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Immune complexes in early arthritis. II. Immune complex constituents are synthesized in the synovium before rheumatoid factors

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SUMMARY

Synovial fluids and paired sera taken from patients either before, after or at the time of diagnosis of definite rheumatoid arthritis (RA) were compared with samples from patients with unclassified inflammatory arthropathies (IA). Raised levels of immune complexes (IC) were detected in some RA patients by C1q binding activity but in the majority of both RA and IA patients by the platelet aggregation test; levels were usually higher in joint fluids than in sera. IgM rheumatoid factors (RF) and IgA RFs were lower in synovial fluids but IgG RF levels were similar in matched samples. Synovial fluid to serum albumin ratios were used to estimate synovial permeability (inflammation) and then to calculate which patients synthesized macromolecules locally in the synovium. Local synthesis of RFs was detected in a greater proportion of RA than IA patients and only two patients formed RFs locally in the first months of symptoms. Half the patients in both groups however appeared to synthesize or trap IC constituents and in many patients there was evidence of local synthesis within 6 months after their symptoms had started. We conclude that local synthesis of large amounts of RFs is uncommon in the early stages of RA but that IC of unknown composition are synthesized or localized in the affected joints of many patients with RA and inflammatory arthropathies shortly after their symptoms appear.

INTRODUCTION

The initial site of rheumatoid arthritis is unknown. Is the putative triggering agent first localized in the affected joints followed later by systemic involvement or is RA a systemic disease which becomes localized in the joints? Once the disease is established the chronic inflammation in the synovium is thought to be the consequence of autoimmunity, namely, rheumatoid factors (antiglobulins) and their antigen–antibody complexes (Vaughan *et al.*, 1979). Large numbers of plasma cells synthesizing immunoglobulins are found in the inflamed synovia of rheumatoid patients and evidence that RFs are synthesized locally was shown by staining the plasma cells in synovial tissues with fluorescent aggregated IgG (Munthe & Natvig, 1972). Additional evidence from the direct plaque-forming cell assay showed RF secretion by synovial fluid lymphocytes (Vaughan *et al.*, 1976) and by lymphocytes teased from synovial membranes (Taylor-Upsahl, Abrahamsen & Natvig, 1977). But the stage in the disease when synthesis of Ig and RF begins in the joint is not known. Lymphocytes are known to have infiltrated the synovium during the first month after

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symptoms appear but no lymphoid follicles or plasma cells were reported at this stage of the disease (Schumacher, 1975).

We have measured the amount of total Igs, RFs and immune complexes in synovial fluids (SF) and paired sera and calculated whether the macromolecules in SF were derived from plasma or synthesized locally. The ratio of albumin concentration in synovial fluid to serum was used to measure synovial permeability. Patients have been studied at various times in early RA and compared with those with inflammatory arthropathies. The number of patients who synthesized Igs, RFs and IC constituents locally was evaluated and then related to the time their symptoms had started. In the early stages more patients appeared to synthesize IC components than synthesized RFs locally. Local synthesis of RFs was detected later.

MATERIALS AND METHODS

Patient selection. Thirty-six patients, 22 with definite RA and 14 with unclassified inflammatory arthropathies (IA) were selected, in most cases at the earliest time in their disease when synovial fluid could be taken. Many patients were taking non-steroidal anti-inflammatory drugs and a few had had intra-articular steroids some time previously. Three patients with RA had had low doses of steroids for a few months beforehand; one had had gold injections for 2 months and one had brief treatment with gold and penicillamine but with side-effects.

Any SFs visibly contaminated with blood were discarded. Those tested were not treated with hyaluronidase beforehand because it was found to be unnecessary (Shakib & Stanworth, 1976; our unpublished data). Fluids and paired sera were stored in aliquots at $-20°C$.

Immunoglobulins and albumin. Ig levels in sera and SF were measured by laser nephelometry and albumin concentrations by radial immunodiffusion.

Rheumatoid factors. IgM RF, IgG RF and IgA RF were measured by the radioimmunoassay for RFs developed by Hay, Nineham & Roitt (1975) with some modifications (Jones *et al.*, 1980). Rheumatoid factor was also measured by latex agglutination (Latex-RF Reagent, Behringwerke AG, Germany).

Assays for immune complexes. Assays for ^{125}I-Clq binding activity (C1q BA) and platelet aggregating activity (PA) of immune complexes are described by Verrier-Jones & Cumming (1977). ^{125}I-C1q binding was measured after PEG precipitation and the normal value was less than 20% binding. The normal value for PA was less than 1:16.

Statistical analysis. Before analysis, PA titres were converted to $-\log_2$. Student's *t*-test was used to compare serum and SF levels of each macromolecule in the two patient groups.

RESULTS

Clinical features

Patients were grouped according to duration of symptoms at the time when SF and serum samples were taken and RA patients were also grouped according to time of diagnosis of RA ($\geq$ five ARA criteria, defined by Ropes, Bennett & Cobb, 1959) at sampling time. At sampling time the number of ARA criteria seen in each patient was also enumerated (Table 1). Four RA patients whose samples were taken before diagnosis was definite had less than five ARA criteria at sampling time. Four IA patients had bilateral synovitis intermittently (ARA criteria 1 to 5) but have not developed definite RA 2–4 years later. At the time when samples were taken more than half the patients had suffered symptoms for less than 1 year and the majority of RA patients had had their disease diagnosed for less than 1 year. Serial samples from individual patients were not included.

Immune complexes

Levels of IC measured by both PA and C1q BA were higher in SF than in paired sera in the majority of RA and IA patients (Fig. 1) and for C1q binding activity these differences were significant ($P < 0{\cdot}05$). Levels of C1q binding IC were higher in RA than in IA patients in both SF and sera

Table 1. Clinical data

		RA	IA
No. of patients		22	14
Age (years): mean (range)		49 (16–80)	36 (19–70)
Males:females		5:17	3:11
No. of patients for whom the time between start of symptoms and taking samples was	1–4 weeks	2	3
	2–6 months	6	4
	7–12 months	6	1
	1–2 years	5	2
	> 2 years	3	4
No. of patients for whom the time between diagnosis of definite RA and taking samples was	1–16 months before	4	
	at diagnosis	10	
	1–12 months after	5	
	> 1 year after	3	
No. of patients with: 1 ARA criterion		0	1
2 ARA criteria		1	1
3 ARA criteria		1	6
4 ARA criteria		2	2
5 ARA criteria		5	4*
6 ARA criteria		9	0
7 ARA criteria		4	0

* Bilateral synovitis.

($P < 0{\cdot}05$) but there was no difference between the groups in mean levels of PA complexes. In general there was no relationship between sero-positivity and levels of IC. The only correlation with RFs was between C1q BA and IgG RF in RA patients' serum ($r = 0{\cdot}51$; $P < 0{\cdot}02$).

Rheumatoid factors

In contrast to IC, amounts of RFs in joint fluids were similar or lower than in paired sera (Fig. 2) but

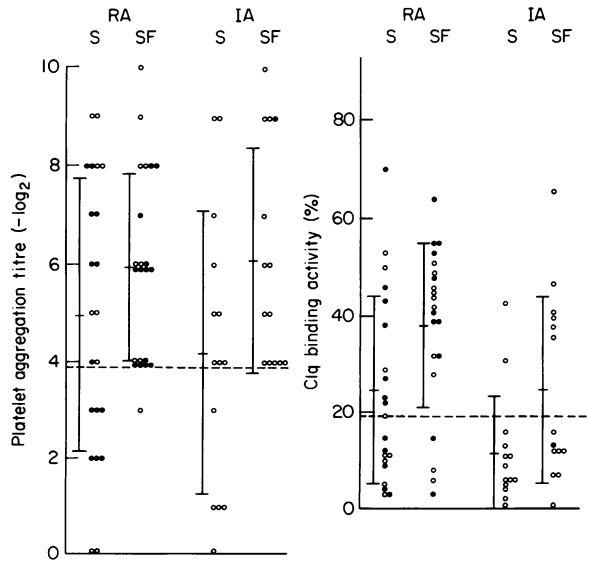

Fig. 1. Immune complex levels in paired synovial fluids and sera from patients with RA and IA (mean ± 1 s.d.). (● = sero-positive; ○ = sero-negative; - - - normal serum range; s = serum).

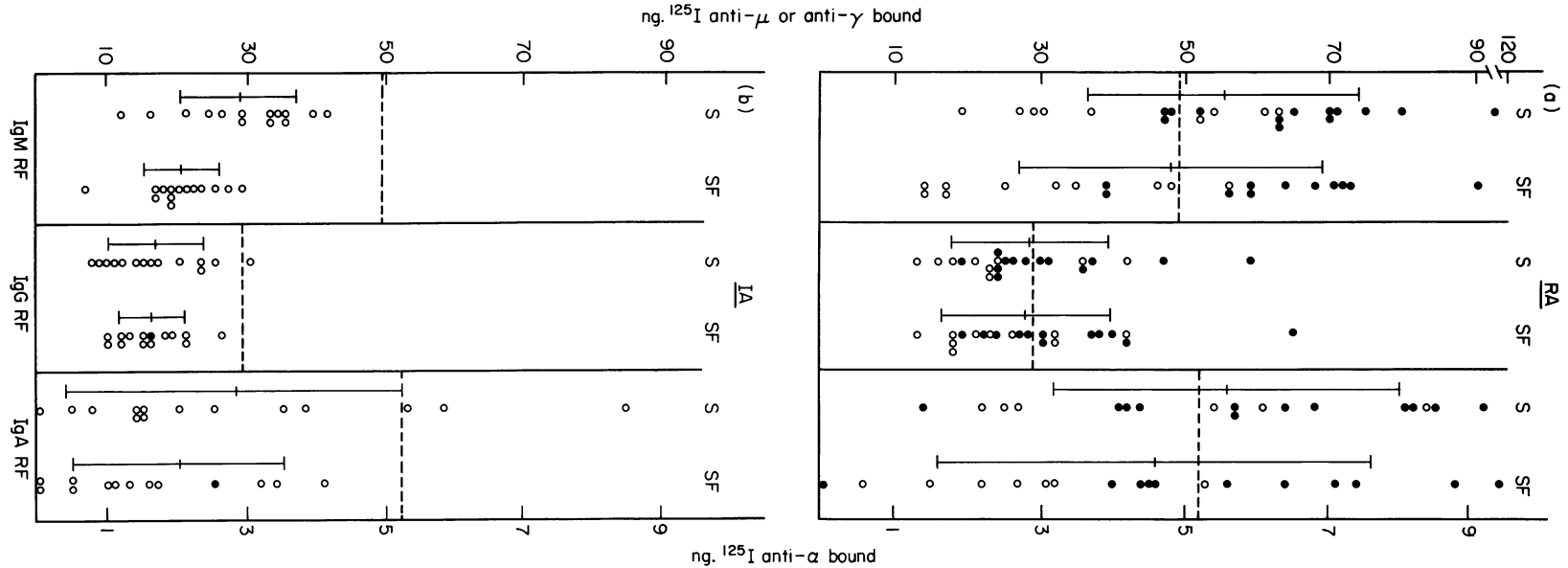

Fig. 2. RF levels in paired synovial fluids and sera from patients with RA and IA (mean ± 1 s.d.): (a) RA patients (b) IA patients. (● = sero-positive; ○ = sero-negative; - - - normal serum range) (mean + 2 s.d.).

differences were not significant. Comparison of RA patients with IA patients showed that, in both SF and sera, RF levels were significantly higher in RAs than in IAs ($P < 0{\cdot}001$ for IgM RF and IgG RF; $P < 0{\cdot}01$ for IgA RF). About half the RA patients had RF levels raised above the normal serum range. No IA patients had raised IgM or IgG RF although three patients had raised IgA RF in their sera. The IA patient with the highest level of IgA RF developed Sjögren's syndrome 1 month later.

Total immunoglobulins

Amounts of IgM, IgG and IgA in SF were approximately half the levels in serum and differences in the mean values were significant. But there were no differences between the two groups of patients either in their serum or SF levels of immunoglobulins, and serum levels were mostly within the normal range.

Albumin concentrations

Albumin concentrations were measured in all SF and serum samples and the SF/serum ratios were calculated for each patient. Mean ratios ($\pm$s.d.) were similar in RAs ($0{\cdot}66 \pm 0{\cdot}18$) and IAs ($0{\cdot}74 \pm 0{\cdot}17$) indicating considerable synovial membrane permeability in both groups of patients.

When synovial fluid albumin and IgG concentrations were compared, there was a fair correlation in IA patients ($r = 0{\cdot}67$; $P < 0{\cdot}02$) but none in RA patients, either for sero-negative or sero-positive patients ($r = 0{\cdot}13$ and $0{\cdot}1$ respectively; Fig. 3).

Synovial synthesis vs permeability

SF/serum ratios of total immunoglobulins, RFs and IC were determined for each patient and the indices calculated using their albumin ratio as denominator. Results are given in Fig. 4 and Fig. 5. Theoretically any index values above $1{\cdot}0$ suggest a local immune response in the synovium, whereas values of $1{\cdot}0$ or below indicate diffusion from the circulation. In practice, we have allowed for technical variations and arbitrarily taken indices above $1{\cdot}1$ for total immunoglobulins, above $1{\cdot}5$ for RFs and above $2{\cdot}0$ for IC as evidence for local synthesis.

A small proportion of RA patients had high Ig indices and three of these had high indices for two of the three Ig classes; the majority were sero-positive. In contrast only one patient of the IA group had a very high Ig index, namely IgM, and coincidentally she was the only IA patient with positive SF but negative serum in the latex agglutination test for RF.

Several RA patients had high RF indices, frequently for all three isotypes, and all but one of these patients were sero-positive in the latex test. Few IA patients on the other hand had high RF indices, mostly for IgG RF.

Indices for C1q binding and platelet aggregating IC are given in Fig. 5. About half the patients, both RA and IA, had high platelet aggregation indices and the majority of patients in both groups had high C1q binding indices. In general, patients with high indices in one test for IC also had high

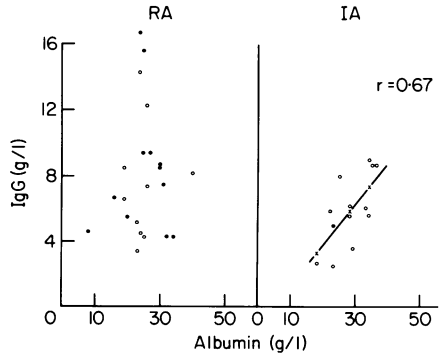

Fig. 3. Correlation between albumin and total IgG in synovial fluids of RA and IA patients. (● = sero-positive; O = sero-negative).

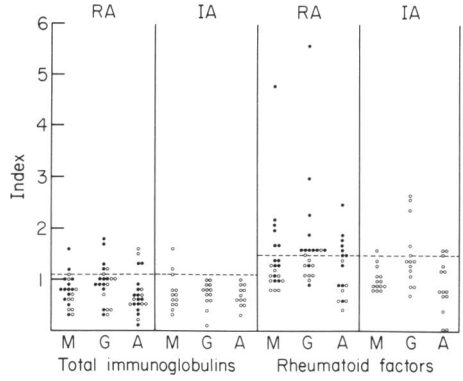

Fig. 4. Indices of total immunoglobulins and rheumatoid factors in patients with RA and IA. - - - indices above the line indicate local synthesis. (● = sero-positive; ○ = sero-negative).

indices in the other test. There was no apparent association between high indices for IC and high indices for RFs or sero-positivity. Also there were no significant differences between the two groups of patients for mean values of Ig, RF or IC indices.

Synovial synthesis related to duration of symptoms

The RA and IA patients were grouped according to the number of months between the onset of rheumatic symptoms and the sampling time. The RAs were also grouped according to whether the samples were taken before, after or at the time when five ARA criteria were present (i.e. definite RA). The number of patients with raised indices for Igs, RFs and ICs at these times are listed in Table 2. Too few patients had raised Ig indices for conclusions to be drawn about the earliest time of immunoglobulin synthesis. The most striking feature in the first 5 months of symptoms was the low number of patients (RA and IA) with raised RF indices (all classes) compared to the high proportion with a raised index for C1q binding IC. More than 1 year after their initial symptoms however, all eight RA patients had evidence for local synthesis of RFs. A similar trend in RA patients was seen when they were grouped according to time of diagnosis but the results were not so clear cut because samples were obtained from only four patients before RA was diagnosed.

The number of RA and IA patients with raised indices for PA IC was fewer than for C1q binding

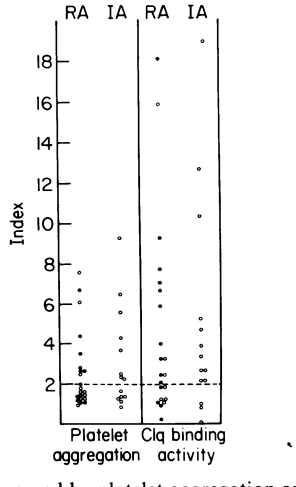

Fig. 5. Indices of immune complexes, measured by platelet aggregation and C1q binding, in patients with RA and IA. - - - indices above the line indicate local synthesis. (● = sero-positive; ○ = sero-negative).

Table 2. The number of patients with evidence of local synthesis in the affected joint; patients are grouped according to duration of symptoms at the time of sampling and *RA patients are also grouped according to time of diagnosis

Duration of symptoms	No. of patients	No. of patients with index raised for:										
		Immunoglobulins				Rheumatoid factors				Immune complexes		
		IgM	IgG	IgA	Total	IgM	IgG	IgA	Total	PA	C1qBA	Total
RAs												
<6 months	8	0	1	2	3	1	1	0	1	2	5	5
6–12 months	6	0	1	0	1	2	3	1	3	2	3	3
>1 year	8	2	3	2	3	3	7	4	8	5	5	7
IAs												
<6 months	6	1	0	0	1	0	1	0	1	1	6	6
6–12 months	2	0	0	0	0	1	1	1	2	1	2	2
>1 year	6	1	0	0	1	0	2	1	2	6	3	6
**RAs*												
before diagnosis	4	0	1	2	2	1	1	1	1	1	2	3
at diagnosis	10	0	1	0	1	3	5	1	5	4	6	6
after diagnosis	8	2	3	2	4	2	5	3	6	4	5	6

IC in the first months. However almost all patients with high PA IC indices had low PA activity in serum but the remaining patients, in whom there was no evidence for local synthesis of IC constituents, had high levels both in serum and in SF. Thus PA IC were formed by most patients in the early stages but we were unable to determine the site of synthesis.

Systemic synthesis related to duration of symptoms

To compare the onset of local synthesis of RFs and IC constituents with systemic synthesis, the number of patients with raised serum levels of these constituents was related to duration of symptoms (Table 3). In the first months more RA patients had raised serum RF levels than a raised RF index. This suggests that more patients synthesize RFs systemically than locally in the early

Table 3. The number of patients with raised levels of RFs and IC in their sera related to disease duration

Duration of symptoms	No. of patients	No. of patients with raised serum levels of:						
		Rheumatoid factors				Immune complexes		
		IgM	IgG	IgA	Total	PA	C1q BA	Total
RAs								
<6 months	8	4	2	3	4	6	3	8
6–12 months	6	4	3	2	4	4	4	5
>1 year	8	6	3	6	7	4	3	7
IAs								
<6 months	6	0	0	2	2	4	1	4
6–12 months	2	0	0	0	0	2	0	2
>1 year	6	0	1	1	2	3	1	4
RAs								
before diagnosis	4	2	1	2	2	3	0	3
at diagnosis	10	6	4	3	6	6	6	9
after diagnosis	8	6	3	6	7	5	4	8

stages. In contrast, in the first months fewer patients had raised C1q binding IC in serum than had raised indices. None of the four RA patients sampled before developing five ARA criteria had circulating C1q binding IC. This suggested that the majority of IA and RA patients synthesized C1q binding IC components locally rather than systemically in the early stages. A high proportion of both RA and IA patients had circulating PA IC early and later in the disease process.

DISCUSSION

To determine whether Igs, RFs and ICs in synovial fluid were derived from plasma or were locally synthesized in the synovium, we have compared the ratio of these macromolecules in SF to serum with the ratio of albumin in SF and serum of individual patients. Previously this approach has been used to demonstrate gammaglobulin synthesis in the synovium of RA patients (Wilkinson & Jones, 1962; Kushner & Somerville, 1971). Others have compared SF with serum concentrations of RFs and ICs usually without adjusting for synovial permeability (e.g. Winchester, Agnello & Kunkel, 1969; Panush, Bianco & Schur, 1971; Shakib & Stanworth, 1978; Hay *et al.*, 1979) and found in general that IgM RF levels were lower and IgG RF and IgA RF levels were similar or higher in SF than in serum but definite conclusions about local synthesis were rarely drawn because differences were mostly not significant. Our results confirm these findings.

Local synthesis of Ig and antibody in articular tissue will raise the concentration of these proteins in SF in relation to that in plasma. Other factors include molecular size of the protein and permeability of the synovial membrane which increases with inflammation. Using the SF/serum ratios of albumin to measure synovial permeability, patients without inflammatory joint disease had mean ratios of 0·43 and those with RA had higher ratios of 0·57–0·7 (Sundblad, Jonsson & Nettelbladt, 1961; Wilkinson & Jones, 1962). Ratios in our patients, both RAs and IAs, were also high (0·66 and 0·74 respectively) indicating considerable joint inflammation in both groups. Albumin with a molecular size closer to IgG will give a more accurate measure of synovial permeability to IgG than to larger molecules. When SF concentrations of these two proteins were compared, there was fair correlation in IA patients but none in RAs implying synovial synthesis of IgG by some RA patients. Indeed five RA but no IA, patients had high IgG indices.

Local synthesis of RFs is most convincingly demonstrated by histology (immunofluorescence) and secretion of RFs from synovial lymphocytes (e.g. Munthe & Natvig, 1971, 1972; Taylor-Upsahl *et al.*, 1977). IgM RF secreting plasma cells were found solely in the synovia of sero-positive RA patients (Munthe & Natvig, 1972) and our evidence for local synthesis of IgM RF was similarly in sero-positive RAs. Apparently IgG RF is formed in synovial plasma cells in both sero-negative and sero-positive RA patients but is present entirely as self-associated immune complexes and cannot be detected until the IC are dissociated by pepsin digestion (Natvig & Munthe, 1975). We have evidence for synovial synthesis of IgG RF in many RA patients but they were, with one exception, sero-positive. Therefore some of the 'free' IgG RF detected might be IgG or IgG RF in a complex with IgM RF (Jones *et al.*, 1980). Dissociation of putative complexes in SF was not attempted because pepsin digestion not only destroys all IgM RF activity but also some IgG RF activity (Carson *et al.*, 1977; our unpublished data).

The high concentrations of C1q binding IC found in joint fluids of RA patients confirm earlier reports (Agnello, Winchester & Kunkel, 1970). The incidence in serum or SF was not as high in IA as in RA patients confirming our earlier results on similar patient groups (Jones *et al.*, 1981). Concentrations of C1q binding IC in RA SF were greater than in companion serum samples and have been attributed to the intra-articular formation of IgG RF (Winchester, Agnello & Kunkel 1970; Hay *et al.*, 1979). We could not confirm the correlation between C1q binding IC and RF levels in joint fluids found by Hay *et al.* (1979) although we found some correlation in serum. This discrepancy might in part be explained by the stage in the disease process studied. For example, there is evidence for local synthesis of C1q binding IC components in many RA and IA patients at a time (less than 6 months after symptoms started) when only two patients had evidence for local synthesis of IgG RF.

High concentrations of IC in the joint space might occur other than by local synthesis of the

constituent antigen or antibody. For example IgG anti-IgG complexes in rheumatoid SF are larger in size as well as in higher concentration than in paired sera (Winchester *et al.*, 1970). Large molecules such as IgM RF, either free or complexed, pass freely into the joint from the intra-vascular space but IgM RF complexes and IgG aggregates apparently are cleared into the lymphatics and do not, like free IgM RF or albumin, return to the blood stream (Zvaifler, 1973). However the rate of catabolism of both free and complexed IgM RF is the same from the joint as from the circulation (Bluestone *et al.*, 1970) and therefore the relative concentrations of IC in SF and serum should not be affected. Marked interaction of hyaluronic acid with IC constituents is unlikely because hyaluronic acid is present in inflamed joints in insufficient amounts to bind large quantities of plasma proteins and does not bind IgG (Schultze & Heremans, 1966).

The high concentrations of platelet aggregating IC in SF and sera of both RA and IA patients also parallel our earlier results on similar patient groups (Jones *et al.*, 1981). As with the C1q binding IC, there was no direct correlation with sero-positivity or RFs. A correlation with IgG RF in RA patients might be hidden however because the material in rheumatoid synovial fluids which releases serotonin from platelets consists of IgG complexes of 450,000 molecular weight which can be blocked by IgM RF (Shapleigh *et al.*, 1980). Platelet aggregating complexes in rheumatoid sera can be blocked similarly (Fink *et al.*, 1979). Local synthesis of PA IC constituents was detected in patients with normal serum but raised SF activity but not in patients with equally high PA activity in both serum and SF. Levels of PA activity are determined by dilution and IC dissociate in high dilution. Thus local synthesis of PA IC components may not be revealed by our techniques.

The time after first symptoms when local synthesis can be detected may vary from patient to patient with speed of onset and severity of symptoms but local synthesis of RFs appears to follow some months after IC components. In the first months more RA patients had raised serum RF levels than had evidence of local synthesis indicating that synovial synthesis of RFs begins after systemic synthesis is amplified. By contrast, a high proportion of patients had evidence of synovial synthesis of C1q binding IC and fewer had raised serum levels in the early months. PA complexes were also present in their joint fluids but with no evidence of synovial synthesis. Whether PA complexes differ from those which bind C1q in their antigenic constitution or simply differ in molecular size, Ig isotype etc. is unknown. It is possible, however, that the arthropathies in IA and RA patients have a similar aetiology and that the local synthesis of IC constituents is one of the earliest signs of an immunological response to the aetiological agent in the affected joints.

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