (Ripley) and Waaler–Rose tests where RF reacts with human and rabbit γG-globulin respectively on the surface of red cells leading to agglutination of the cells. This agglutination is inhibited by the presence of low concentrations of human

γG-globulin in aggregated form. In addition, the SHC (Ripley) test—but usually not the Waaler–Rose test—is inhibited by non-aggregated (native) γG-globulin (for reference see Aho, 1961).

A *high* ratio for RF γM-globulin compared to other γM-globulins as observed in case No. 3 and in other studies (Krehl *et al.*, 1957; Guariglia *et al.*, 1960; Bland & Clark, 1963) may result from either: (1) selective retention of RF in the joint, or (2) local synthesis in the synovial membrane. Theoretically, RF bound to γG-globulin might be retained in the joint because of the larger size of such a complex. However, there is no evidence that the 22S complex—the only known soluble complex of RF γM-globulin that is serologically active—is present in higher concentration in the joint fluid than in the circulation (Rodnan *et al.*, 1963). In all other known interactions between RF and γG-globulin, precipitates are formed which are chemotactic (Hollander *et al.*, 1965) and phagocytosed by granulocytes (Parker & Schmid, 1962). Thus a *low* rather than high ratio for RF would be expected to result from such interactions. On the other hand, local synthesis of RF in the rheumatoid synovial membrane is well established (Mellors *et al.*, 1959) and seems to be the most likely explanation for the high RF ratios. In our case No. 3, the decline of the RF ratio following synovectomy and the presence of an abundance of fluorescing plasma cells in the synovial membrane after staining with fluorescein-labelled aggregated γG-globulin (Hannestad, unpublished observations) furnish additional evidence in support of this explanation.

A *lower* ratio for RF γM-globulin than for other γM-globulins, as observed in cases Nos. 6, 7 and 10, may result from either: (1) the presence of excess soluble aggregates of γG-globulin causing agglutination inhibition, or (2) precipitation or adsorption of RF with soluble or solid forms of aggregated γG-globulin leading to a local consumption of RF, or a combination of the two. Direct evidence for the presence of components in inflammatory joint fluids precipitating with RF is presented in the accompanying paper (Hannestad, 1967). That the low RF ratio was not due to a low concentration of total γM-globulin in the synovial fluid was conclusively shown by the other γM-globulin ratios. Inhibition by high concentration of γG-globulin was excluded by demonstrating that the γG-globulin concentration of the synovial fluids with low RF ratio was equal to or lower than that of the corresponding sera. Inhibition of joint fluid RF agglutinations by complement components, known to occur in the latex fixation test (Schubart, 1959), is also unlikely, since complement activity in rheumatoid joint fluids is usually lower than in serum (Hedberg, 1963; Pekin & Zvaifler, 1964).

In the present study a low synovial fluid/serum ratio for RF was detected more often by the SHC (Ripley) than by the Waaler–Rose test. Such low ratios have been noted in other investigations using latex particles coated with Cohn Fraction II (Guariglia *et al.*, 1960; Rodnan *et al.*, 1963; Bland & Clark, 1963), but rarely with the Waaler–Rose test (Krehl *et al.*, 1957; Peltier, Coste & Delbarre, 1966). Although no systematic study of this point is available, it appears from these data that a number of rheumatoid synovial fluids are negative or low titred compared to serum using RF tests with human γG-globulin as reactant, without such a discrepancy being detected as frequently with rabbit γG-globulin (the Waaler–Rose test).

In most of the seropositive cases, the ratio for RF was of the same magnitude as for the other γM-immunoglobulins. This indicates that the amount of RF synthesized locally in

the joint and the amount removed locally by aggregated γG-globulin is usually small compared with the quantities of RF delivered to the synovial fluid from the circulation.

REFERENCES

AHO, K. (1961) The problem of the antibody nature of the rheumatoid factor. *Ann. med. exp. Fenn.* **39,** suppl. 7.

BARNETT, E., BIENENSTOCK, J. & BLOCH, K. (1966) Antinuclear factors in synovia. *J. Amer. med. Ass.* **198,** 143.

BLAND, J.H. & CLARK, L. (1963) Rheumatoid factor in serum and joint fluid. *Ann. intern. Med.* **58,** 829.

EDELMAN, G.M., KUNKEL, H.G. & FRANKLIN, E.C. (1958) Interaction of the rheumatoid factor with antigen-antibody complexes and aggregated gamma globulin. *J. exp. Med.* **108,** 105.

FAHEY, J.L. & MCKELVEY, E.M. (1964) Quantitative determination of serum immunoglobulins in antibody-agar plates. *J. Immunol.* **94,** 84.

FRANKLIN, E.C., HOLMAN, H.R., MÜLLER-EBERHARD, H.J. & KUNKEL, H.G. (1957) An unusual protein component of high molecular weight in the serum of certain patients with rheumatoid arthritis. *J. exp. Med.* **105,** 425.

FUDENBERG, H.H. & KUNKEL, H.G. (1957) Physical properties of red cell agglutinins in acquired hemolytic anemia. *J. exp. Med.* **106,** 689.

GRUBB, R. & SWAHN, B. (1958) Destruction of some agglutinins but not of others by two sulphydryl compounds. *Acta path. microbiol. scand.* **43,** 305.

GUARIGLIA, E., BERKOWITZ, S. & STEINBROCKER, O. (1960) Latex agglutination in serum and synovial fluid (Abstract). *Arthr. and Rheum.* **3,** 447.

HANNSTAD, K. (1967) Presence of aggregated γG-globulin in certain rheumatoid synovial effusions. *Clin. exp. Immunol.* **2,** 511.

HARBOE, M. (1961) Interactions of rheumatoid factor with immune-precipitate containing antibody of human origin. *Ann. rheum. Dis.* **20,** 363.

HEDBERG, H. (1963) Studies on the depressed hemolytic complement activity of synovial fluid in adult rheumatoid arthritis. *Acta rheum. scand.* **9,** 1965.

HOLLANDER, J.L., MCCARTY, D.J., ASTORGA, G. & CASTRO-MURILLO, E. (1965) Studies on the pathogenesis of rheumatoid joint inflammation. I. The 'R.A.' cell and a working hypothesis. *Ann. intern. Med.* **62,** 271.

KIPNIS, D.M. & SACKS, M.S. (1955) Observations on an agglutinin in human serum for periodate-treated erythrocytes. *J. Lab. clin. Med.* **45,** 632.

KREHL, W.A., BOISVERT, P.L., FOREST, G.K. & MUCCI, M.B. (1957) The rheumatoid factor in serum and synovial fluid. *Yale J. Biol. Med.* **30,** 30.

MACKIEWICZ, S. & FENRYCH, W. (1961) Immuno-electrophoretic analysis of proteins in serum and synovial fluid in rheumatoid arthritis and ankylosing spondylitis. *Ann. rheum. Dis.* **20,** 265.

MELLORS, R.C., HEIMER, R., CORCOS, J. & KORNGOLD, L. (1959) Cellular origin of rheumatoid factor. *J. exp. Med.* **110,** 876.

MELLBYE, O.J. (1965) Reversible agglutination of trypsinised red cells by normal human sera. Identification of the agglutinating factor as a γM-globulin. *Scand. J. Haemat.* **2,** 318.

MELLBYE, O.J. (1966) Reversible agglutination of trypsinised red cells by γM-globulin synthesized by the human foetus. *Scand. J. Haemat.* **3,** 310.

MORTON, J.A. & PICKLES, M.M. (1947) Use of trypsin in the detection of incomplete anti-Rh antibodies. *Nature (Lond.),* **159,** 779.

PARKER, R.L. & SCHMID, F.R. (1962) Phagocytosis of particulate complexes of gamma globulin and rheumatoid factor. *J. Immunol.* **88,** 519.

PEKIN, T.J. & ZVAIFLER, N.J. (1964) Hemolytic complement in synovial fluid. *J. clin. Invest.* **43,** 1372.

PELTIER, A., COSTE, F. & DELBARRE, F. (1966) Études sur le complément hémolytique dans certaines maladies ostéoarticulaires. *Presse méd.* **74,** 1523.

PODLIACHOUK, L., EYQUEM, A. & JAQUELINE, F. (1958) Le diagnostic de la polyarthrite chronique évolutive par agglutination des globules rouges humains sensibilisés. *Ann. Inst. Pasteur,* **94,** 659.

RAWSON, A.J., ABELSON, N.M. & HOLLANDER, J.L. (1965) Studies on the pathogenesis of rheumatoid joint inflammation. II. Intracytoplasmic particulate complexes in rheumatoid synovial fluids. *Ann. intern. Med.* **62**, 281.

RODNAN, G.P. & MACLACHLAN, M.J. (1960) The absorption of serum albumin and gamma globulin from the knee joint of man and rabbit. *Arthr. and Rheum.* **3**, 152.

RODNAN, G.P., EISENBEIS, C.H. & CREIGHTON, A.S. (1963) The occurrence of rheumatoid factor in synovial fluid. *Amer. J. Med.* **35**, 182.

ROPES, M.W., BENNETT, G.A., COBB, S., JACOX, R. & JESSAR, R.A. (1959) 1958 revision of diagnostic criteria for rheumatoid arthritis. *Arthr. and Rheum.* **2**, 16.

SCHUBART, A.F. (1959) Latex-fixation test in rheumatoid arthritis. II. Characterization of the thermolabile inhibitor by a serologic study. *New Engl. J. Med.* **261**, 579.

SCHUR, P.H. & SANDSON, J. (1963) Immunologic studies of the proteins of human synovial fluid. *Arthr. and Rheum.* **6**, 115.

VAUGHAN, J.H. (1956) Behavior of the rheumatoid arthritis agglutinating factor with immune precipitates. *J. Immunol.* **77**, 181.

YACHNIN, S. & GARDNER, F.H. (1961) pH dependent hemolytic systems. III. The physical properties of the serum factors involved, with some observations on their occurrence in various disease states in man. *Blood*, **18**, 349.

ZUCKER-FRANKLIN, D. (1966) The phagosomes in rheumatoid synovial fluid leucocytes: A light, fluorescence, and electron microscope study. *Arthr. and Rheum.* **9**, 24.