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Research Article

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Metabolism of the Fifth Component of Complement, and its Relation to Metabolism of the Third Component, in Patients with Complement Activation

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ABSTRACT The metabolism of the fifth component of complement (C5), and its relationship to metabolism of the third component of complement (C3), has been studied in normal subjects and patients by simultaneous administration of radioiodine labeled C5 and C3. In seven normal subjects the fractional catabolic rate of C5 ranged from 1.5 to 2.1% of the plasma pool/h and extravascular/intravascular distribution ratio from 0.22 to 0.78, these values being similar to those obtained for C3, and synthesis rate from 71 to 134 μg/kg per h. In patients with complement activation the increase in fractional catabolic rate of C5 was nearly always less than that of C3. The data also showed that there was increased extravascular distribution of C3 and C5 in most patients and considerable extravascular catabolism of both proteins in some. However, there were differences in metabolic parameters between patients with different types of complement activation. In patients with systemic lupus erythematosus, fractional catabolism and extravascular distribution of C3 and C5 were both increased, and there was marked extravascular catabolism of both proteins. There was increased fractional catabolism and extravascular distribution of C3 in patients with mesangiocapillary nephritis and (or) partial lipodystrophy, and fractional catabolism of C5 was also increased in three of six studies although distribution of C5 was always within the normal range; however, in two patients with nephritic factor in their serum fractional catabolism of C5 was normal despite markedly increased C3 turnover, suggesting that in patients with alterna-
tive pathway activation by nephritic factor little or no C5 convertase is generated.

INTRODUCTION

The fifth component of complement (C5)[1] is the major component of the final common pathway of complement activation—the membrane attack complex C5-9. The cleavage of C5—the final enzymatic step in the complement sequence—yields a larger fragment, C5b, which binds C6-9 to form the membrane attack complex, and a smaller fragment, C5a, which has anaphylatoxic and chemotactic activity.

The subunit structure of C5 is very similar to that of the third component (C3) (1), and this is reflected by the similarity of the C3 and C5 cleaving enzymes (convertases) which are generated after activation of complement by the classical or alternative pathways. Thus the classical pathway C3 convertase, $\overline{C4b2a}$, binds the major cleavage fragment of C3, C3b, to become a C5 convertase $\overline{C423b}$.

The mechanism of formation and composition of the alternative pathway C3 and C5 convertases has been the subject of much recent investigation. Upon activation of the alternative pathway C3b is generated and interacts with factors B and $\bar{D}$ ($\bar{D}$ cleaving Factor B) in the C3b feedback or amplifica-

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[1] *Abbreviations used in this paper*: B, Factor B (or C3 proactivator or glycine-rich B glycoprotein) of the alternative pathway; C3, third component of complement; C3bINA, C3b inactivator; C5, fifth component of complement; CVF, cobra venom factor; EV/IV ratio, extravascular/intravascular distribution ratio; FCR, fractional catabolic rate; HBAg, hepatitis B antigen; MCGN, mesangiocapillary nephritis; NeF, the C3 nephritic factor; PLD, partial lipodystrophy; SLE, systemic lupus erythematosus; U/P ratio, urine/plasma ratio of radioactivity.

tion cycle to generate the bimolecular C3 convertase $\overline{C3bBb}$. $\overline{C3bBb}$ is also a C5 convertase, but this activity is only expressed if the convertase is generated on a cell surface (rather than in the fluid phase) and contains additional C3b molecules (2, 3). $\overline{C3bBb}$ loses activity by intrinsic decay, but it has recently been shown that the plasma protein β1H accelerates this decay by dissociating Bb from the complex and rendering the residual C3b accessible to the C3b inactivator (C3bINA) (4, 5, 6); the additional C3b in the solid phase C5 convertase $\overline{C3bBb}$ is apparently already susceptible to C3bINA, which causes the enzyme to revert to a C3 convertase only (3).

The half-life of $\overline{C3bBb}$ is prolonged by activated properdin, which binds to C3b, and by the C3 nephritic factor (NeF) which binds to $\overline{C3bBb}$ (7, 8). The convertase thus stabilized by activated properdin is still susceptible to β1H but that stabilized by NeF is relatively resistant to β1H (4). Furthermore, in the presence of NeF a C3 convertase can be generated, $\overline{C3bB}$, which contains uncleaved B (9–11); it is not established whether this latter enzyme is able to cleave C5.

To determine the extent of C5 activation in disease, and to examine the relationship of these in vitro observations to mechanisms of complement activation in vivo—particularly as C5 concentrations are frequently normal in patients with complement activation—we have studied the metabolism of simultaneously administered radioiodinated C5 and C3 (or in a few cases Factor B) in normal subjects, and in patients with various diseases, including those known to involve complement activation.

METHODS

Purification of complement proteins. C5 was purified from pooled normal human serum by the method of Nilsson et al. (12). The final preparation contained no C3 (or other proteins) on immunoelectrophoresis and Ouchterlony gel analysis against antiwhole human serum (The Wellcome Foundation, Ltd., The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, England) and antihuman C3 antisera and gave a single band on polyacrilamide disk gel electrophoresis and sodium dodecyl sulphate polyacrilamide disk gel electrophoresis. Immunization of rabbits with this preparation of C5 produced an antiserum monospecific for C5 and showing no reactivity against C3.

Factor B and C3 were prepared as previously described (13). Factor B contained no other proteins by immunoelectrophoresis against antiwhole human serum. All of the several C3 preparations used contained a trace contaminant (probably βIH [14]) and one a trace amount of IgG, but no other proteins by immunoelectrophoresis against antiwhole human serum.

Radioiodination of proteins. C5, C3, and Factor B were trace labeled by a modification of the chloramine-T method as previously described (15, 16). C5 was always labeled

with ^{125}I, and C3 and Factor B with ^{131}I. Efficiency of labeling ranged from 20 to 40% and specific activity of the labeled proteins up to 1 μCi/μg of protein. Purified human serum albumin (Lister Institute of Preventive Medicine) was added to all preparations to a concentration of 10 mg/ml immediately after labeling. Free radioactivity was removed by one passage down an anion-exchange resin column followed by dialysis until 1% or less of total radioactivity was nonprotein bound. Labeled proteins were subjected to immunoelectrophoresis against antisera to C5, C3, or Factor B and whole human serum, followed by autoradiography. All preparations contained a single radiolabeled protein arc except for one C3 preparation which contained a trace of labeled IgG—this was removed by a Sepharose-coupled monospecific antihuman IgG antibody. (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) Labeled proteins were sterilized by Millipore filtration (Millipore Corp., Bedford, Mass.) and pyrogen-tested in rabbits as previously described (16).

Functional activity of radiolabeled proteins. C5 and Factor B were functionally assayed by a hemolytic plate technique (13, 17) and C3 by hemolytic titration (13). No, or only slight, reduction in functional activity per unit of protein occurred on labeling. However because these proteins were trace labeled it could not be assumed that the total functional capacity of a labeled preparation was necessarily representative of the small number of labeled molecules. Therefore, as a further functional test of the labeled molecules, their ability to be turned over by cobra venom factor (CVF) was shown by in vivo turnover studies in rabbits as previously described (18). Rapid acceleration in turnover of radiolabeled C5, C3, and Factor B occurred after administration of CVF to rabbits which had previously been injected with these proteins (see Fig. 1). No such acceleration was seen if inactivated components were used.

Complement component assays. Plasma C5 concentrations were measured by electroimmunoassay ('rocket' technique) with a monospecific rabbit antihuman C5 antibody; concentrations of C1q, C4, C3, and Factor B were measured by single radial immunodiffusion. Factor D was measured functionally by a hemolytic plate technique (17). C3 splitting activity was detected by crossed immunoelectrophoresis (19) and its capacity to activate the alternative pathway (NeF activity) examined in magnesium EGTA as previously described (20). The presence of circulating C3d was detected by immunoelectropheresis of fresh EDTA plasma against an antiserum with specificity for C3d (21).

Protocol for turnover studies. Informed consent was obtained from all patients and control normal subjects. Thyroid uptake of radioiodine was blocked by administration of potassium iodide 180 mg three times daily for 3 days before and during the study or (in iodine-sensitive subjects) of potassium perchlorate 1 g 1 h before starting, and 200 mg three times daily during the study. Subjects were given 10–15 μCi of ^{125}I-C5 and 2–12 μCi of ^{131}I-C3 or Factor B intravenously, syringes being weighed before and after injection to calculate the dose of isotope. Venous blood samples were taken into EDTA at 10 min, 4–6 h, 12–30 h, and 26–28 h after injection and daily thereafter for 6–8 days. Blood samples were processed and counted and free and protein bound radioactivity calculated as previously described (16). Continuous urine collections were obtained throughout the study. In some patients whole body radioactivity for ^{131}I was measured with a whole body counter (10 6-inch sodium iodide crystals).

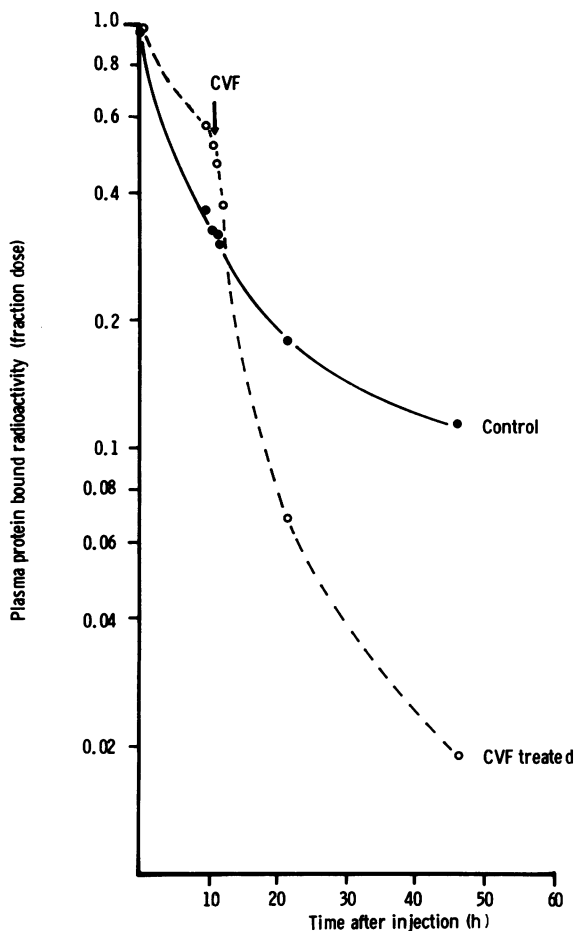*Analysis of metabolic data.* Metabolic parameters (frac-

FIGURE 1 Effect of a single decomplementing dose of CVF on plasma disappearance curve of ^{125}I-C5 in normal rabbit. There was accelerated plasma disappearance of ^{125}I-C5 in the CVF treated rabbit compared to the saline injected control.

tional catabolic rates [FCR], synthesis rates and extravascular/intravascular pool ratios [EV/IV] ratios) were derived by exponential analysis of the plasma disappearance curve (Matthews' method) (22), by the metabolic clearance method (urine to plasma [U/P] ratios) and from the integrated rate equations method of Nosslin (23), as previously described (16, 21). Nosslin's method utilizes both plasma and urine data and offers theoretical advantages over the other two methods, particularly in studying proteins with short half-lives (23). Unless otherwise stated, and except in patients with impaired renal function, the values for FCR and EV/IV ratios given are those calculated by Nosslin's method. Synthesis was calculated by assuming the synthetic rate was equal to the absolute catabolic rate, provided that plasma concentrations of the protein were stable throughout the study.

Subjects. 28 subjects were studied. Seven were normal volunteers from the medical and technical staff. 16 were patients with diseases associated with complement activation; 7 with clinically active systemic lupus erythematosus (SLE); 4 with mesangiocapillary (membranoproliferative) nephritis (MCGN), 2 of whom had associated

partial lipodystrophy (PLD); 1 with PLD alone; 1 with acute poststreptococcal nephritis; 1 with recurrent skin lesions, angioedema and classical pathway complement activation (previously reported by us [24]); 1 with subacute Q Fever (Coxiella) endocarditis, and 1 with essential mixed cryoglobulinemia. Of the remaining 5 patients, 2 had end stage chronic renal failure without evidence of complement activation, 1 minimal-change nephritis, and 1 myasthenia gravis. Patient 17 had chronic active hepatitis and hepatitis B (HBAg) antigenemia, and was given two intravenous infusions of hepatitis B antibody during the period of study in a therapeutic attempt to eliminate the HBAg.

Five of the above subjects (one normal, one SLE, one MCGN, and the patients with angioedema and mixed cryoglobulinemia) were injected with [131]I Factor B and ^{125}I-C5; all the remaining subjects were injected with ^{131}I-C3 and ^{125}I-C5.

RESULTS

Normal subjects (Table I). The metabolic data for C5 in seven and for C3 in six normal subjects are given in Table I. The values for FCR, EV/IV ratios, and $t\frac{1}{2}$ were similar for both proteins; it can be seen that the three methods of analysis used gave very similar values for FCR, and EV/IV ratios calculated by Matthews' and Nosslin's methods were also similar. The values obtained for C3 (and the value obtained in a single normal for Factor B) fall within the ranges previously established in this laboratory for normal subjects (21). The lower plasma concentration of C5 as compared to C3 is a reflection of the difference in synthetic rates for the two proteins. From the integrated rate equations method the rate constants for extravascular catabolism of C5 and C3 were near zero indicating that under normal circumstances their catabolism is mainly intravascular. Plasma volumes calculated from the initial plasma sample in normal subjects (and patients) were usually close to expected values, arguing against the presence of significant amounts of denatured or aggregated proteins (which would artefactually elevate plasma-volume values because of their early rapid removal).

Patients. The metabolic data for patients are given in Table II. Metabolic parameters given are those calculated by Nosslin's integrated rate equations method, except in patients with impaired renal function when only exponential analysis of the plasma curve was possible. The serum concentrations of complement components in patients are shown in Table III.

Patients with SLE. All seven patients with SLE had clinically active disease and all but two (patients 3 and 6) had low plasma C3 concentrations at the time of study. All patients, including the two with normal C3 concentrations, had increased FCR for C5 and for C3 (or Factor B in patient 3). For

TABLE I

Metabolism of C5 and C3 in Normal Subjects

Subject	Protein	Plasma volume	Plasma half life	FCR			EV/IV Ratio		Serum concentration	Synthesis rate
				Matthews' method	U/P ratios	Nosslin's method	Matthews' method	Nosslin's method		
		ml/kg	h	% plasma pool/h					µg/ml*	µg/kg/h
1	C5	46	59	1.60	1.58	1.58	0.258	0.42	103	75
	C3	49	58	1.56	1.25	—	0.238	—	725	518
2	C5	46	68	1.61	1.59	1.64	0.405	0.560	100	77
	C3	46	88	1.52	1.55	1.67	0.658	0.356	600	461
3	C5	40	63	1.65	1.68	1.72	0.358	0.397	118	81
	C3	48	76	1.54	1.44	1.32	0.562	0.551	810	429
4	C5	51	65	1.60	1.66	1.71	0.517	0.224	126	111
	C3	49	82	1.40	1.46	1.75	0.414	0.178	1,280	1,157
5	C5	39	63	1.83	1.69	1.94	0.550	0.547	104	78
	C3	41	74	1.58	1.39	1.58	0.505	0.612	1,400	854
6	C5	47	68	2.04	1.96	2.13	0.676	0.784	134	134
	C3	40	63	1.63	1.72	1.70	0.332	0.455	1,430	1,143
7	C5	39	55	1.86	1.52	—	0.311	—	99	71
	B	32	62	2.14	2.06	—	0.630	—	200	166
Range	C5	39–51	55–68	1.6–2.04	1.52–1.96	1.58–2.13	0.258–0.676	0.224–0.784	99–134	71–134
Mean		44	63	1.74	1.67	1.79	0.439	0.489	112	90
Range	C3	40–49	58–88	1.4–1.63	1.25–1.72	1.32–1.75	0.238–0.658	0.178–0.612	600–1,430	429–1,161
Mean		46	74	1.54	1.47	1.60	0.452	0.43	1,040	759
Range	B‡		61–74			1.7–2.2		0.81–1.31		140–210
Mean			66			1.98		1.07		180

* Normal ranges for C3 and C5 concentrations given in Table II.
‡ Normal metabolic data for Factor B taken from reference 21.

three patients (the three with clinically more severe disease) the increase in FCR of C3 was greater than that for C5 whereas the FCRs in the others were similar. There was an increased EV pool and increased EV catabolism of both proteins in most of these patients (see below), except for patient 5 in whom Nosslin's analysis could not be applied and thus no estimate of EV catabolism was possible— the EV/IV ratio calculated by Matthews' method was normal in this patient.

Patients with MCGN and (or) PLD. All of the four patients with MCGN studied had increased catabolism of C3 or (Patient 9) Factor B. Two had an associated increase in catabolism of C5, very markedly so in patient 8 in whom precise calculation of the FCR was not possible because of a non-linear plasma disappearance curve and impaired renal function; however in patients 9 and 11 C5 catabolism was within the normal range. Because of these findings patient 11 was studied twice: on

the first occasion there was increased C3 catabolism (FCR 3.2%/h) but C5 catabolism was in the low normal range (FCR 1.5%/h); on the second occasion when his disease was clinically more active and serum C3 concentration lower, C3 catabolism was further increased (FCR 4.0%/h) and C5 catabolism had also increased (FCR 2.4%/h, normal 1.5–2.1%/h).

Patient 12 had PLD with no clinical evidence of nephritis and a low serum C3 concentration. This patient had the highest FCR for C3 in the series (8.02%/h) but C5 turnover was normal (1.9%/h).

NeF activity was detectable in the serum of patient 11 on both occasions, and was strongly positive in patient 12. Although there was some C3-splitting activity in the serum of patient 8, this was heat labile and did not act in Mg^{++} EGTA (i.e. was not acting via the alternative pathway) in contrast to that in the other two patients; there was also a high titer of C1q binding material (presumed to be immune complexes) as detected by the C1q deviation test

TABLE II

Metabolism of C5 and C3 (or Factor B) in 21 Patients

Patient number	Diagnosis	Sex/Age	Protein studied	Plasma volume	Half life	FCR	EV/IV ratio	Plasma concentration*	Synthesis rate	Foot- notes
				ml/kg	*h*	*%/h*		*μg/ml*	*μg/kg/h*	
1	SLE	F 23	C5	32	43	2.23	1.55	128	91	
			C3	34	45	2.09	1.28	273	182	
2	SLE	F 31	C5	46	50	2.13	1.24	86	84	
			C3	48	70	2.2	0.861	176	177	
3	SLE	F 22	C5	37	64	2.68	0.902	127	127	
			B	34	41	4.03	1.29	280	422	
4	SLE	F 35	C5	41	48	2.35	1.00	68	65	
			C3	44	—	4.7	1.6	420	809	‡
5	SLE	F 13	C5	43	49	2.20	0.407	172	163	§
			C3	52	43	2.87	0.454	633	788	§
6	SLE	F 33	C5	52	54	2.21	1.04	99	116	
			C3	51	50	2.18	1.22	755	870	
7	SLE	F 12	C5	31	34	2.83	1.40	163	149	
			C3	32	36	4.28	1.74	550	730	
8	MCGN	M 34	C5	47	—	—	—	15	—	‡‖
			C3	50	—	—	—	250	—	‡¶
9	MCGN	M 18	C5	43	47	1.96	0.738	108	91	
			B	31	42	2.94	0.834	320	405	
10	MCGN	M 30	C5	38	42	2.60	0.702	122	119	
			C3	35	29	2.73	0.796	648	666	
11a	MCGN and PLD	M 62	C5	65	60	1.49	0.715	106	103	**
			C3	71	—	3.23	0.903	300	630	‡¶
11b			C5	55	65	2.48	0.601	137	188	**
			C3	70	—	4.0	2.30	192	420	‡¶
12	PLD No nephritis	F 51	C5	41	52	1.93	0.686	144	114	**
			C3	45	—	8.02	1.18	250	821	‡¶
13	Acute nephritis (poststreptococcal)	F 45	C5	43	39	3.90	0.838	68	115	
			C3	48	—	3.57	0.673	325	308	‡
14	Skin lesions and angioedema	F 53	C5	35	54	1.92	0.643	103	68	
			B	30	57	2.98	1.09	260	271	
15	Mixed cryoglobulinemia	F 62	C5	43	75	1.67	0.561	120	87	§
			B	37	61	2.38	0.733	210	217	§
16	Subacute infective endocarditis	F 41	C5	70	52	1.94	0.356	144	196	§
			C3	70	48	2.54	0.344	800	1,422	§
17	Chronic active hepatitis	M 40	C5	45	44	2.32	0.436	130	137	‡‡
			C3	43	49	1.92	0.360	780	682	
18	Minimal change nephritis	F 27	C5	52	30	2.73	0.852	111	162	
			C3	51	50	3.31	0.699	750	1,291	
19	Myasthenia gravis	F 32	C5	43	45	2.47	0.532	141	150	
			C3	39	45	2.34	0.766	1,300	1,182	
20	Chronic renal failure	M 58	C5	44	66	1.50	0.396	102	68	§
			C3	46	53	2.10	0.576	560	517	§

TABLE II (Continued)

Patient number	Diagnosis	Sex/Age	Protein studied	Plasma volume	Half life	FCR	EV/IV ratio	Plasma concentration*	Synthesis rate	Foot-notes
				ml/kg	*h*	*%/h*		*μg/ml*	*μg/kg/h*	
21	Chronic renal failure	M 59	C5	60	55	1.99	0.447	131	158	§
			C3	67	53	1.94	0.370	1,200	1,397	§

Values for FCR and EV/IV ratios calculated by Nosslin's method unless otherwise stated.

* Normal ranges (mean±2 SD): C5 86–132 μg/ml, C3 676–1,796 μg/ml, B 100–500 μg/ml.

‡ Plasma disappearance curve did not reach final monoexponential. FCR and EV/IV ratio calculated from first 72 h data only. $t_\frac{1}{2}$ reduced but not calculable.

§ FCR and EV/IV ratios calculated by Matthews' method because of impaired renal function or incomplete urine collections.

‖ No values for FCR and EV/IV ratios could be calculated in this patient because of renal failure and nonlinear plasma disappearance curves.

¶ C3d detected in EDTA plasma.

** NeF detected in serum.

‡‡ Base-line values for FCR and EV/IV ratios calculated by exponential analysis. See Fig. 2 for further details of patient 17.

(25) in the serum of patient 8 whereas 11 and 12 were negative. NeF was not detected in the sera of any of the other 18 patients.

Other patients. The one patient with acute nephritis (patient 13) had the highest FCR for C5 observed in this series (3.9%/h). Patient 14 with angioedema and low C3, had slightly increased turnover of Factor B but normal C5 catabolism, as did patient 15 with mixed cryoglobulinemia. Patient 16, with subacute infective endocarditis due to Q fever

TABLE III
Serum Concentrations of Complement Components in Patients

Patient	C1q %RNS*	C4 %RNS*	C3	C5	Factor B %RNS	NeF
			μg/ml			
1	38	16	273	128	100	−
2	35	45	176	86	76	−
3	40	62	747	127	100	−
4	22	26	420	68	40	−
5	60	16	633	172	64	−
6	60	90	755	99	100	−
7	41	60	550	163	65	−
8	35	70	250	15	90	−
9	60	100	320	108	100	−
10	130	200	648	122	100	−
11	100	80	300	106	45	+
12	52	90	250	144	100	++
13	100	58	325	68	100	−
14	10	38	600	100	100	−
15	55	46	810	118	72	−
16	100	15	1,280	126	100	−
17	75	110	1,400	104	140	−
18	80	90	1,430	134	85	−
19	76	42	1,300	99	100	−
20	80	90	560	102	75	−
21	110	120	1,200	131	80	−
Normal ranges	60–110	50–210	676–1,796	86–132	40–210	

* %RNS, Percent reference normal serum.
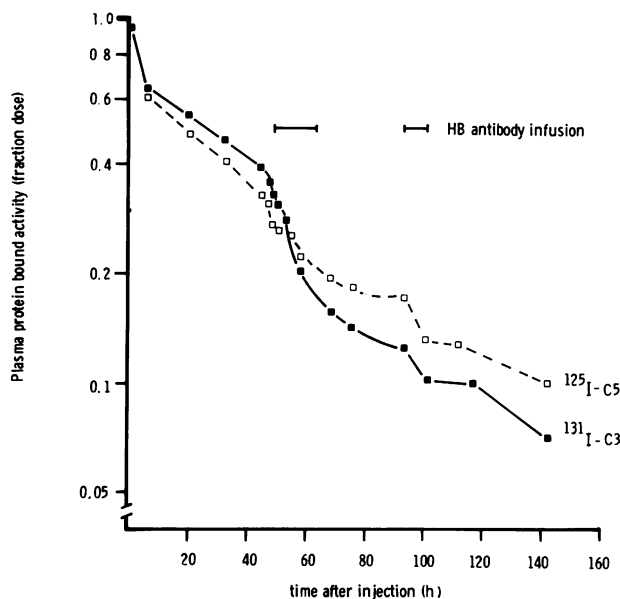
FIGURE 2 Plasma disappearance curves of ^{125}I-C5 and ^{131}I-C3 in patient 17. This patient with chronic active hepatitis and HB antigenaemia was given two infusions of HB antibody during the turnover study. Acceleration in C5 and C3 turnover occurred with each infusion.

showed normal C5 despite increased C3 turnover; patient 19, with myasthenia gravis, showed slightly increased turnover of C3 and C5. The two patients with chronic renal failure showed normal C5 and C3 metabolism.

A patient with minimal change nephritis (patient 18) had increased C5 and C3 turnover—this was the only patient in whom protein bound radioactivity was present in the urine. Correction of the fractional turnover rate by subtraction of the fractional proteinuric rate still revealed increased fractional catabolism; this raises the possibility that increased turnover was at least partly due to renal tubular catabolism of filtered labeled protein in this particular patient.

Patient 17 with HB antigenemia was infused with hepatitis B antibody during the turnover study. As is apparent from Fig. 2 there was increased turnover of C3, and to a lesser extent C5, during the two periods of infusion (presumably related to the formation of circulating immune complexes). This study also provided additional in vivo evidence of the functional activity of both proteins.

GENERAL CONCLUSIONS

C5 and C3 catabolism. There was generally good agreement between the values obtained for FCR for each protein in individual patients by the three methods of analysis, as shown for the normal sub-

jects in Table I. However, an exception was shown by some patients (patients 4, 8, 11, 12, and 13) in whom U/P ratios for C3 decreased progressively throughout the study; in these patients there was also a tendency for their plasma disappearance curves not to reach a final monoexponential slope. This has been previously described by us and attributed to the presence of labeled C3d in the circulation (21). However, this phenomenon was never observed with C5, U/P ratios remaining relatively constant, although a nonlinear plasma disappearance curve was observed in patient 8 in whom C5 catabolism was very rapid. In this patient values for FCR and U/P ratios could not be calculated because of impaired renal function. Fig. 3 shows that, while there is in general a correlation between FCR of C5 and C3, striking disparities between the two may occur, and a similar disparity was also observed between C5 and Factor B turnover. It is also apparent that at higher FCRs of C3 there is generally proportionately less increase in the FCR of C5 (except for patients 8 and 13—see Table II). With the exception of patient 13 no patient had an FCR for C5 above 3%/h, although several had FCRs for C3 above this value. The total pool size for C3 was always greater than that for C5 and thus absolute catabolism of C3 was always considerably greater than that of C5.

C5 concentrations. Plasma concentrations of C5 were below normal in only three of the patients studied (patients 4, 8, and 13) and markedly reduced in only one of these (patient 8). There was a closer correlation between plasma C5 concentration and C5 synthesis than FCR of C5 (see Figs. 4a and 4b).
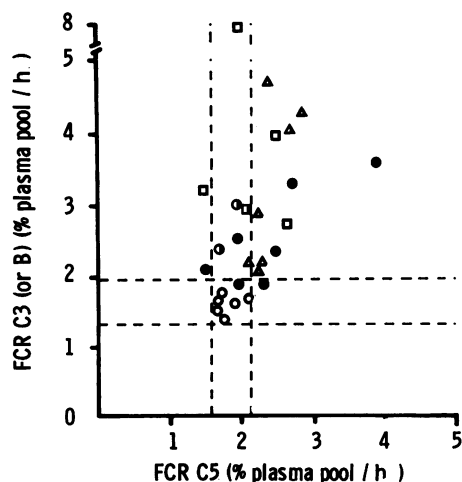

FIGURE 3 Relationship between FCR of C5 and C3. Broken lines indicate normal ranges. ○ Normal subjects; △ SLE; □ MCGN/PLD; ● other disease; ▲ ◨ ◑ patients with SLE, MCGN, or other disease in whom C5 and B turnover was studied.
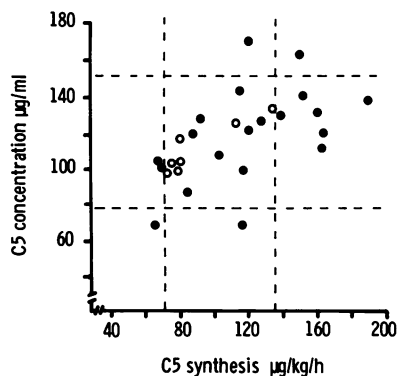
FIGURE 4a Relationship between synthesis and plasma concentration of C5. Broken lines indicate normal ranges. ○ normal subjects; ● patients; r = 0.671; P = <0.001.
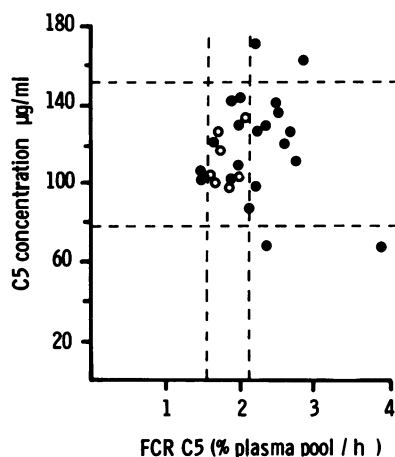

FIGURE 4b Relationship between FCR and plasma concentrations of C5. Broken lines indicate normal ranges. ○ normal subjects; ● patients; there is no significant correlation.

No patient had values for C5 synthesis unequivocally below the normal range but three (patients 1, 2, and 13) had low values for C3 synthesis.

Distribution of C5 and C3. EV/IV ratios were calculated by the methods of Matthews and Nosslin; there was less agreement between the values obtained by these two methods in patients than in normals. In Nosslin's analysis the size of the EV pool is derived by subtraction of plasma radioactivity from whole body activity—the latter being obtained by subtraction of cumulative urine excretion of free radioiodine from the dose of isotope (23). The value for the EV/IV ratio obtained by Matthews' method is dependent on the shape of the initial portion of the plasma disappearance curve and urine data are not used (22). The presence of aggregated protein would increase the value, and it is necessary to obtain multiple early blood samples to plot the initial portion of the curve accurately. Thus, in studying distribution, values obtained by Nosslin's analysis have been used as being theoretically more accurate—furthermore only patients with accurate urine collections and with no cumulation of free plasma radioiodine have been included (iodide retention due to impaired renal function, or incomplete urine collections would tend to artefactually increase the size of the EV pool). In five patients where whole body radioactivity for ^{131}I was measured with the whole body counter there was close agreement with the values obtained with urine excretion of iodide as described above.

The relationship between the distribution of C3 and C5 with values thus obtained, is shown in Fig. 5. There was always increased EV distribution of C3 compared to controls and in patients with SLE there was a similar increase in EV distribution of C5. However in patients with MCGN there was increased EV distribution of C3 but the EV/IV ratios for C5 all fell within the normal range (al-

though the actual values were all higher than all but one of the normal values) the disparity being particularly marked in patient 11B. The amount of the total C3 and C5 pool (milligram per kilogram body weight) which was intra- and extra-vascular is shown in Figs. 6a and b. It can be seen that the alteration in distribution of C5, and sometimes of C3, occurred despite the total pool being similar to that in normals.

Site of catabolism. The equivalent two pool model used for the integrated rate equations method includes a rate constant for catabolism from the lumped EV pools (23)—an indication of whether
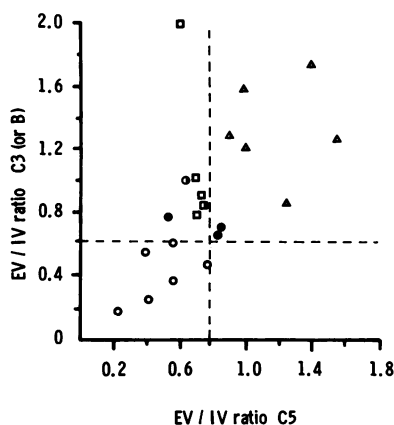

FIGURE 5 Relationship between EV/IV ratio of C5 and C3 (or B). Broken lines indicate upper limit of normal ranges. ○ normal subjects; △ SLE; □ MCGN/PLD; ● other disease; ▲ ◩ ◑ patients with SLE, MCGN, or other disease in whom C5 and B turnover was studied. r = 0.518; P = <0.05 Only values derived by Nosslin's method have been included. Note the complete separation between patients with SLE and other patients.

Metabolism of C5 and C3 711
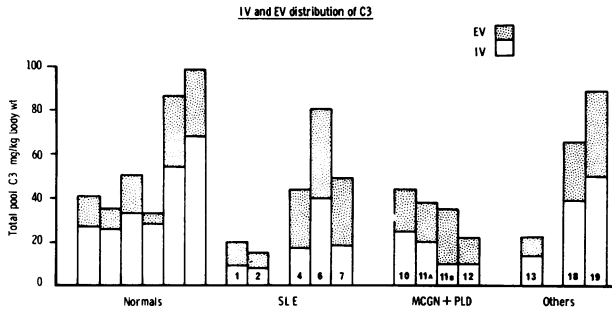
IV and EV distribution of C3

FIGURE 6a IV/EV distribution of total C3 pool milligram/kilogram body weight. Values derived by Nosslin's integrated rate equations method. Each bar represents one patient—the numbers refer to the patients in Table II.

IV and EV distribution of C5

FIGURE 6b IV/EV distribution of total C5 pool milligram/kilogram body weight. Values derived by Nosslin's integrated rate equations method. Each bar represents one patient—the numbers refer to the patients in Table II.

EV catabolism is occurring can thus be obtained and values for the rate constant may be calculated. Most other methods of analyzing protein turnover data assume catabolism is intravascular and do not allow any assessment of EV catabolism. Calculated values for the percentage of total catabolism of C5 and C3 which was intra and extravascular are shown in Fig. 7. These indicate an increase in EV catabolism in a number of patients, especially those with SLE where catabolism appeared to be largely extravascular in some patients. By contrast in two patients with MCGN and PLD with NeF there was no EV catabolism of C5.

DISCUSSION

These studies show that fractional catabolism of C5 is similar to that of C3 in normal subjects but
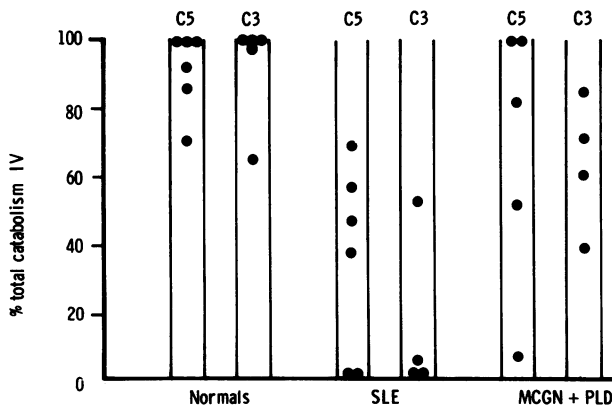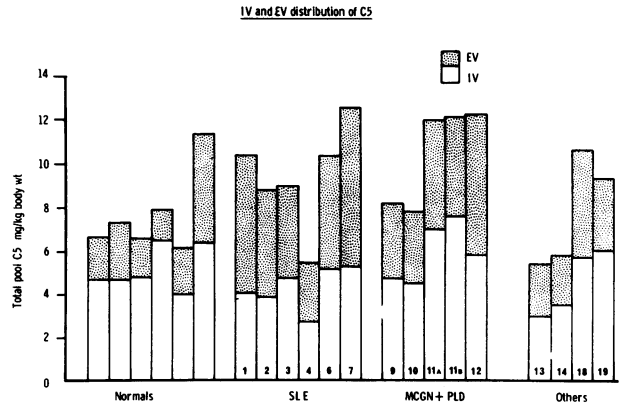

FIGURE 7 Percentage of total catabolism of C5 and C3 which was intravascular. Values derived from Nosslin's integrated rate equations method, which allows differentiation between intra and extravascular catabolism. Catabolism of both proteins was largely extravascular in some patients with SLE. In the MCGN and PLD group the two patients with NeF are those with 100% IV catabolism of C5 but the lowest two values for IV catabolism of C3. Each point represents the value for one patient.

nearly always less than that of C3 in patients with complement activation. They also show that C5 turnover may be normal, or near normal, despite greatly increased C3 turnover, particularly in patients with NeF in their serum. Finally they show that in many patients with complement activation there is increased extravascular distribution of C5, and C3, and strongly suggest that there is also extravascular catabolism of C5 and C3, particularly in patients with SLE.

C5 and C3 catabolism. There has to our knowledge been no previous study of the relationship between C3 and C5 turnover, or any studies of C5 turnover apart from those of Ruddy et al. (26). In a large series of complement protein turnovers the latter authors include C5 turnovers in three normal subjects and five patients. The FCR of C5 in our normal subjects accords with their findings and is similar to that found for C3 (21, 26–29), Factor B (21), and C4 (26) in previous studies by ourselves and others; complement proteins have a rapid turnover relative to other serum proteins, the mechanism of this normal turnover being unknown although there is some evidence against it being due to low level activation of the system (21).

The catabolism of labeled proteins is obviously affected by the presence of denatured and altered protein and the difficulties of assessing the functional activity of trace labeled complement proteins have already been discussed. However, we are confident that the radioiodinated C5 used in these studies contained little or no denatured material in view of the tests already mentioned, the narrow normal range for FCR, expected plasma volumes and capacity of the labeled protein to show accelerated turnover in vivo as shown in Fig. 2.

Synthesis, rather than catabolism, appears to be

the principal determinant of variation in C5 concentration, as has been observed for other complement proteins (21, 26). Although generally of lesser extent than C3 turnover, it is of interest that C5 turnover is nevertheless increased in patients even when its plasma concentration is normal, and sometimes when C3 concentration is normal, as in two of the SLE patients.

Differences between C5 and C3 turnover. The lower fractional catabolism of C5 compared to C3 in patients with complement activation is also of interest. This disparity may be explained on the basis of in vitro observations on C3 and C5 convertase generation. Thus when C3 is cleaved by the classical pathway C3 convertase (C$\overline{4b2a}$) not all the resultant C3b will necessarily bind to form the classical pathway C5 convertase, C$\overline{4b2a3b}$:C3b will bind to surfaces around the site of complement activation, and possibly to receptors on other cells, and some fluid phase C3b may quickly be inactivated by the C3b inactivator. Hence although the steps before C3 conversion are amplification steps the finding that C5 and C3 are not turned over on an equimolecular basis is not unexpected; indeed as the plasma concentration of C5 is so much less than that of C3, turnover on an equimolecular basis would be expected to result in very low C5 concentrations—an unusual finding in patients with complement activation as illustrated by this and other series of patients (17).

In fact there is great difficulty in relating the absolute amounts of C3 and C5 turned over in vivo, since for each protein there will be a normal route of catabolism which is probably distinct from complement activation (the argument that this is the case for C3 has been previously discussed—21); absolute catabolic rates will reflect both normal and abnormal catabolism and cannot readily be compared in states of C3 activation since the absolute amount of C3 turned over may be reduced because of impaired C3 synthesis. However elevation of fractional catabolic rates appears to be a better guide to abnormal catabolism, as is the case generally for plasma proteins. One way of approaching this problem would be to restrict analysis to EV catabolism, which appears only to occur to any extent in disease. Dissociation of EV C3 and C5 catabolism can be detected as shown in our studies, lending support to there being a real difference in metabolic behavior of the two proteins in disease.

The mechanism of generation and composition of the alternative pathway C3 and C5 convertases is the subject of considerable recent and current research, as outlined in the introduction. The principal C3 convertase of the alternative pathway and the C3b feedback cycle is C$\overline{3bBb}$ and, as discussed, additional C3b is required for this enzyme to become a C5 convertase and the two forms of the enzyme differ in their susceptibility to C3bINA. The mechanisms by which the alternative pathway is activated, and the initial C3b generated, are still incompletely understood. At present it appears that native C3, trace amounts of C3b, Factor B, and Factor D interact together to generate a supply of C3b (11, 30); another protein which may be involved in alternative pathway activation is a β-pseudoglobulin present in normal serum, which shows some analogies to NeF and has recently been described by Schreiber and colleagues (31) who have termed it initiating factor.

These experimental observations suggest several factors, in addition to those already mentioned in the context of the classical pathway, which might theoretically favor greater C3 than C5 conversion by the alternative pathway. These are: firstly that fluid phase C$\overline{3bBb}$ is not a C5 convertase (2); secondly that additional C3b is required for C$\overline{3bBb}$ to become a C5 convertase (2, 3)—consumption or reduced synthesis of C3, by whatever mechanism, might thus limit its availability for C5 convertase formation; and thirdly that the additional C3b in the C5 convertase is susceptible to C3bINA, while that in the bimolecular convertase is not (3), which might tend to accelerate decay of the C5 convertase, relative to the C3 convertase, in vivo.

Hence several different situations might theoretically exist in vivo with respect to C3, Factor B, and C5 turnover, and our studies suggest that this is in fact the case. Thus the patients with SLE all showed increased fractional catabolism of C3 and C5, presumably resulting from activation of the classical pathway: however in three patients with evidence of classical pathway activation (low Clq or C4 in patients 14, 15, and 16) C5 turnover was normal despite moderately increased turnover of C3 or B—the reason for this discrepancy is unclear. Some of the patients with MCGN also showed increased C5 as well as increased C3 or B turnover; in one (patient 8) this was associated with evidence of classical pathway activation by immune complexes, and it has previously been shown that some patients with MCGN, particularly the type associated with subendothelial deposits on electron microscopy, have evidence of classical pathway activation (20). The only patients in this series with unequivocal alternative pathway activation are the two with MCGN or PLD, and NeF in their serum, and it is noteworthy that in two studies in these patients C5 turnover was normal despite greatly increased fractional turnover of C3. This suggests that, for some of the reasons mentioned above, little or no C5 convertase is being generated in these cases; in particular NeF would tend to generate C$\overline{3bBb}$ (NeF) in the fluid

phase, and any cell bound C5 convertase formed and stabilized by NeF would still be susceptible to C3bINA, whereas C3 convertase stabilized by NeF would be resistant to the effects of β1H and C3bINA (3, 4). A further theoretical possibility is that the enzyme $\overline{C3bB}$, containing uncleaved B and therefore unlikely to convert C5, is being generated by NeF. Although it seems unlikely that the latter enzyme could exist for long in vivo in the presence of the B cleaving enzyme Factor $\bar{D}$, which was present in normal amounts in these patients' serum, we have previously reported a patient with MCGN and NeF who had normal Factor B turnover, despite increased C3 turnover (21).

These differences in C3 and C5 metabolism in patients with MCGN emphasize that they are a heterogeneous group, and that this histological category embraces nephritis which may be produced by differing pathogenic mechanisms and accompanied by differing types of complement activation (20).

Distribution of C3 and C5. The increased EV distribution of C5 in patients with complement activation is of interest and was associated with increased EV distribution of C3, the latter also having been observed in our previous study (21). We recognize that there are difficulties in deriving reliable estimates of pool size and distribution from protein turnover studies (as discussed above and in reference (23). However the advantages of Nosslin's method of analysis and the exclusion of patients with iodide retention or incomplete urine collections increase the validity of these observations. This increased EV distribution, even when the total pool of C5 was apparently normal, was particularly marked in patients with SLE and was the most consistently abnormal parameter in these patients; there was also evidence of increased EV catabolism of C5 and C3 in this group.

In patients with MCGN there was a lesser increase in EV distribution of C3 (with the exception of one patient) and the values for EV/IV ratios for C5 fell within the normal range. Where EV catabolism could be estimated it was increased in only one case for C3, and two for C5 (Fig. 7); in the two patients with MCGN or PLD and NeF (11A and 12), who had normal FCR of C5, there was no EV catabolism of C5 despite EV catabolism of C3, presumably reflecting the absence of C5 activation. This disparity in EV distribution of C5 between patients with MCGN and those with SLE, even where the FCR of C5 is comparable, may reflect differences in the mode of complement activation in the two groups. The scale of the increase in EV distribution in patients with SLE, and the disparity observed between EV/IV ratios for C3 and C5 in some patients with MCGN and PLD indicate that these altera-

tions in distribution are specific consequences of the underlying immunopathological process in SLE and MCGN, rather than nonspecific changes resulting from a generalized increase in vascular permeability to plasma proteins.

CONCLUSION

These differences in metabolic parameters for C5 between patients with SLE and those with MCGN and NeF presumably reflect the different mechanisms of complement activation in these two groups. In SLE the increased catabolism and EV distribution of C3 and C5 suggest that both proteins are playing a role in inflammatory tissue injury, whereas the failure to activate C5 and normal C5 distribution, in the patient with PLD may possibly explain the lack of overt tissue inflammation in such cases (32).

Combination of such in vivo studies as these, with in vitro work with isolated proteins, should enhance understanding of the relevance of both types of experimental approach to complement metabolism.

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