Clin. exp. Immunol. (1970) **6,** 407–411.

STUDIES OF THE THIRD COMPONENT OF COMPLEMENT IN SYNOVIAL FLUID FROM ARTHRITIC PATIENTS

I. IMMUNOCHEMICAL QUANTITATION AND RELATION TO TOTAL COMPLEMENT

B. LUNDH, H. HEDBERG AND ANNA-BRITA LAURELL

Department of Internal Medicine, Department of Rheumatology and Institute for Medical Microbiology, University Hospital, University of Lund, Sweden

(*Received* 20 *June* 1969)

SUMMARY

In a study of forty-one patients, most of whom had longstanding rheumatoid arthritis, a comparison was made in serum and synovial fluid between total complement activity (CH_{50}) and C3, measured immunochemically. Nineteen out of twenty-seven patients with rheumatoid arthritis and four out of four cases of systemic lupus erythematosus (SLE) or SLE-like syndromes had a depressed CH_{50} value in the synovial fluid, while ten cases with a non-rheumatoid form of arthritis all had normal activity.

The total C activity in serum was normal or increased in most cases. While no correlation was found between CH_{50} and C3 in serum, there was a statistically highly significant correlation in synovial fluid, indicating that C3 determinations can be used to reveal alterations in C activity of synovial fluid.

INTRODUCTION

A suppression of the total complement (C) activity of synovial fluid has been shown to be a characteristic phenomenon of rheumatoid arthritis and systemic lupus erythematosus (for references see Hedberg, 1967). In fact, the phenomenon appears in rheumatoid arthritis with the same frequency as a positive test for rheumatoid factors (Hedberg, 1969).

Monospecific antisera have been used for immunochemical quantitation of some of the C components in human whole serum (e.g. Clq, C3, C4 and C5, see Kohler & Müller-Eberhard, 1967). Immunochemical quantitation of C3 in synovial fluid has been used in a few preliminary reports (Bailey, 1967; Hanson *et al.*, 1968).

In the present study the concentration of C3, as determined by electrophoresis in agarose gel containing antibodies (Laurell, 1966), was compared with the total C activity in serum and synovial fluid from arthritic patients.

Correspondence: Dr B. Lundh, Department of Internal Medicine, University Hospital, Fack, 220 05 Lund, Sweden.

MATERIALS AND METHODS

The *clinical material* consisted of forty-one patients with active arthritis, twenty-seven of whom had longstanding rheumatoid arthritis (RA), four SLE or SLE-like syndromes (all receiving corticosteroids) and ten various forms of non-rheumatoid arthritis (see Table 1). The patients were selected and the arthritis classified as described elsewhere (Hedberg, 1967).

For the determination of C3, freshly obtained *blood and synovial fluid* were immediately mixed with Na_2EDTA (final concentration 10^{-2} M) (Laurell & Lundh, 1967). The samples were centrifuged and the supernatants frozen within $1\frac{1}{2}$ hr and stored at $-80°C$ until analysed. The synovial fluid was treated with a hyaluronidase preparation, known to be free of proteolytic activity (HYALAS®, which was kindly supplied by Dr B. Högberg, AB Leo, Hälsingborg, Sweden). For determination of CH_{50} units blood and synovial fluid without EDTA were used, centrifuged and the supernatants frozen within $1\frac{1}{2}$ hr.

The *content of* C3 in EDTA plasma and EDTA synovial fluid was estimated by electrophoresis in agarose gel according to Laurell (1966), containing antiserum to C3 and its conversion products. A standard curve was prepared using three dilutions of a pool of normal sera. The concentration of C3 in the experimental samples was calculated with the aid of this curve, and the results were expressed as a percentage of the C3 concentration of the serum pool. Duplicate analyses were made for every determination.

The standard error of a single determination, calculated from duplicate determinations performed on different days, was found to be 9·2% for serum (27 × 2 determinations) and 13·5% for synovial fluid (15 × 2 determinations). The standard error for the determination of the CH_{50} units in serum and synovial fluid was 7·3% and 6·9% respectively (Hedberg, 1967).

The total C activity of synovial fluid and rheumatoid factor titres were assayed as described previously (Hedberg, 1967).

For expressing the degree of correlation Spearman's rank-order correlation coefficient 'r_S' (Siegel, 1956) was calculated.

RESULTS

The relation between the C3 concentration and the number of CH_{50} units/ml in synovial fluid and in serum is shown in Fig. 1. There was no correlation between the two parameters in serum (Fig. 1b), while there was a statistically highly significant correlation in synovial fluid ($r_S = +0·83$***, Fig. 1a). Similarly, the synovial fluid-serum ratio for the C3 concentration correlated with the synovial fluid-serum ratio for CH_{50} units/ml ($r_S = +0·83$***, Fig. 2). The finding of low ratios could not be explained by a low protein content of the synovial fluids (Table 1). Positive tests for rheumatoid factors (in serum and synovial fluid) were most common at low ratios.

In Table 1 the cases are grouped according to the total C activity of synovial fluid, estimated as described elsewhere (Hedberg, 1967) using the confidence limits obtained on a large number of non-rheumatoids (Hedberg, 1969). As in previous studies (e.g. Hedberg, 1967, 1969), the lowest values of the synovial fluid C activity were obtained in cases of RA (notably those with positive tests for rheumatoid factors) and in cases of SLE or SLE-like syndromes (Table 1).

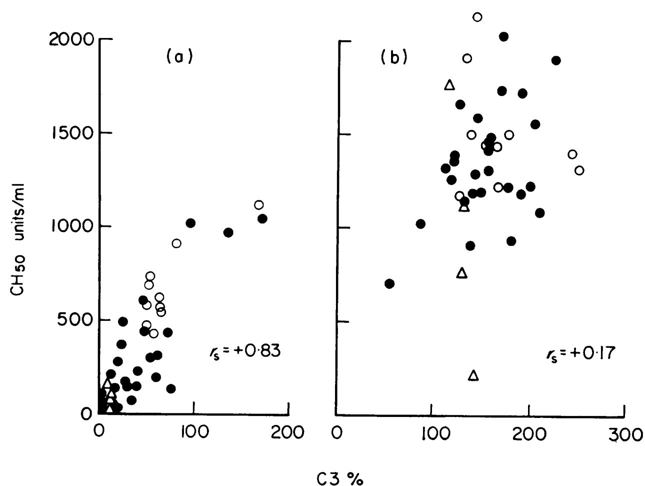

FIG. 1. The relation between total C (CH_{50} units/ml) and C3 (immunochemically quantitated) in synovial fluid (a) and serum (b). The C3 values (C3 %) are expressed as a percentage of the value of a pool of normal sera. ●, Rheumatoid arthritis; △, SLE or SLE-like syndromes; ○, non-rheumatoid arthritis (see Table 1), r_S = Spearman's rank-order correlation coefficient.

TABLE 1. The synovial fluid-serum ratios for C3 and CH_{50} units and the protein content of synovial fluid ('TP_{SF}') at different levels of the total C activity of synovial fluid

Total C activity of synovial fluid	Synovial fluid—serum ratio for		TP_{SF} (g%)	Total	No. of patients		
	C3	Total C			Non-rheumatoid arthritis*	Rheumatoid arthritis	SLE or SLE-like
Normal (140–56)	0·40	0·42	4·8	18	10	8	0
Probably suppressed (55–42)	0·14	1·20	4·0	5	0	4	1
Significantly suppressed (<42)	0·12	0·09	4·3	18	0	15	3

* Reiter's disease, two; psoriatic arthropathy, five; juvenile oligo-arthritis (Ansell & Bywaters, 1962), two; and ankylosing spondylitis, one.

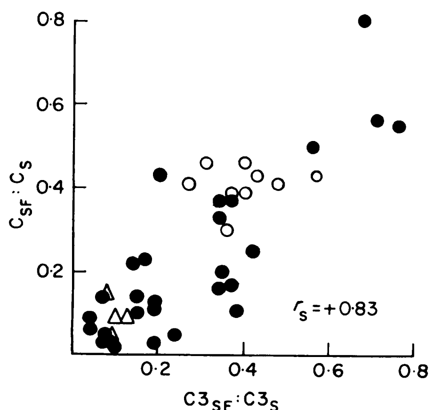

FIG. 2. The synovial fluid-serum ratio for total C (C_{SF}:C_S) plotted against the synovial fluid-serum ratio for C3 ($C3_{SF}$:$C3_S$). Symbols as in Fig. 1.

When additional specimens of synovial fluid and serum specimens from seventeen patients were studied, the results conformed essentially with those obtained on the primary specimens.

DISCUSSION

In synovial fluid the concentration of C3, often markedly decreased, was found to correlate with the number of CH_{50} units/ml. In serum, however, with its normal or increased C activity, no correlation was found. In a group of patients with SLE, nephrotic syndrome and hereditary angioneurotic oedema Klemperer *et al.* (1965) showed that in the two first mentioned conditions the C activity of *serum* was depressed only when the C3 concentration was below a certain value. Our findings seem to indicate a similar relationship.

In a previous report (Hedberg, 1967) an estimate of the synovial fluid C activity was used, defined as the synovial fluid-serum ratio for total C (CH_{50} units) divided by the protein content of synovial fluid. By use of this estimate the variations of the serum C level and the protein content of synovial fluid were taken into account. In the present study the synovial fluid-serum ratio for total C proved to correlate with the synovial fluid-serum ratio for C3. This might indicate that the C3 ratio may reveal a disturbance involving the C system in synovial fluid. The immunochemical determination of this parameter is rapid, inexpensive and performed with a methodological error comparable to that for the determination of the number of CH_{50} units. However, it has to be observed that spontaneous conversion of C3 may affect the result (Lundh, 1965; Kohler & Müller-Eberhard, 1967) since immunochemical determination of the concentration of C3 does not differentiate between the haemolytically active C3 and its inactive conversion products. However, in synovial fluid the concentration correlated with the number of CH_{50} units/ml; and among the synovial fluid samples with a high concentration of C3 there was none in which the total C activity was suppressed. This argues against the possibility that a high C3 concentration in synovial fluid would represent predominantly conversion products. The extent to which conversion appears at different synovial fluid C activity levels will be described in a forthcoming article. Attempts to avoid spontaneous conversion *in vitro* were made by immediate mixing of the sample with EDTA, freezing within 1½ hr and storage at $-80°C$.

The immunochemical technique used, i.e. electrophoresis in agarose gel containing antibodies, has certain advantages compared with the Oudin (1952) technique, e.g. the results can be obtained within a few hours and the agarose plates can be preserved after drying and staining.

Previous reports have shown the haemolytically determined titres of C1, C4 and C2 to be low in rheumatoid synovial fluid (Zvaifler & Pekin, 1963; Fostiropoulus *et al.*, 1965). Thus, together with the demonstration of a decreased concentration of C3, available evidence indicates an involvement of the first four C components (C1, C4, C2 and C3), which is the rule in C fixation, e.g. to antigen–antibody complexes. C3 and C4 have been shown *in vivo* to be bound to the rheumatoid synovial membrane (Fish *et al.*, 1966; Rodman *et al.*, 1967). The results support the idea of the binding of C components to the rheumatoid synovial membrane as a cause of the decrease of total C and C components in rheumatoid synovial fluid. Nevertheless, a decrease of these C components might also be due to an enzymatic activation of C1, e.g. through the effect of kallikrein (Gigli *et al.*, 1968) or other enzymes (Ratnoff & Naff, 1967; Laurell, 1968).

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council (Grant No. B 69-16x-68-05A), 'Konung Gustaf V:s 80-årsfond' and 'Alfred Österlunds Stiftelse.'

REFERENCES

ANSELL, B.M. & BYWATERS, E.G.L. (1962) Diagnosis of 'probable' Still's disease and its outcome. *Ann. rheum. Dis.* **21,** 253.

BAILEY, J.P. (1967) Evaluation of a semi-quantitative $beta_{1C}/beta_{1A}$ complement assay. *Arthr. and Rheum.* **10,** 263 (Abstract).

FISH, A.J., MICHAEL, A.F., GEWURZ, H. & GOOD, R.A. (1966) Immunopathologic changes in rheumatoid arthritis synovium. *Arthr. and Rheum.* **9,** 267.

FOISTIROPOULUS, G., AUSTEN, K.F. & BLOCH, K.J. (1965) Total hemolytic complement ($C'H_{50}$) and second component of complement ($C'2^{hu}$) activity in serum and synovial fluid. *Arthr. and Rheum.* **8,** 219.

GIGLI, I., MASON, J.W., COLMAN, R.W. & AUSTEN, K.F. (1968) Interaction of kallikrein with the C'1 esterase inhibitor. *J. Immunol.* **101,** 814.

HANSON, V., REED, G., KAPLAN, J.M. & KORNREICH, H. (1968) Studies of synovial fluid and synovia in juvenile rheumatoid arthritis. *Arthr. and Rheum.* **11,** 486 (Abstract).

HEDBERG, H. (1967) Studies on synovial fluid in arthritis. *Acta med. scand.* Suppl. 479.

HEDBERG, H. (1969) The suppressed synovial fluid complement activity and its close association with seropositivity in arthritis. *Arthr. and Rheum.* (In press).

KLEMPERER, M.R., GOTOFF, S.P., ALPER, C.A., LEVIN, A.S. & ROSEN, F.S. (1965) Estimation of the serum $beta_{1C}$ globulin concentration: Its relation to the serum hemolytic complement titer. *Pediatrics,* **35,** 765.

KOHLER, P.F. & MÜLLER-EBERHARD, H.J. (1967) Immunochemical quantitation of the third, fourth and fifth components of human complement: concentrations in the serum of healthy adults. *J. Immunol.* **99,** 1211.

LAURELL, A.-B. (1968) Effect of trypsin on complement components in normal human serum. *Acta path. microbiol. scand.* **72,** 139.

LAURELL, C.-B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analyt. Biochem.* **15,** 45.

LAURELL, C.-B. & LUNDH, B. (1967) Electrophoretic studies of the conversion products of serum β_{1C}-globulin. *Immunology,* **12,** 313.

LUNDH, B. (1965) Studies on serum β_{1C}-globulin. *Acta univ. Lund.* Section II, No. 27.

OUDIN, J. (1952) Specific precipitation in gels and its application to immunochemical analysis. *Methods in Medical Research,* Vol. 5, p. 335. The Year Book Publishers, Chicago.

RATNOFF, O.D. & NAFF, G.B. (1967) The conversion of C'1s to C'1 esterase by plasmin and trypsin. *J. exp. Med.* **125,** 1967.

RODMAN, W.S., WILLIAMS, R.C., JR, BILKA, P.J. & MÜLLER-EBERHARD, H.J. (1967) Immunofluorescent localization of the third and the fourth component of complement in synovial tissue from patients with rheumatoid arthritis. *J. Lab. clin. Med.* **69,** 141.

SIEGEL, S. (1956) *Nonparametric statistics for the Behavioral Sciences.* McGraw-Hill, New York.

ZVAIFLER, N. & PEKIN, T.J., JR (1963) Complement components in synovial fluid. *Clin. Res.* **11,** 180.