Behaviour of synovial complement C3 and C4 components in inflammatory and degenerative joint diseases, before and after synoviorthesis

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Gabay, R., Micheli, A., and Fallet, G. H. (1975). *Annals of the Rheumatic Diseases*, 34, 166. Behaviour of synovial complement C3 and C4 components in inflammatory and degenerative joint diseases, before and after synoviorthesis. The synovial fluid β_1A (C3) and β_1E (C4) (expressed by their ratio to corresponding serum concentrations) were studied in 31 cases of seropositive rheumatoid arthritis (RA+), 5 cases of seronegative rheumatoid arthritis (RA-), and 15 cases of osteoarthritis (OA) before osmic acid synoviorthesis on knees. This was repeated after synoviorthesis in the synovial fluid (SF) of 24 RA+, 4 RA-, and 10 OA patients.

The following studies were undertaken: (a) the relationship between these components; (b) their correlation with the SF protein concentration and rheumatoid factor titre, when present.

This analysis led us to the following conclusions. (1) *Before synoviorthesis* (a) The SF β_1E is significantly lower than β_1A in RA+. In OA, an inverse phenomenon is observed. (b) The concentration of β_1A and β_1E are proportional to the protein concentration in the SF of OA. A significant inverse relationship between β_1A , β_1E , and the titre of rheumatoid factor is found. (2) *After synoviorthesis* The same studies performed on knees at the time of one or more relapses shows that the same pathogenetic process is involved and that the immunological mechanism is little influenced by this treatment. In OA also the relapse differs very little from the initial process observed before synoviorthesis.

Many authors have described a rise in total haemolytic complement in the synovial fluid (SF) of articular inflammatory processes, with the exception of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (Pekin and Zvaifler, 1964; Peltier, Coste, and Delbarre, 1966; Hedberg, 1967; Sonozaki and Torisu, 1970; Kourilsky, Peltier, and de Sèze, 1972). Other investigators have shown an inverse correlation between the fluid complement activity and the titre of the latex test for rheumatoid factor in the SF and serum of RA patients (Hedberg, 1967; Vaughan, Barnett, Sobel, and Jacox, 1968; Lundh, Hedberg, and Laurell, 1970; Townes and Sowa, 1970; Hedberg, 1971; Hedberg and Laurell, 1972). It has also been shown that the decrease in components C2 and C4 was more marked than that of C3 in the SF from RA

and SLE patients (Peltier and others, 1966; Ruddy, Britton, Schur, and Austen, 1969; Sonozaki and Torisu, 1970; Peltier and de Sèze, 1971; Ruddy, Matsuura, Stillmann, and Austen, 1971).

The first objective of our study was to determine the concentrations of the components C3 (β_1A) and C4 (β_1E), expressed as the ratio of their SF concentration to serum concentration in the following three entities: (i) seropositive rheumatoid arthritis, RA+; (ii) seronegative rheumatoid arthritis, RA-; (iii) osteoarthritis, OA.

We also investigated the relationship between SF protein concentrations, the SF latex levels, on one hand, and the SF/serum ratio of β_1A and β_1E , on the other.

Another of our objectives was the investigation of possible changes in our initial data that appear during a relapse, after an unsuccessful synoviorthesis with

osmic acid. For this purpose C3, C4, and their relationship to different SF parameters mentioned above were analysed.

Material and methods

We studied 31 samples of SF in RA+ (from 25 knees) before synoviorthesis, and 24 samples (from 15 knees) after unsuccessful synoviorthesis (carried out by intra-articular injections of 100–200 mg osmic acid) (Boussina, Kuzmanovic, Esselinckx, and Fallet, 1974; Micheli, Boussina, and Fallet, 1975). The same study was repeated on 5 samples of RA– (5 knees) before and 4 samples (3 knees) after synoviorthesis.

These determinations were also carried out on 15 samples from 13 OA knees before synoviorthesis and 10 samples collected at the time of repeated relapses after synoviorthesis from 3 knees of OA patients.

The β_{1A} (C3) and the β_{1E} (C4) were measured by the radial immunodiffusion method of Mancini, Carbonara, and Heremans (1965) by using the specific antisera of Dutch Red Cross anti- β_{1A} (KH 41-10-P1) and anti- β_{1E} (KH 12-6-P1). The SF protein concentration was determined by the biuret method and the rheumatoid factor sought by the quantitative latex fixation test according to Rheins, McCoy, Burrell, and Buehler, (1957).

The SF concentration is expressed as the ratio of C3 (β_{1A}) and C4 (β_{1E}) SF concentration to serum concentration measured simultaneously (β_{1A}/β_{1A}^s and β_{1E}/β_{1E}^s). For simplification, these ratios will be expressed, in this study, as β_{1A} and β_{1E} .

Results

A The mean concentrations and standard deviations of β_{1A} and β_{1E} in RA+, RA–, and OA, before and after synoviorthesis, are shown in Table I. This table clearly shows a lower level of β_{1E} in RA+ before and after synoviorthesis compared to the RA– and OA fluids. This difference, which is significant in RA+ and OA before synoviorthesis, may be expressed as

the ratio β_{1A}/β_{1E} indicated in Table I, II. Before synoviorthesis, the three conditions can be differentiated in a significant manner by this ratio. These differences persist after synoviorthesis but according to a statistical analysis, they are significant only between RA+ and OA.

B Fig. 1 illustrates the relationship between the level of SF protein concentration and the levels of β_{1A} and β_{1E} in RA+, RA–, and OA. In OA the concentration of β_{1A} and β_{1E} increases as the concentration of the fluid protein rises. However, this correlation does not seem to exist in RA+ nor in RA–.

C According to Hedberg (1967), the 'complement activity' in SF may be expressed by the following equation:

$$\text{Complement activity} = \frac{C}{C^s} \times \frac{10}{\text{protein}} \times 100$$

where C^f is the SF complement concentration, C^s the serum concentration measured simultaneously, and 'protein' is the SF protein concentration in g/100 ml. By introducing into this equation the ratios of $\beta_{1A}^f/\beta_{1A}^s$ or $\beta_{1E}^f/\beta_{1E}^s$, derived from measurements by the method of Mancini and others (1965), the values indicated in Table II were obtained. In comparing RA+, RA–, and OA, it can be seen that for β_{1E} RA+ gives obviously lower values than OA. RA– has intermediate values. For β_{1A} , the differences between these various conditions are minimal. However, the comparison of components β_{1A} and β_{1E} , expressed by the values obtained from the above equation, had the same relationship as that calculated in Table I, II.

D Fig. 2 shows the relationship between the rheumatoid factor (latex test) and levels of β_{1A} and β_{1E} in RA+ before and after synoviorthesis. A significant inverse correlation exists between the titre of rheuma-

Table I Levels of C3 and C4 and their relationship in RA+, RA–, and OA

Group	Synoviorthesis	No.	I			II	
			C3*	C4*	Difference between C3 and C4	Ratio C3/C4	Significance v.
RA+	Before	31	0.41 ± 0.20	0.29 ± 0.20	P < 0.001	1.5 ± 0.4	$\begin{cases} \text{RA- P} < 0.005 \\ \text{OA P} < 0.0001 \\ \text{RA- NS} \\ \text{OA P} < 0.01 \end{cases}$
	After	23	0.45 ± 0.18	0.36 ± 0.17	NS	1.4 ± 0.7	
RA–	Before	5	0.61 ± 0.15	0.63 ± 0.22	NS	0.98 ± 0.05	See above and below
	After	4	0.62 ± 0.18	0.63 ± 0.25	NS	0.99 ± 0.08	
OA	Before	15	0.39 ± 0.14	0.51 ± 0.16	P < 0.001	0.77 ± 0.09	$\begin{cases} \text{RA+ P} < 0.0001 \\ \text{RA- P} < 0.0005 \\ \text{RA+ P} < 0.01 \\ \text{RA- NS} \end{cases}$
	After	10	0.46 ± 0.12	0.56 ± 0.23	NS	0.82 ± 0.18	

* The concentrations of synovial fluid C3 and C4 are expressed as the ratio $\beta_{1A}^f/\beta_{1A}^s$ and $\beta_{1E}^f/\beta_{1E}^s$, respectively.

FIG. 1 Relationship between SF protein and β_{1A} , β_{1E} in RA+, RA-, and OA

toid factor (latex test) and β_{1A} and β_{1E} values: as the titre of rheumatoid factor increases fewer complement components appear in the SF. After synoviorthesis this correlation is not significant.

E A statistical analysis was made using the values of $\beta_{1A}^f/\beta_{1A}^s$ and $\beta_{1E}^f/\beta_{1E}^s$ in RA+, RA-, and OA before and after synoviorthesis, in an effort to discover any changes due to treatment. No statistically

Table II Concentration of synovial fluid C3 and C4 corrected for serum levels, and for synovial protein concentration

Group	Synoviorthesis	No.	C3*	C4*
RA+	Before	31	98	69
	After	24	108	87
RA-	Before	5	144	149
	After	4	126	128
OA	Before	15	132	173
	After	10	114	139

* Values derived from the equation $(C^f/C^s \times 10/\text{prot}) \times 100$ (Hedberg, 1967) where C^f and C^s are the values found by Mancini test in synovial fluid (f) and serum (s), respectively; 'prot' is the concentration of protein in synovial fluid, in g/100 ml.

significant differences were found. In other words, almost the same values were observed when a relapse of arthritis occurred in a joint treated by synoviorthesis.

Discussion

Several authors have concluded that C4 decreases much more than C3 in the SF of adult and juvenile RA patients (Peltier and others, 1966; Ruddy and others, 1969; Sonozaki and Torisu, 1970; Peltier and De Sèze, 1971). We obtained the same results in RA+. Ruddy and others (1969) reported a moderate depletion of C4 in SF of RA- as compared to that of OA. Similarly, we found a slight decrease of the β_{1E} and not of the β_{1A} ; however, SF protein concentrations must be taken into consideration. It is important to note that the anti- β_{1A} sera used in this work cannot distinguish immunologically the β_{1A} from

FIG. 2 Relationship between SF rheumatoid factor (latex test) and complement components β_{1A} , β_{1E}

β_1C . Therefore, by the method of Mancini and others (1965), it is not possible to evaluate the degree of activation or degradation of C3.

Compared to degenerative SF, the SF protein level and SF β_1A and β_1E are higher in RA-. In OA fluid a relationship exists between the value of these components and the protein level. This correlation does not appear in RA-, which suggests a certain consumption of complement in the synovial space. This impression is confirmed when 'the complement activity' is calculated according to Hedberg's (1967) equation (Table II). This formula utilizes the coefficient of SF 'protein concentration'. As shown in Table II, important differences in the β_1E appear between RA+, RA-, and OA. The indicated values, obtained from mean concentrations of each group, show a low consumption of β_1E in RA-, at least in some cases. Concerning β_1A , the differences between these three conditions are slight, particularly between RA- and OA.

On further consideration of the β_1A concentration, a phenomenon was encountered in our study which might have a certain practical or theoretical value: in RA+ fluids the β_1E concentration is much more depleted than that of β_1A ($P < 0.001$), whereas in OA this phenomenon is significantly reversed (Table I, II). If one considers that the majority of complement components pass from the blood to the synovial space, it is logical to assume that β_1E depletion reflects a local consumption, which is greater than that for β_1A , as we have previously shown. In OA the lower level of β_1A as compared to β_1E might signify a preferential permeability of the synovial membrane for C4. This probably does not depend on its molecular weight (slightly larger than that of C3), but rather on its larger glucidic moiety. In OA, C3 does not seem to be involved in a 'by-pass' activation pro-

cess (P. H. Lambert, personal communication, 1974; Tesar, Kazmar, and George, 1973; Götze, Zvaifler and Müller-Eberhard, 1972). It is probable that C4 is produced in synovial tissue (macrophages), which might account for its relative increase in OA in the absence of consumption.

Many published reports, except that of Sonozaki and Torisu (1970), have pointed out an inverse correlation between total haemolytic complement or C3 and C4 on the one hand, and SF rheumatoid factor on the other. We found a statistically significant inverse correlation between synovial β_1A , or β_1E and rheumatoid factor.

Recurrence of inflammation in the treated joint causes no significant difference in the concentration of both β_1A and β_1E , compared to the findings before synoviorthesis, in the three conditions considered: β_1E was relatively lower than β_1A in RA+; the inverse being true in OA, although the difference was not statistically significant.

In conclusion, this work confirms the consumption of synovial complement in RA+, which is also observed in RA- to a lesser degree. A peculiar concentration of synovial C4 compared to C3 was found in OA, which does not appear to be caused by an 'alternate pathway', lowering C3, but rather by a definite relative increase of C4.

In addition, the present study suggests that in RA when local relapses occur after osmic acid synoviorthesis the initial pathogenetic process is maintained and that the immunological mechanisms are barely influenced by this treatment. In OA also a recurrence differs very little from the inflammatory process observed before synoviorthesis.

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Note added in proof

Since this paper was submitted for publication, these studies were extended to a total of 45 RA+, 8 RA-, and 19 OA patients. The values found at this stage, in each group of patients, for the ratio C3/C4 were 1.46 ± 0.44 , 0.96 ± 0.10 , and 0.76 ± 0.11 , respectively, before synoviorthesis. These values show highly significant differences between groups at levels which confirm those indicated in Table I, II.