Clin. exp. Immunol. (1976) 26, 11–20.

Activation of the alternative complement pathway in systemic lupus erythematosus

M. R. WILSON, C. M. ARROYAVE, R. M. NAKAMURA, J. H. VAUGHAN & E. M. TAN *Department of Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California, U.S.A.*

Received 26 *February* 1976

SUMMARY

Serum factors activating the alternative pathway of complement *in vitro*, independent of classical pathway activation were demonstrated in six of eleven patients with systemic lupus erythematosus (SLE). These serum factors were detected by lysis of gluthathione-sensitized human erythrocytes and by C3 and factor B conversion in the presence of EGTA (10 mM) and $MgCl_2$ (0·3 mM), conditions which blocked activation of the classical pathway but permitted activation of the alternative pathway. In order to determine if *in vivo* activation of the alternative pathway of complement was present in SLE, highly-purified factor B was labelled with radioactive iodine (^{125}I), and its metabolism studied in the eleven patients with SLE and in twelve control subjects. All six patients with serum factors capable of activating the alternative pathway *in vitro*, had *in vivo* evidence of alternative pathway activation as measured by increased fractional catabolic rate (FCR) of factor B. Two patients without demonstrable alternative pathway activating factors in their sera had an elevated FCR of factor B. Six of the patients with increased FCR of factor B had disease limited to skin or joint and one had lupus nephritis which was inactive at the time of study. One of the four patients who were in clinical remission had elevated FCR. This study demonstrates that a significant number of patients with SLE of relatively mild disease activity had evidence of alternative complement pathway activation. This activation did not appear to be limited to patients with lupus nephritis and raises the possibility that it could also be related to some of the extra-renal manifestations of SLE.

INTRODUCTION

The complement system is a multimolecular protein sequence which is one of the major mediators of tissue injury in autoimmune diseases. Present evidence indicates that the classical pathway of complement is activated in patients with systemic lupus erythematosus (SLE). The serum levels of total haemolytic complement and specifically of components C1q, C4 and C3 are reduced, usually when SLE is complicated by renal involvement (Gewurz *et al.*, 1968; Kohler & ten Bensel, 1969, Hunsicker *et al.*, 1972). Studies of metabolism using classical pathway proteins labelled with radioactive iodine have shown hypercatabolism of these proteins in SLE during periods of increased disease activity, especially, when nephritis is present (Alper & Rosen, 1967; Hunsicker *et al.*, 1972). Therefore, decreased levels of complement have been explained by the increased rate of catabolism of these proteins during active disease states. In addition, the hypocomplementaemia associated with active SLE has been attributed to a decreased rate of synthesis of complement components (Sliwinski & Zvaifler, 1972). It has been postulated that in certain cases split products of activated C3 may have a suppressive effect on the synthesis rate of native C3 protein (Charlesworth *et al.*, 1974). Thus, the hypocomplementaemic state in certain SLE

Correspondence: Dr Eng M. Tan, Scripps Clinic and Research Foundation, 476 Prospect Street, La Jolla, California 92037, U.S.A.

patients has been postulated to be due to at least two factors, depression of the synthesis rate and hypercatabolism of C3.

The alternative pathway of complement which bypasses the early components (C1, C4, C2) is being investigated actively in patients with SLE (Perrin *et al.*, 1973; Provost & Tomasi, 1973; Rothfield *et al.*, 1972). Many investigators have used serum levels of various proteins of the alternative complement pathway as an indication of activation of this pathway. Serum levels of factor B, a protein of this pathway, were depressed in SLE patients with renal involvement during periods of increased disease activity (Hunsicker *et al.*, 1972; Perrin *et al.*, 1973). In a study of metabolism conducted by Charlesworth and co-workers (1974), factor B labelled with ^{125}I was used to measure the relationship between activation of C3 and recruitment of the alternative pathway by way of the C3b feedback cycle in twenty-two patients with various diseases associated with hypocomplementaemia and eleven patients with various renal diseases without hypocomplementaemia. In the C3b feedback cycle, C3b which is generated by activation of native C3 combines with factor D to produce activation of factor B. This results in the production of a split product $\overline{Bb}$, which is C3 convertase. Charlesworth observed that increased factor B catabolism was usually associated with an increased C3 catabolism, and suggested that the alternative pathway had been activated via the C3b feedback cycle.

In this study, we determined the metabolism of injected ^{125}I-labelled factor B in a group of eleven SLE patients. Some of these patients had been shown by an earlier study (Arroyave, Wilson & Tan, 1976) to have serum factors which were capable of activating the alternative pathway of complement *in vitro* independent of classical pathway activation. We showed an increased catabolism of factor B in eight of the eleven SLE patients. Six of the eight patients with increased factor B catabolism had serum factors which activated the alternative pathway of complement.

MATERIALS AND METHODS

Subjects. Twelve healthy individuals were used as control subjects and compared to eleven patients with SLE. All patients fulfilled the diagnostic criteria for SLE proposed by the American Rheumatism Association (Cohen & Canoso, 1972). One SLE patient had no detectable C2 by radial immunodiffusion and haemolytically-active C2 was not detectable in her serum; two SLE patients with a partial deficiency had less than 30% haemolytically-active C2 in serial determinations. Family studies of these patients revealed that their family members had partial C2 deficiency, so that the C2 deficiency in the three patients were inherited C2 deficiencies of the type described by Agnello, De-Bracco & Kunkel (1972).

Stages and activity of disease in SLE patients from this series varied. All patients were clinically stable during the study period and had been maintained on the same therapeutic regimens for at least 6 weeks prior to study. Eight patients were receiving low doses of corticosteroids (prednisone: 10–20 mg/24 hr), and one patient was treated with an immunosuppressive agent (azathioprine) in addition to corticosteroids. Two patients were untreated when studied. Patients J.T. and E.B. had persistent hypocomplementaemia for 7 and 4 years respectively; the latter patient also had membranoproliferative glomerulonephritis demonstrated by renal biopsy. He previously had 4–5 g of proteinuria per 24 hr but at the time of the study, this had decreased to 1·4 g per 24 hr. Two other patients (L.G. and N.H.) previously had mild proteinuria (less than 300 mg/day) but no proteinuria during the study period.

Reagents. Reduced gluthathione was purchased from Boeringer Mannhein Corporation, New York. Inulin was obtained from Pfanstiehl Laboratories, Waukeegan, Illinois. Factor B-depleted serum was produced by incubating normal human serum with insolubilized monospecific antiserum to factor B. The insolubilized antibody was prepared with ethylchloroformate (Avrameas & Ternynck, 1967). The antiserum was raised in goats by injection of factor B isolated according to methods previously described (Götze & Müller-Eberhard, 1971). The antiserum gave a single precipitin line in immunoelectrophoretic assay against whole human serum or partially purified factor B. The alternative pathway of complement could not be activated by inulin in the sera depleted of factor B by this antisera. However, the activity could be restored to 100% by the addition of purified factor B.

Antinuclear antibodies (ANA) testing, and deoxyribonucleic acid (DNA) binding. The serum of each patient was tested for the presence of ANA by methods described by Northway & Tan (1972). The sera were also tested for binding to single-stranded deoxyribonucleic acid (ssDNA) and double-stranded deoxyribonucleic acid (dsDNA) (Picazo & Tan, 1975). Further differentiation of specificities of ANA were performed by analysing for antibodies to the Smith antigen (Sm) and nuclear ribonucleoprotein (RNP), two acidic nuclear protein antigens (Northway & Tan, 1972).

Assays for activators of the alternative pathway. The serum of each patient was tested by two different methods for the presence of factors which might activate the alternative pathway of complement when added to fresh normal human serum. The first assay was a determination of the capacity of tested serum to cause lysis of gluthathione-sensitized human erythrocytes (GSHE) (Arroyave, Vallota & Müller-Eberhard, 1974). Briefly, GSHE (5×10^7 cells) were incubated with 5 μl of

patient's serum and 20 μl of normal serum in the presence of 10 mM ethylene glycol tetra-acetic acid (EGTA) and 0·0003 M $MgCl_2$. These conditions have been shown to block classical pathway activation but permit activation of alternative pathway (May, Rosse & Frank, 1973). After incubation in a water bath at 37°C for 60 min, 2 ml of cold saline were added, and after mixing and centrifugation, the amount of free haemoglobin was determined spectrophotometrically at 412 nm. Normal human serum activated by inulin in the presence of EGTA and Mg^{2+} was used as the standard for 100% lysis. Sera from forty normal individuals were used to establish the normal levels of GSHE lysis. Less than 5% lysis was noted for all of our normal sera.

The second method of detecting alternative pathway activation, factor B and C3 conversion, was performed by incubating 5 μl of patient's serum and 20 μl of normal human serum in the presence of EGTA (10 mM) and $MgCl_2$ (0·0003 M) at 37°C for 30 minutes. The mixture was then electrophoresed on a microscope slide in 1·5% agarose using veronal buffer pH 8·6 and ionic strength 0·05. The slide was then developed with monospecific antisera to factor B or C3. Conversion of factor B indicating alternative pathway activation was detected by the appearance of split products with α and γ mobility. Conversion of C3 was considered positive if split products with anodal migration appeared (Bokish, Müller-Eberhard & Cochrane, 1969).

Serum complement determinations. Functional levels of total complement (CH50), C2 (C2H50), and C3 (C3H50) were measured by standard techniques (Mayer, 1961; Cooper, Polley & Müller-Eberhard, 1970; Müller-Eberhard *et al.*, 1966). Functional levels of factor B were determined on diffusion plates by using 1% agarose in veronal buffer (pH 7·5, ionic strength 0·15) containing normal human sera (final concentration 1:30) heated at 50°C for 20 min to inactivate factor B and to provide other complement components in excess. Guinea-pig erythrocytes (0·5%) were used as the indicator cells. EGTA (10 mM) was added to block classical pathway activation, and $MgCl_2$ (5 mM) was added to enable activation of factor D. This mixture was poured into plates, 3-mm wells were cut and 10 μl samples of patient's sera were applied to the wells. The plates were incubated at 4°C for 12 hr and at 22°C for 4 hr; each area of haemolysis was then measured and compared to a standard curve constructed from dilutions of fresh normal human serum (we are indebted to Dr Peter Lachmann for this technique).

Immunochemical quantitation of C2, C3, factor B and C3b inactivator (C3bI) were performed by using single radial diffusion (Mancini, Carbonara & Heremans, 1965). Monospecific antiserum to C3bI was kindly supplied by Dr Enrique Vallota, Children's Hospital, Cincinnati, Ohio.

Isolation of factor B and ^{125}I labelling technique. Factor B was isolated from human serum as described by Götze & Müller-Eberhard (1971). Three separate preparations of purified factor B were trace-labelled with ^{125}I by the chloramine T method (McConahey & Dixon, 1966). The iodinated protein was dialysed for 40 hr against 3 × 10l of normal saline containing 200 mg of chloramphenicol. The protein mixture was centrifuged for 60 min at 45,000 rev/min in a SW 50·1 rotor in an L2 ultracentrifuge (Spinco Division, Beckman Instruments, Inc., Palo Alto, California), and then passed through a Millipore filter (0·45 μm pore size). Sterile human albumin in a final concentration of 1 mg/ml was added to radiolabelled factor B to provide a buffer solution. The material was stored in rubber-stoppered vials at 4°C until used. It was tested for bacterial contamination in aerobic and anaerobic cultures and for endotoxin by the limulus assay (Levin *et al.*, 1972). As a further test for endotoxin and pyrogens, two rabbits were each injected with a human dose equivalent and a rise in temperature of 1°C within 3 hr was considered a positive test for pyrogenicity. Only samples of ^{125}I-labelled factor B free of bacterial and endotoxin contamination by all these methods were injected into patients after authorized informed consents were obtained.

The specific activities of the three different preparations of labelled factor B were 0·59, 1·35 and 2·0 μCi/μg. The haemolytic activity of ^{125}I-labelled factor B was assayed by incubating serum depleted of this protein plus isolated ^{125}I-labelled factor B in the presence of inulin and GSHE cells. The activity was approximately 90% when compared to the protein before it was labelled. Ninety-four percent of the radioactivity could be precipitated by monospecific antisera to factor B or by 10% trichloracetic acid (TCA). Immunoelectrophoresis of the labelled protein was performed to determine the presence of split products. The ^{125}I-labelled factor B was electrophoresed in 1·5% agarose for 90 min; the agarose was sliced into 1-mm sections and each section counted in a Nuclear-Chicago automatic gamma scintillation counter (Nuclear-Chicago, Des Plaines, Illinois) equipped with a 2-inch sodium iodide crystal. A single peak of the labelled protein in the β region was observed when curves were constructed from the counts of each segment.

Administration of the ^{125}I-labelled factor B and collection of samples. The control subjects and SLE patients were given 10 drops of Lugol's solution 24 hr before injection of ^{125}I-labelled factor B, and daily during the study to prevent thyroid uptake of the labelled ^{125}I. A dose of 30–35 μCi of labelled factor B was administered to each individual intravenously through a cannula. Ten to 15 ml of normal saline was used to irrigate the syringe and cannula to ensure delivery of the entire dose. The amount of radioactivity retained in the syringe after this procedure was determined to be approximately 1% of the total calculated dose prepared for injection.

After injection of the ^{125}I-labelled protein, blood samples were drawn from the opposite arm at 2, 5, 10, 20 and 30 min, at 4, 8, 24 hr and then daily for 5 days. Sera and plasma were stored at −70°C, and all samples from each patient were analysed at the same time on the last day of each study. Urine samples were collected at 4, 8 and 24 hr and daily thereafter for the period of study, and stored at 4°C until analysed with the plasma samples. The radioactivity was measured in duplicate in 1 ml samples of plasma or urine in a Nuclear-Chicago gamma scintillation counter. Less than 2% of the counts in the urine were precipitated in 10% TCA suggesting that very little radioactivity in the urine was bound to protein. No radioactivity could be detected in washed red blood cells from the patients or controls. The counts were corrected for background activity and radioactive decay.

Data analysis. The plasma volumes were calculated by dividing the total injected dose of radioactivity by the radioactivity computed by extrapolating the counts at 2, 5, 10, 20 and 30 min after injection back to zero time. Total body radioactivity was equal to the radioactivity injected minus the cumulative radioactivity excreted in the urine. Extravascular radioactivity was calculated by subtracting the sum of the plasma radioactivity and the cumulative urine radioactivity from the total injected dose at each time period. The plots of plasma radioactivity, extravascular radioactivity, and total body radioactivity were constructed on semi-logarithmic paper. The half-life of the protein was determined graphically. The synthesis rate was computed from the plasma volume, the mean serum level of factor B and FCR of factor B. The FCR and extravascular: plasma (E:P) ratios were determined by Nosslin's method for integrated rate analysis (Nosslin, 1973). The Student's *t*-test and the Pearson product-moment correlation coefficient (r) were used to analyse the data statistically (Colton, 1974).

RESULTS

Clinical data

Using the classification of disease activity suggested by Rothfield & Pace (1962) all patients were classified on a scale of 0–3+ activity. This classification is based on history and physical findings of the patients at the time of study as follows: 0 = no clinical evidence of disease activity (remission); 1+ = active disease in only one system; 2+ = active disease in more than one system without fever or in one system with fever; 3+ = active disease in two or more systems with fever. Four patients were judged to be 0 or in clinical remission; seven patients had 1+ activity.

ANA profiles of SLE patients

The ANA profiles of the SLE patients at the time of study are summarized in Table 1. ANA were positive in ten of eleven patients. The one patient (MG) who was negative at the time of study, had been positive prior to treatment with corticosteroids. Antibodies to ssDNA were present in nine patients, and to dsDNA in one patient. Antibodies to RNP were present in one patient, and three patients had anti-

TABLE 1. Clinical and laboratory data

Patient	Age	Sex	ANA§	ssDNA (% binding)¶	dsDNA (% binding)¶	RNP antibody	Sm antibody
M.H.	44	F	1:16 Speckled	14·8	7·6	–	–
D.L.	19	F	1:16 Mixed	24·0	8·3	–	–
E.B.	24	M	1:16 Speckled	11·5	3·2	–	+
J.T.	69	F	1:16 Homogeneous	19·6	0·9	–	+
K.R.	33	F	1:16 Patchy	14·0	0·7	–	–
A.M.	47	F	1:64 Homogeneous	29·7	0·7	–	+
M.C.	27	F	1:4 Speckled	6·6	4·7	–	–
T.K.	38	F	1:4 Rim	34·9	34·9	+	–
M.G.*	15	F	Negative‡	22·9	9·2	–	–
L.G.†	24	F	1:16 Patchy	37·5	5·3	–	–
N.H.†	19	F	1:16 Mixed	7·9	0·7	–	–

* Complete C2 deficiency.

† Partial C2 deficiency.

‡ Patient was negative at the time of study, but positive at 1:4 mixed pattern previously.

§ ANA expressed in titre and staining pattern.

¶ Less than 10% binding considered normal for ssDNA (single-stranded DNA) and dsDNA (double-stranded DNA).

bodies to Sm antigen. Antibodies to dsDNA are generally present in SLE patients with either severe disease activity or renal disease (Schur & Sandson, 1968). The fact that most of our patients did not have detectable antibodies to dsDNA is in agreement with the clinical classifications described above.

Complement profiles of the SLE patients

The haemolytic and quantitative serum complement determinations are shown in Table 2. The CH50 was below normal in seven of eleven, including those patients with C2 deficiency. The same seven patients

TABLE 2. Complement profiles of SLE patients

Patients	Haemolytic activity				Radial immunodiffusion		
	CH50 (u)	C2H50 (u)	C3H50 (u)	Factor B (percentage of control)	C3 (mg%)	Factor B (mg%)	C3bI (percentage of control)
M.H.	**12**‡	**240**	**930**	100	**80**	**22·5**	95
D.L.	**32**	**330**	**1700**	100	120	**19·0**	100
E.B.	**34**	**780**	**1675**	**63**	110	**19·0**	90
J.T.	**47**	**570**	**1970**	70	100	**17·0**	95
K.R.	75	2340	**3080**	**60**	100	**16·0**	92
A.M.	80	1859	3900	100	118	**21·0**	100
M.C.	85	1728	4275	**60**	160	**15·0**	90
T.K.	85	1680	4150	100	110	**25·5**	98
M.G.*	**0**	**0**	**985**	**35**	110	**10·0**	85
L.G.†	**12**	**434**	**1310**	**25**	100	**12·0**	95
N.H.†	**18**	**100**	**1585**	**33**	110	**12·0**	85
Normal values: (±2 s.d.)	65–107	1550–2540	3750–5250	70–130	100–190	15–30	80–120

* Complete C2 deficiency.
† Partial C2 deficiency.
‡ In this and Table 3, numbers in bold type indicate values outside normal range.

TABLE 3. Metabolism of factor B in SLE patients

Patients	FCR (%/hr)	T 1/2 (hr)	Synthesis rate (mg/kg/hr)	E:P ratio	Alternative pathway activators		
					GSHE (percentage lysis)‡	Factor B and C3 conversion	Disease activity§
M.H.	**2·19**	**48**	0·187	**0·79**	4	−	1+
D.L.	**2·72**	**34**	0·193	**0·44**	27	+	1+
E.B.	**2·20**	**59**	0·149	**0·66**	32	+	1+
J.T.	1·51	90	**0·102**	0·95	0	−	0
K.R.	**2·16**	**48**	0·125	**0·58**	19	+	1+
A.M.	1·78	72	0·152	0·91	2	−	0
M.C.	1·85	76	0·121	**0·75**	0	−	0
T.K.	**2·59**	**34**	**0·242**	**0·53**	24	+	1+
M.G.*	**2·42**	**53**	**0·103**	0·93	21	+	1+
L.G.†	**2·04**	**65**	**0·105**	1·05	4	−	0
N.H.†	**2·82**	**48**	0·128	0·93	28	+	1+
Normal:							
Mean	1·68	75	0·161	1·17			
Range (±2 s.d.)	1·55–1·81	67–90	0·117–0·203	0·80–1·54			

* Complete C2 deficiency.
† Partial C2 deficiency.
‡ Greater than 5% lysis is considered abnormal.
§ Determination of disease activity see text (clinical data).

also showed decrease in the C2H50. The C2H50 was 6% and 25% of normal in the two patients with partial C2 deficiency (N.H. and L.G.) and completely absent in the patient with complete deficiency (M.G.). The C3H50 was decreased in eight patients. However, only one patient showed a decreased serum level of C3 by radial immunodiffusion. This feature will be discussed later.

The assay of factor B indicated that six of eleven patients had abnormally low haemolytic activity of factor B whereas only three had low levels by radial immunodiffusion. The three patients with C2 deficiency had the lowest activity.

Metabolism of factor B in SLE patients

The plasma volumes ranged from 34–43 ml/kg in the subjects studied. The data on the metabolism of factor B in SLE patients are presented in Table 3. The mean FCR in twelve control subjects was 1·68% of the plasma pool per hour with a range (±2 s.d.) of 1·55–1·81%. Eight of eleven SLE patients had an elevated FCR of factor B and an abnormally shortened T 1/2 of the protein. There was good correlation ($r = 0{\cdot}91$, $P<0{\cdot}001$) between the FCR of factor B which was determined by Nosslin's integrated rate analysis and the T 1/2 which was determined graphically on semi-logarithmic paper. The mean FCR of the control and SLE groups were tested by the *t*-test for independent means, and were significantly different ($t = 3{\cdot}83$, $P<0{\cdot}001$). All seven patients with 1+ disease activity had elevated FCRs of factor B. One patient in clinical remission (L.G.) also had an increased FCR of factor B. Three other patients in remission had a normal FCR of factor B.

The synthesis rate of factor B was decreased in three SLE patients: one patient (M.G.) with a complete C2 deficiency and an elevated FCR of factor B, one patient (L.G.) with a partial C2 deficiency and an elevated FCR, and one patient (J.T.) with normal FCR. One patient (T.K.) had significantly increased synthesis (0·242 mg/kg/hr) of factor B in addition to an elevated FCR.

The mean E:P ratio for the control subjects was 1·17 with a range (±2 s.d.) of 0·80–1·54. This ratio was below the normal range in six of eleven SLE patients, and five of these six patients had an abnormally high FCR of factor B. The E:P ratio was calculated from the rate of change of factor B in the extravascular pool versus that of the plasma pool. There was little change in the plasma pool volume with respect to time and hence the fall in E:P ratio was attributed to decreases in the extravascular pool volume. This suggests increased extravascular catabolism of factor B in SLE patients.

The serum of six of eleven SLE patients contained a factor or factors which activated the alternative complement pathway, independent of classical pathway activation. As can be seen in Table 3, the serum of six patients produced greater than 5% lysis of GSHE cells and conversion of factor B and C3 when added to fresh normal human serum in which the classical pathway had been blocked. The serum factors of the patients have been characterized by sucrose density gradient ultracentrifugation and immunochemical analysis. Also a serum factor of one patient (E.B.) was isolated by column chromatography. These data have been reported by Arroyave *et al.* (1976). In the present study, all six patients with serum factors which activated the alternative complement pathway had an abnormally elevated FCR of factor B. Only two patients (M.H. and L.G.) with an elevated FCR did not have an alternative complement pathway activating factor in their serum.

The *in vivo* behavior of the ^{125}I-labelled protein was significantly different between the SLE patients with an elevated FCR and the control subjects. The plasma and extravascular radioactivity curves are shown in Fig. 1. The ^{125}I-labelled protein was cleared more rapidly from the plasma space in the SLE patients than in the control subjects. Only 9–15% of the injected dose remained in the plasma space of the SLE patients at 96 hr. The increased catabolism of factor B is even more dramatic when the extravascular radioactivity curves are examined. Twenty-nine to 38% of the injected dose equilibrated in this space within the first 24 hr after injection in both patients and control subjects. However, it was cleared more rapidly in this space by the SLE patients after the first 24 hr.

This increased clearance of factor B by the SLE patient is again observed when the mean total body and mean plasma radioactivity retention curves of all the normal subjects and of all SLE patients were compared in Fig. 2. The radioactivity was cleared only slightly faster in the plasma space of the SLE

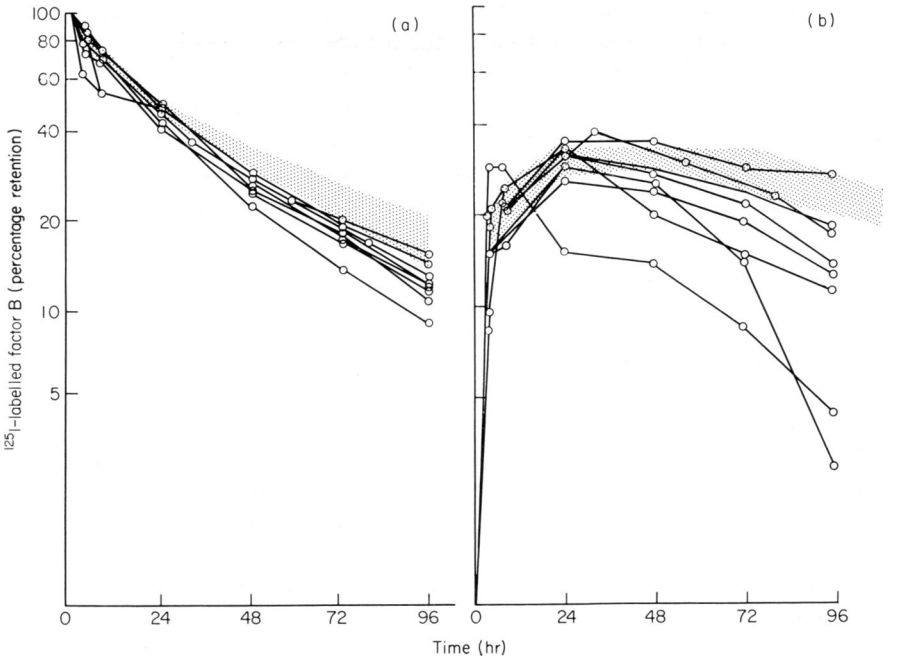

FIG. 1. Metabolism of injected radio-labelled factor B, presented as percentage factor B retention in (a) plasma space and (b) extravascular space. Radioactivity in plasma space was obtained from direct measurement and that in extravascular space from subtraction of plasma and urine radioactivity from total injected dose. The shaded areas represented the range of twelve control subjects (mean$\pm$2 s.d.). The studies from eight SLE patients with elevated fractional catabolic rates are shown. Although the difference between SLE and normals was noted in the plasma retention curves, this difference was more striking in the extravascular space retention curves (see text for explanation).

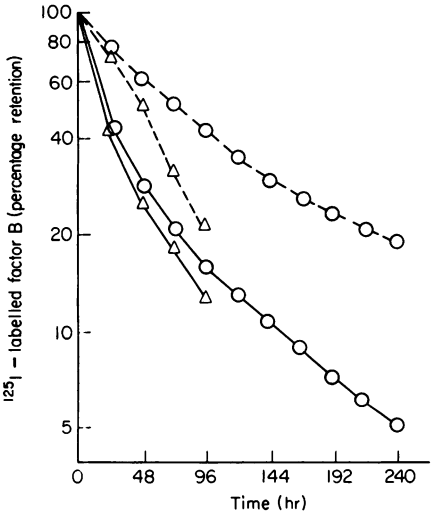

FIG. 2. Metabolism of factor B in the same subjects shown in Fig. 1, represented here as comparison of retention in total body space versus plasma space. Each point was the mean value of each group (twelve normals and eight SLE patients). The significant difference is readily observed in the total body retention curves. This was demonstrated to be related to increased extravascular catabolism in the SLE patients. (○) Controls; (△) patients; (△ — —△) and (○ — — ○) total body; (△ — △) and (○ — ○) plasma.

patients than in the control subjects. However, when the mean total body curves for both groups are compared, the difference between the SLE patients and the control subjects is distinctly greater.

Nine of the eleven SLE patients were taking corticosteroids daily (prednisone 10–20 mg). Two patients were untreated at the time of study. Both of the untreated patients (D.L. and N.H.) had elevated FCRs of factor B. There was no correlation between the dosage of corticosteroid taken and the FCR or synthesis of factor B. Three patients (J.T., A.M., M.C.) who were taking corticosteroids had normal FCRs of factor B.

DISCUSSION

In the present study, we have shown an increased FCR of factor B in eight of eleven SLE patients. An increased FCR of factor B can occur either by direct activation of the alternative pathway by way of properdin and factor D or by recruitment from the classical pathway through the C3b feedback cycle. It was our original intention to dissect the mode of complement activation in each patient by simultaneously injecting a protein of the alternative pathway and a protein of the classical pathway labelled with radioactive iodine. We intended to use ^{131}I-labelled C4 in addition to ^{125}I-labelled factor B. However, because of the instability of purified C4, it was not possible to label the protein with ^{131}I without causing a significant loss in functional activity. This problem has been noted previously in studies of C4 metabolism performed in patients with renal allograft rejection and patients with hereditary angioedema (Carpenter *et al.*, 1969), who reported non-specific activation and occasionally denaturation of protein in seven of nine radioactively labelled samples of C4. C1q might be another component of the classical pathway which could have been used simultaneously with factor B. However, because C1q combines directly with immune complexes and with nucleic acids, any such reaction *in vivo* would have complicated the interpretation of results. We therefore, limited our studies using only functionally active factor B.

An interesting finding in our studies was the correlation of serum factors which activated the alternative pathway and an increased FCR of factor B in our patients. Complement activating factors of the 7S and 19S type have been reported in the pericardial fluid of two SLE patients by Hunder, Mullen & McDuffie (1974). The pericardial fluid could also produce immunoelectrophoretic conversion of factor B when added to fresh normal human serum. Agnello *et al.* (1971) also have described 7S, 19S and heavier factors which could precipitate with C1q in the sera of hypocomplementaemic lupus patients. We have demonstrated in a previous study (Arroyave *et al.*, 1976) that the sera of approximately 30% of our SLE patients contained factors which activated the alternative pathway of complement when added to fresh normal human serum. These serum factors were detected by *in vitro* assays (GSHE lysis and immunoelectrophoretic conversion of factor B) in which the classical pathway was blocked by chelation of calcium ions by EGTA or by using C2-deficient serum. Therefore, these serum factors caused activation of the alternative pathway independent of classical pathway activation. The serum factors could be separated into 7S and 19S types by sucrose density gradient ultracentrifugation. The 7S type activating factor which was isolated in purified form in two SLE patients was found to be identical to C3 nephritic factor previously described in the sera of patients with chronic hypocomplementaemic glomerulonephritis (Vallota *et al.*, 1974) but the character of the 19S factors was not identified. In the present study, we have demonstrated that in all six SLE patients who had a serum factor which activated the alternative pathway, there was an increased FCR of factor B. In these six patients, increased catabolism of factor B could be related entirely or in part to these serum factors. A contribution to catabolism of factor B by way of the C3b feedback cycle from classical pathway activation however, cannot be excluded. In the two patients without alternative pathway activating factors the increased B catabolism could be due entirely to C3b feedback activation by the classical pathway.

The finding of decreased serum levels of factor B in our C2 deficiency patients was interesting. These levels were noted to be decreased by immunochemical quantitation and by haemolytic assay. Decreased serum levels of factor B to C2-deficient patients have been noted by others (Sussman *et al.*, 1973; Osterland *et al.*, 1975), and have been interpreted as evidence of alternative pathway activation. Our

data shows that in addition to an increased FCR of factor B, two of our three patients with C2 deficiency (M.G. and L.G.) had decreased synthesis of this protein and the third C2-deficient patient (N.H.) had a synthesis rate at the lower limits of normal. There were five SLE patients without C2 deficiency who had increased FCR of factor B but normal synthesis rates. This raises the question that decreased synthesis of factor B and deficiency of C2 in the latter patients may be more than a chance relationship.

Another interesting aspect of this study was the apparent discrepancy between the serum levels of C3 measured by haemolytic assay and C3 quantitated by radial immunodiffusion. Eight of eleven patients had decreased levels of haemolytically active C3, and only one patient had an abnormally low serum C3 level measured by radial immunodiffusion. This difference may be explained by the fact that antisera to C3 recognizes native C3 protein as well as split products of C3 activation. Thus, the radial immunodiffusion technique will not differentiate between native protein and the split products which share antigenic determinants. In contrast, the haemolytic assay measures only native C3 protein which is haemolytically active. Therefore, it is possible that some of our SLE patients may have had products of C3 activation in circulation which would cause measurement of C3 serum levels by radial immunodiffusion to remain within normal limits. Charlesworth *et al.* (1974) and Perrin, Lambert & Miescher (1975) have detected C3d in the sera of SLE patients associated with increased C3 consumption.

In a previous study of factor B and C3 metabolism, Charlesworth and coworkers (1974) studied twenty-two patients with diseases associated with hypocomplementaemia and eleven patients with renal disease with normal serum levels of complement. Included in this study were patients with mesangiocapillary glomerulonephritis, partial lipodystrophy, acute glomerulonephritis and five patients with SLE. An increased catabolism of factor B was observed in ten of twenty-three patients. Recently, Ziegler and associates (1975) have reported a study of metabolism using ^{125}I-labelled properdin in five patients with SLE and nephritis. All five patients had an elevated FCR of properdin. In our studies only one of our patients (E.B.) had renal disease. Yet, eight of eleven patients had evidence of an increased FCR of factor B. These data suggest that activation of the alternative pathway in SLE is not necessarily limited to renal disease.

These studies demonstrated increased catabolism of factor B which occurred mainly in the extravascular compartment. This extravascular compartment may be considered a tissue space, at least in part. Our findings are supported by the reports of other investigators who have demonstrated the fixation of alternative pathway proteins such as properdin and factor B in skin and kidneys of patients with SLE (Rothfield *et al.*, 1972; Provost & Tomasi, 1973). Others have pointed out the importance of a relationship between activation of the alternative pathway and renal disease (Charlesworth *et al.*, 1974; Ziegler *et al.*, 1975). We have shown that activation of this pathway can be observed in a number of patients without renal disease, and this phenomenon may also be important in some of the extra-renal manifestations of SLE.

This is publication number 993. This work was supported by research grants from the National Institutes of Health AM 12198, AI 10386, AI 12594 and a General Clinical Research Center grant RR 0083 from the Public Health Service. Doctor Wilson is a post-doctoral trainee supported by a grant from the National Institutes of Health AI 00214. Dr Arroyave is a senior investigator of the Arthritis Foundation.

We should like to express our appreciation to Dr Peter Lachmann and Dr Hans Müller-Eberhard for their advice and criticism during this study. We would like to thank also Dr David De Heer for his help in the computer analysis of the data, and Ms Edith Mödder and Ms. Kathy Pattison for their technical assistance.

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