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Metabolism of Factor B of serum complement in rheumatoid arthritis

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SUMMARY

An increased rate of catabolism of radio-iodinated Factor B has been shown in five out of ten patients with rheumatoid arthritis. Serum levels of Factor B were normal, the increased catabolism being matched by increased synthesis. The patients showing high catabolic rates had more manifestations of extra articular disease than did those with normal catabolic rates and they had higher rheumatoid factor titres. In seven patients, the catabolic rate for Factor B correlated significantly with the rate of IgG catabolism. In this series, the Raji-cell assay for immune complex-like material was in the normal or near normal range in all but one patient.

INTRODUCTION

Considerable evidence has implicated complement-fixing immune complexes in the pathogenesis of rheumatoid arthritis (RA). Intracytoplasmic inclusions in leucocytes from synovial fluid have been shown to contain immunoglobulins, especially IgG and IgM, and these have frequently been associated with complement components C1q, C4 and C3 (Hollander *et al.*, 1965; Vaughan, Jacox & Noell, 1968; Brandt, Cathcart & Cohen, 1968; Britton & Schur, 1971; Rodman *et al.*, 1967). Immunoglobulin and complement deposits also have been demonstrated in synovial tissues, and in blood vessel walls of patients with necrotizing vasculitis (Pernis, Ballabio & Chiappino, 1963; Conn, McDuffie & Dyck, 1972).

Lower joint fluid haemolytic complement levels (CH_{50}) were shown to occur in RA by Hedberg (1963) and Pekin & Zvaifler (1964), and the presence of large amounts of soluble IgG complexes in joint fluids was shown by Hannestad (1968). That the lowered joint fluid complement levels were due to activation of complement by the classical pathway has been indicated by low levels of both C2 and C4 (Ruddy & Austen, 1970; Gabay, Micheli & Fallet, 1975; Peltier & De Seze, 1971). Involvement of the alternative pathway also has been suggested by decreased Factor B and properdin levels and by the appearance of conversion products of Factor B and properdin in joint fluids (Zvaifler, 1974; Ruddy, Fearon & Austen, 1975; Lambert *et al.*, 1975).

Complement levels in the sera of patients with RA usually have been normal or elevated (Vaughan, Bayles & Favour, 1951; Schubart *et al.*, 1965; Williams & Law, 1958). Low CH_{50} titres have been noted, however, in patients with very severe disease, especially in those with generalized necrotizing vasculitis (Mongan *et al.*, 1969; Britton & Schur, 1971). Catabolism of a specific complement component, C3, has been studied by classical turnover techniques (Alper & Rosen, 1967; Weinstein *et al.*, 1972; Ruddy *et al.*, 1975) and found to be elevated in six out of seventeen cases; three of the six cases were reported to have vasculitis complicating their RA (Alper & Rosen, 1967; Weinstein *et al.*, 1972). In

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another study (Versey, Hobbs & Holt, 1973), fifteen out of twenty RA patients were found to exhibit conversion products of C3 in their plasmas by immunoelectrophoresis; eleven of the twenty patients exhibited conversion products of C4, a component of the classical complement pathway. In contrast, there have been no reports of the behaviour of Factor B or properdin, both components of the alternate complement pathway, in blood plasma in patients with RA.

The present study was designed to measure the fraction catabolic rate (FCR) of Factor B in patients with RA and to determine the quantitative relationship between the rate of Factor B catabolism and: (a) catabolism of IgG (Catalano *et al.*, 1977); (b) presence of rheumatoid factor (RF) complexes or other immune complexes in the blood; and (c) clinical manifestations of disease.

MATERIALS AND METHODS

Subjects. Patients with classical RA as defined by the American Rheumatism Association (American Rheumatism Association Committee, 1959) were studied (Tables 1 and 2). All patients were hospitalized in the Clinical Research Centre for a period of 3 weeks. Duration of arthritis ranged from 2 to 12 years. All patients had RF titres of 1:320 or greater plus clinical evidence of joint inflammation. Routine anti-inflammatory medications were continued unchanged throughout the study and five patients were maintained on their regular doses of Prednisone.

Groups of one to three RA patients were studied concurrently with one or two normal healthy controls. Normal subjects included those of the present study as well as those from a previous study (Wilson *et al.*, 1976). This provided eighteen normal subjects, including hospital and laboratory personnel, spouses of patients, and unrelated friends. The ages of the normal controls ranged from 21 to 82.

Serum complement determinations. Levels of haemolytic complement (CH_{50}) were determined by the technique described by Mayer (1961). Factor B and C3 concentration was measured by radial immunodiffusion analyses (Mancini, Carbonara & Heremans, 1965). Haemolytic Factor B was determined by the technique previously reported by Wilson *et al.* (1976).

Immune complexes. Sera were assayed by the Raji-cell method (Theofilopoulos, Wilson & Dixon, 1976) for the presence of soluble immune complexes.

Isolation of serum components. Factor B was isolated from normal human serum as previously described (Götze & Müller-Eberhard, 1971). The preparation showed a single line by immunodiffusion and polyacrylamide gel electrophoresis, and was fully active haemolytically when tested against a Factor B deficient serum and was free of C2 activity by haemolytic titration. Normal human IgG was isolated at pH 7·5 from a serum pool from five normal persons by DE52 column chromatography in 0·01M tris. It gave a single line in immunodiffusion.

Iodination. Factor B was trace labelled with ^{125}I or ^{131}I by the chloramine T method (McConahey & Dixon, 1966) or by the iodomonochloride method of McFarlane, as modified by Helmkamp *et al.* (1960). The latter method more frequently provided undamaged protein as judged by disappearance rates in the normal controls. IgG was iodinated by the iodomonochloride method (Helmkamp *et al.*, 1960). Sterile human albumin was added to protect against self-irradiation. The iodinated proteins were dialysed exhaustively against normal saline. Factor B preparations were deaggregated by centrifugation at 150,000 *g* for 1 hr at 4°C and sterilized by passage through a 0·45 μm Millipore filter. The IgG preparations were not centrifuged, but were shown to be free of aggregates by sucrose density gradient analyses (Kunkel, 1960).The preparations were stored at 4–5°C until used,which was usually within 24 hr. All preparations were pyrogen-free by rabbit tests (Kenne,

TABLE 1. Clinical features of patients studied

	Age	Duration of RA	No. of active joints	Extra articular disease	Prednisone mg/d.	Aspirin gm/d.	Other medications
K.M.	65	2	34	Nodules, Carpal tunnel	5	3·6	—
A.C.	54	6	48	0	5	4·5	—
F.R.	38	3	19	Sicca	—	3·6	—
P.F.	61	2	36	Nodules	—	4·2	—
I.B.	57	2	15	0	15	4·8	—
C.V.	77	20	55	Nodules, livedo reticularis	—	—	Penicillamine, Naproxen
M.G.	66	10	12	Nodules, sicca	—	3·6	—
E.B.-1	71	2	17	Nodules, Felty's, neuropathy	—	2·4	—
V.M.	52	22	30	0	10	—	Penicillamine, Ifupropin
E.B.-2	72	3	30	Nodules, Felty's, neuropathy	10–0	3·0	—
P.J.	68	12	33	Nodules, rash, neuropathy	—	3·6	—

Silberman & Landy, 1961), and 95–98% of the radioactivity was precipitable with 20% trichloroacetic acid. The haemolytic activities of the labelled complement preparations were preserved to approximately 90% when compared to the protein prior to labelling.

Study protocol. To prevent thyroid uptake of the radioactive iodine, controls and patients were given ten drops of saturated solution of potassium iodide (SSKI) solution daily beginning 1 day before injection of radiolabelled Factor B. Ten to 30 μCi of labelled Factor B was administered intravenously and plasma samples were drawn from the opposite arm, 5, 10, 15, 30 and 60 min later, 8 and 24 hr later, and daily thereafter for 7–10 days. Urine samples were collected at 8, 16 and 24 hr and on a 24-hr basis thereafter.

In selected patients the rate of IgG catabolism was determined immediately following the completion of the Factor B study. In such instances the Factor B was labelled with ^{131}I and the IgG with ^{125}I. The residual serum radioactivity from the Factor B was, on the average, only 2% of that of the injected IgG.

All studies in which the control subjects showed a rapid loss of radioactivity from the plasma (P) to the urine (U) as represented by a U/P during the first 1–2 days, twice or more the average U/P for the remainder of the study were considered invalid on the assumption that this indicated denaturation of the Factor B during its isolation or labelling. These normal subjects and the patients studied with them were eliminated from further consideration. Five groups involving twelve patients were thus eliminated. Eleven patient studies in five groups were satisfactorily completed.

Data calculation and analysis. Daily urine and plasma samples were counted at the completion of the study using a Nuclear Chicago 1195 gamma scintillation counter. Fractional catabolic rates for the entire periods of study were calculated by the Nosslin integrated rate equation (Nosslin, 1973). Daily FCRs also were calculated as described by Campbell *et al.* (1956).

Synthesis rates (SR) were derived from the formula:

$$SR = \frac{FCR \times \text{plasma volume} \times \text{plasma concentration}}{kg}.$$

RESULTS

The individual and mean FCRs for Factor B calculated by the Nosslin method are presented in Tables 2 and 3. The mean FCR in patients with RA was 2·01±0·55% per hr, while that in the normal subjects was 1·60±0·26. Half of the RA patients had FCRs which were higher than those observed in any of the eighteen normal controls, with individual values ranging up to 187% of the normal mean. The mean FCR calculated by the Campbell method (U/P) (Campbell *et al.*, 1956) was 2·65±0·58 for the AR patients and 1·85±0·42 for the controls. The values obtained by the Nosslin method are regarded as more reliable, however, as they are derived from integrated equations which utilize more information. The mean plasma disappearance curves for the five hypercatabolic RA patients are shown in Fig. 1. The more rapid disappearance of radiolabelled Factor B in the RA group was seen throughout the study. The calculated slopes of the straight line portions of the plasma disappearance curves of RA and normal individuals were significantly different ($P<0·01$).

TABLE 2. Laboratory features of RA patients

Subject	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	ESR (mm/hr)	1/RF	Immune complexes (μg IgG/ml)	CH_{50} (u/ml)	C3 (mg/dl)	Factor B Conc. (mg/dl)	Factor B FCR (%/hr)	Factor B Synthesis (mg/kg/hr)
K.M.	510	220	195	17	640	26	36	120	22·2	1·29	12·51
A.C.	3375	300	440	106	640	26	83	147	28·7	1·37	17·17
F.R.	2050	120	225	17	640	n.d.	52	95	11·6	1·48	7·50
P.F.	1300	108	190	27	320	39	60	134	20·0	1·54	13·45
I.B.	800	196	180	14	1280	0	53	186	29·0	1·75	22·16
C.V.	1440	138	380	94	2560	17	46	166	31·8	2·21	30·69
M.G.	1350	355	355	17	1280	23	76	177	21·7	2·32	21·98
E.B.-1	2250	875	205	84	10,240	115	36	164	18·5	2·37	19·14
V.M.	820	120	225	27	1280	0	65	221	26·6	2·38	27·65
E.B.-2	2000	2700	410	100	163,840	280	45	146	27·2	2·49	29·58
P.J.	1050	198	130	87	5120	0	41	130	29·9	2·96	38·64

TABLE 3. Mean values for RA patients and normal controls

		IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	CH_{50} (u/ml)	C3 (mg/dl)	Factor B Conc. (mg/dl)	Factor B FCR (%/hr)	Factor B Synthesis (mg/kg/hr)
RA (11)	Means	1554	484	267	54	153	24·3	2·01	21·86
	s.d.	839	77	108	16	34	6·1	0·55	9·20
Normal (18)	Means	1030	136	191	50	154	19·9	1·60	13·76
	s.d.	442	60	108	12	35	5·5	0·26	5·60

When the FCRs were compared to the latex fixation titres for RF (Table 2 and Fig. 2), significantly higher titres were found in those with FCR values $>2\%$ per hr ($P<0{\cdot}05$ by Wilcoxon's two sample rank test). The median titre in patients with FCRs $>2\%$ per hr was 2560–5120. With FCRs $<2\%$ per hr it was 640.

In seven patients FCRs were determined both for Factor B and homologous IgG. A significant correlation ($P<0{\cdot}05$) was found between the two (Fig. 3). When the patients' sera were assayed for immune complex-like material by the Raji-cell assay (Theofilopoulos *et al.*, 1976), only one case was found with very high values (Fig. 4). This was E.B., who on two occasions studied had values five and ten times the upper limit of most normal populations ($\leqslant 25$ μg/ml aggregated IgG equivalent).

The rates of synthesis of Factor B were calculated. In the normal subjects, the mean rate of synthesis was $13{\cdot}76 \pm 5{\cdot}60$ mg/kg/hr (Tables 2 and 3). In the RA patients the rate was $21{\cdot}86 \pm 9{\cdot}20$ mg/kg/hr.

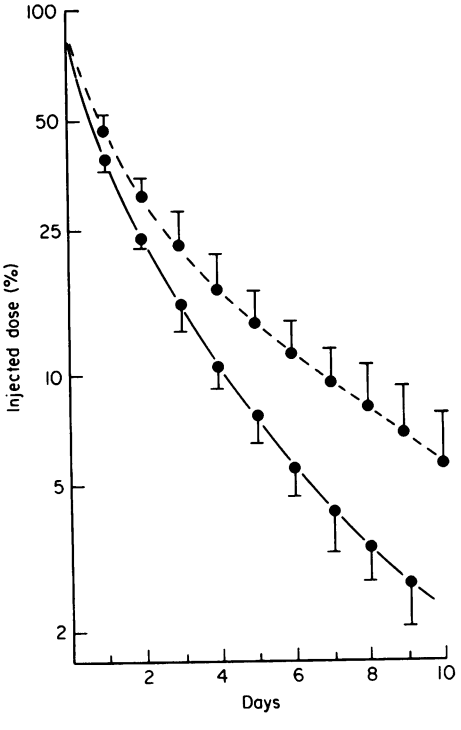

FIG. 1. Disappearance of labelled Factor B from the plasma in normal persons (- - - -) and in five patients with rheumatoid arthritis (——). A more rapid rate of disappearance for the patients was seen throughout the period of study.

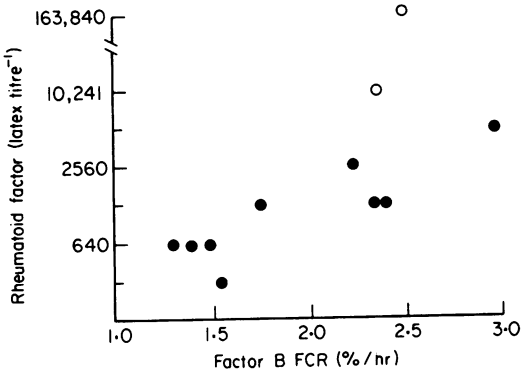

FIG. 2. Comparison of Factor B catabolic rate with rheumatoid factor titre. The open circles represent determinations on the patient E.B. done two years apart.

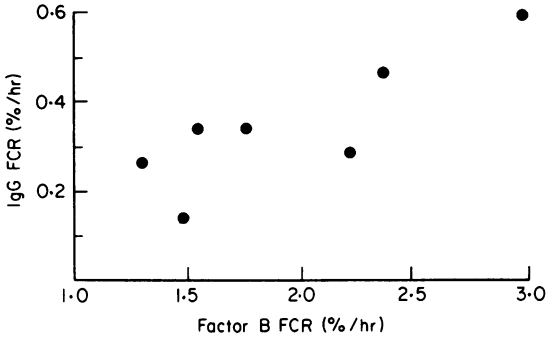

FIG. 3. Comparison of IgG and Factor B catabolic rates in rheumatoid arthritis. This was determined by sequential turnover studies in seven patients.

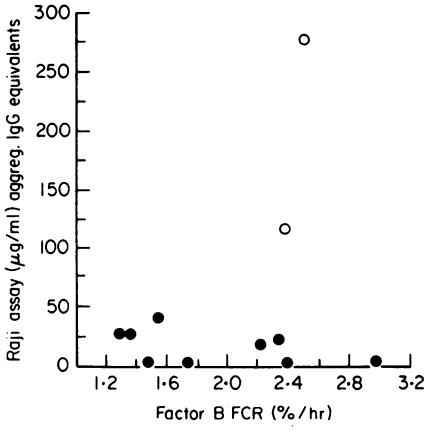

FIG. 4. Relation of Raji-cell assay to Factor B catabolic rate. The two studies of patient E.B. are indicated in open circles (○).

It is apparent that the increased FCR for Factor B in patients with RA was mostly balanced by an increased rate of synthesis. The single patient who showed a low serum Factor B level (F.R.) owed this to a low rate of synthesis rather than a high rate of catabolism.

Since the rate of Factor B activation through the amplification loop is controlled by two inhibiting proteins, C3bINA and β_1H (Lachmann & Halbwachs, 1975; Forristal *et al.* 1977, and Pangburn *et al.*,

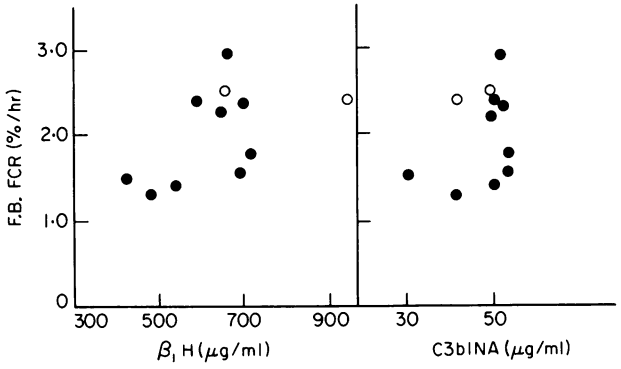

FIG. 5. Relation of the FCR for Factor B with serum levels of C3b INA and β_1H. Studies of patient E.B. are indicated in open circles.

1977) the FCR of Factor B was compared to the serum levels* of those proteins determined by radial diffusion analysis (Fig. 5). The FCR had no statistically significant relation to the levels of either. If anything, the correlation tended to be positive, rather than negative as would be expected if the FCR were determined by relation deficiency of either of the proteins.

DISCUSSION

This is the first evaluation by the turnover technique of Factor B in rheumatoid arthritis. We have found increased catabolism in half the patients we studied. The increased catabolism was matched by an increase in synthesis in all, so that there were no instances in which increased catabolism led to low serum levels of Factor B. Those patients showing increased catabolism were patients with multiple signs of extra-articular disease and with higher serum RF titres. The increased catabolism of Factor B also correlated with increased IgG catabolism in seven patients in whom turnover studies were performed with both proteins.

Based on the correlation between rate of Factor B catabolism and amount of RF by latex titre, it appeared that increased Factor B catabolism may have occurred as a result of alternative pathway complement activation by rheumatoid factor complexes. However, Tanimoto *et al.* (1975) observed that the ability of IgM RF to fix and activate complement depended upon the presence of all classical pathway complement components, thereby indicating that IgM RF activated complement only through the classical pathway. It is unlikely, therefore, that IgM RF is directly involved in Factor B activation. The possibility that it activates Factor B secondarily through the amplification loop (Götze & Müller-Eberhard, 1971; Goodofsky & Lepow, 1971) was considered, but IgM RF measured directly by radio-immunoassay (Carson, *et al.*, 1977) did not correlate with Factor B turnover ($r = 0{\cdot}364$, $t = 1{\cdot}17$, $P > 0{\cdot}05$). Little information is available on complement activation by IgA or IgG RF. Winchester, Agnello & Kunello (1969) reported a correlation between lowered joint fluid complement titres and intra-articular complexes composed predominantly of IgG RFs. Tanimoto *et al.* (1976) have reported the presence of complement dependent, haemolytic RFs in sera depleted of IgM RF. However, Pope, Teller & Mannik (1975) have presented evidence that intermedially sedimenting, self-associating IgG RFs do not fix complement. A single monoclonal IgA RF studied by us (Tanimoto *et al.*, 1975) was found to be haemolytically active. From these observations it seems likely that, if RF contributes to Factor B activation in RA, it most likely does so through IgA RF, or through very large complexes of IgG RF.

It is important to establish which immune complexes are involved in activating complement in RA. The Raji-cell assay detects immune complexes onto which complement components have become fixed, and therefore this assay should detect complexes capable of increasing complement component catabo-

* We are indebted to Dr M. Pangburn for these determinations.

lism. The assay has not, however, demonstrated as high titres of immune complex-like material in the serums of patients with RA as it does in systemic lupus erythematosus (A.N. Theofilopoulos, personal communication). In the present study, only one patient (E.B. 1 and 2) showed a very high titre. The other patients had normal or near normal values. The one patient (E.B.) was one of the five with an elevated FCR for Factor B. She had Felty's syndrome with peripheral neuropathy. Although she had the highest titres for RF of any of the patients we examined, she did not have the fastest catabolic rate for Factor B. We do not know whether the Raji-cell assay detected complement fixing RF complexes, or other complexes in this patient.

It appears likely that several types of immune complexes may independently contribute to complement activation in patients with RA. These include RF complexes, complexes formed by other autoantigen-autoantibody systems and possibly virus-antibody complexes. The most damaging of these complexes would probably be those causing the greatest amount of activation of complement. The large amounts of RF complexes present in the tissues may give RF complexes greater significance than the others.

Other mechanisms are known to increase Factor B catabolism in various experimental situations. Inulin and other polysaccharides including endotoxin (Osler & Sanberg, 1973), radiocontrast media (Arroyave, Bhat & Crown, 1976), and aspirin (C.M. Arroyave, unpublished observations) are among agents that have been reported to activate Factor B. In our patients, there was no correlation between FCR for Factor B and aspirin dosage or serum salicylate levels, so we doubt that this drug was a determinant in the current studies. There is no evidence of endotoxemia in patients with RA, although this possibility has not to our knowledge been directly studied. It is possible that unknown mechanisms are operative in patients with RA, like those causing activation of the alternative pathway in hypocomplementemic nephritis (Vallota *et al.*, 1974), or activation of complement by factors released following tissue damage as described by Giroud & Willoughby (1969). The increased FCR could not be attributed to deficiency in either of the modulating proteins, C3bINA or β_1H (Fig. 5).

The rate of Factor B catabolism correlated with rate of IgG catabolism. We take this to be consistent with activation of the Factor B by immune complexes. Increased IgG catabolism in RA occurs predominantly in the extravascular compartment (Catalano *et al.*, 1977) and we believe it probable that increased catabolism of Factor B also occurs predominantly in the extravascular space, although with the numbers of patients we examined this could not be shown statistically.

A knowledge of the relative degrees of activation of the alternative and classical complement pathways in RA will be important to establish. This can be provided by turnover studies such as these. The mean synthetic rates for Factor B are 515 mg/kg/day and 330 mg/kg/day, respectively, for RA and normal subjects (Table 2). The differences, 195 mg/kg/day, is the mean quantity of Factor B consumed in the hypercatabolic process. In our previous studies of IgG in RA (Catalano *et al.*, 1977), the mean synthetic rates for IgG were 60 mg/kg/day and 14 mg/kg/day in RA and normals, respectively, or 46 mg/kg/day of IgG consumed in the hypercatabolic process. Studies are currently underway with complement component C4 to get an estimate of the quantity of this factor of the classical pathway that is utilized in the hypercatabolic process.

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