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Metabolism of human β1H: studies in man and experimental animals

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SUMMARY

Metabolic studies were performed with a purified, functionally-active preparation of human β1H. In seven normal human subjects, the half-life ranged from 66–87 hr with fractional catabolic rates (FCR) of 1·04–1·63%/hr. Synthesis rates were 0·22–0·57 mg/kg/hr and extravascular distribution ratios were 0·34–0·67. There was evidence of extra-vascular catabolism in each subject. In sixteen patients with immunological disease four showed hypercatabolism of β1H. However, three patients with C3 nephritic factor (NeF) had normal β1H turnover despite profound reduction in C3 concentration; it is suggested that the reaction of β1H with the $\overline{C3b.Bb}$ convertase exposes it to a catabolic site and that in the NeF patients the NeF stabilized convertase prevents such exposure. Studies of the acute phase response were carried out in nine patients following elective surgery, with C-reactive protein (CRP) used as the control protein: six patients showed no rise in β1H levels and three showed a small (20%) rise whereas all exhibited a gross rise in CRP.

Pre-incubation of ^{125}I-β1H with NHS, with NHS in the presence of NeF and with C3b+C3b INA caused no change in β1H turnover in animals despite demonstrable total C3 conversion with the NeF.

INTRODUCTION

Activation of the third component of complement (C3), whether by the classical or alternative pathway, yields two biologically active cleavage-products C3a and C3b. C3b then generates further C3 converting activity by complexing with Factor B to form the highly labile enzyme $\overline{C3b.Bb}$, the stability of which is increased by binding with activated properdin ($\overline{P}$) (Fearon & Austen, 1975) or C3 nephritic factor (NeF) (Daha, Fearon & Austen, 1976). The activity of C3b is controlled by several serum proteins and two of these have been extensively studied: firstly, the C3b inactivator (C3b INA), a protease which facilitates cleavage of the C3b molecule (Ruddy & Austen, 1971); and secondly β1H globulin, previously known as C3b inactivator accelerator, which both enhances the action of C3b INA (Whaley & Ruddy, 1976) and binds directly to C3b, thus interfering with the formation of $\overline{C3b.Bb}$ (Conrad, Carlo & Ruddy, 1978).

Detailed investigation of β1H behaviour in human disease has not been performed but knowledge of its properties *in vitro* suggests that such studies may be of clinical importance. In order to examine further the kinetics of β1H during complement activation we performed turnover studies using a radio-labelled, biologically active, purified preparation of this protein. Healthy controls and patients with a variety of immunological diseases were examined. Turnovers were also carried out in rabbits to examine the effects on *in vivo* β1H behaviour of *in vitro* exposure to NHS, NeF and C3b.

The response of β1H to an acute phase stimulus was studied by examining in nine patients the effect of elective surgery on β1H and C-reactive protein concentrations before surgery and in the immediate post-operative period.

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PATIENTS AND METHODS

Subjects. Normal controls: seven healthy members of the medical and technical staff received labelled β1H.

Patients. Seventeen patients were studied. The diagnoses were: systemic lupus erythematosus (SLE): four patients; rheumatoid arthritis (RA): two patients; partial lipodystrophy (PLD) with circulating NeF: three patients; chronic glomerulonephritis (CGN): two patients; polyarteritis nodosa (PAN): one patient; Wegener's granulomatosis (WG): one patient; mixed connective tissue disease (MCTD): one patient; anti-glomerular basement membrane (GBM) disease: one patient. In addition, two patients with stable chronic renal failure were studied: one with amyloid disease and one with diabetic nephropathy.

Acute phase response. In nine patients undergoing elective surgery serum β1H concentrations were measured before operation and on days, 2, 4 and 6 after surgery.

Preparation of β1H, C3, NeF, C3b INA and C3B. β1H was purified from the euglobulin precipitate of hepatitis B antigen-free serum and further purified by DEAE cellulose chromatography (Lachmann & Hobart, 1978). The major peak of β1H was eluted with a linear salt gradient immediately before the major peak of C3. It was further purified by hydroxyl apatite chromatography in 0·015 M KPO4, pH 7·9 with a linear gradient (final concentration of 0·25 M KPO4). The peak of β1H protein eluted after a minor peak of contaminating C3. Immunochemical purity was tested by polyacrylamide gel electrophoresis in buffers containing sodium dodecyl sulphate (PAGE-SDS) and Ouchterlony double diffusion using a monospecific antiserum to β1H and an anti-whole human serum. Antiserum to β1H was provided by Dr R. Harrison. PAGE-SDS of the purified β1H showed a major component of approximately mol. wt 140,000 and traces of residual C3. These findings were supported by Ouchterlony gel analysis.

C3 and C3b INA were prepared from fresh normal human serum as described by Lachmann & Hobart (1978). C3b was prepared from C3 using a cobra venom factor-sepharose convertase. The C3b was then separated from the C3a by hydroxyl apatite chromatography (Harrison & Lachmann 1979). C3 nephritic factor was isolated from a patient with PLD as previously described (Amos *et al.*, 1976).

Radio-iodination. β1H and C3 were labelled by a modification of the chloramine T method (McConahey & Dixon 1966; Charlesworth *et al.*, 1973). Human albumin was added to reduce self-irradiation. Preparations were sterilized by Millipore filtration and pyrogen-tested in rabbits.

Radioautography of β1H in PAGE/SDS showed approximately 95% of radioactivity to reside in a single band corresponding to β1H (Fig. 1). Following reduction with dithiothreitol two bands were observed, one of reduced electrophoretic mobility. Incubation of labelled protein with an optimum amount of monospecific antibody and human serum as a carrier led to precipitation of more than 90% of the radioactivity.

Assessment of biological activity. Functional activity was measured before and after radioiodination: this assay was carried out using a modification of the method described by Whaley & Ruddy (1976). EAC43b cells (1%) were prepared with limiting C3, as described by Lachmann & Hobart (1978). 100 μl cells were incubated with two-fold dilutions of β1H and 18 μg C3b INA in a final volume of 160 μl CFD (complement fixation diluent) at 37°C for 30 min. The cells were centrifuged and the residual C3 sites determined by resuspending the cells in 30 μl CFD containing 1·4 μg Factor B 0·29 μg Factor D and 50 μg NeF (these were purified as described by Amos *et al.*, 1976). After incubation at 37°C for 5 min 100 μl C-EDTA (NHS diluted 1:10 in PBS—0·04 M EDTA) was added to supply C3–C9 and the cells incubated for a further 30 min at 37°C. 0·6 ml cold CFD was then added and after centrifugation the extent of lysis was measured from the optical density of the supernatant at 412 nM the results being expressed as percent inhibition of lysis. The specific activity was determined by the amount of β1H required to inhibit 50% of the C3b sites on the EAC43b cells. β1H functional activity was also measured by its ability to accelerate the decay of the C5 convertase. This was kindly performed by Dr K. Whaley.

Functional activity of C3 was tested in a haemolytic assay as previously described (Charlesworth *et al.*, 1973).

Measurement of complement components and immune complexes. C1q, C4, C3 and β1H were measured by radial immunodiffusion using monospecific antisera (Mancini, Carbonara & Heremans, 1965). Total haemolytic complement activity (CH_{50}) C2 and total alternative pathway function were measured by haemolytic assays described by Lachman & Hobart (1978). C-reactive protein (CRP) was measured by an electroimmunoassay as described by Pepys *et al.* (1978). Rabbit factor B was measured on a haemolytic plate as described by Martin *et al.* (1976.) Immune complexes were detected by a C1q-binding assay (C1qBA) (Pussell *et al.*, 1978). NeF was detected by two-dimensional electrophoresis. (Laurel, 1965; Peters *et al.*, 1972).

Metabolic studies. Human subjects: seven normal volunteers and seventeen patients each received approximately 10 μCi of ^{125}I-β1H. In one patient with PLD a simultaneous turnover with ^{131}I-C3 was performed. Informed consent was obtained from each person before commencing the study.

Thyroidal uptake of iodide was blocked by the oral administration of 180 mg potassium iodide daily for three days prior to the injection of radioactivity and throughout the period of sampling. Each study lasted 5–7 days and plasma and urine samples were processed as described by Charlesworth *et al.* (1974). In patients with proteinuria, urinary protein bound radioactivity was assessed by counting precipitable activity in a 2 ml aliquot of urine using horse serum as carrier. In three patients red cell-bound radioactivity was measured by counting 2 ml aliquots of red cells washed thrice in CFD. Serum concentrations of β1H were determined on at least three occasions during each study to assure a steady state.

Experimental animals: turnovers with human ^{125}I-β1H were performed in NZ white rabbits (2·0–2·5 kg). Two groups of 3 rabbits were used: in group A the ^{125}I-β1H was preincubated for 3 hr at 37°C with buffer; in group B the ^{125}I-β1H was

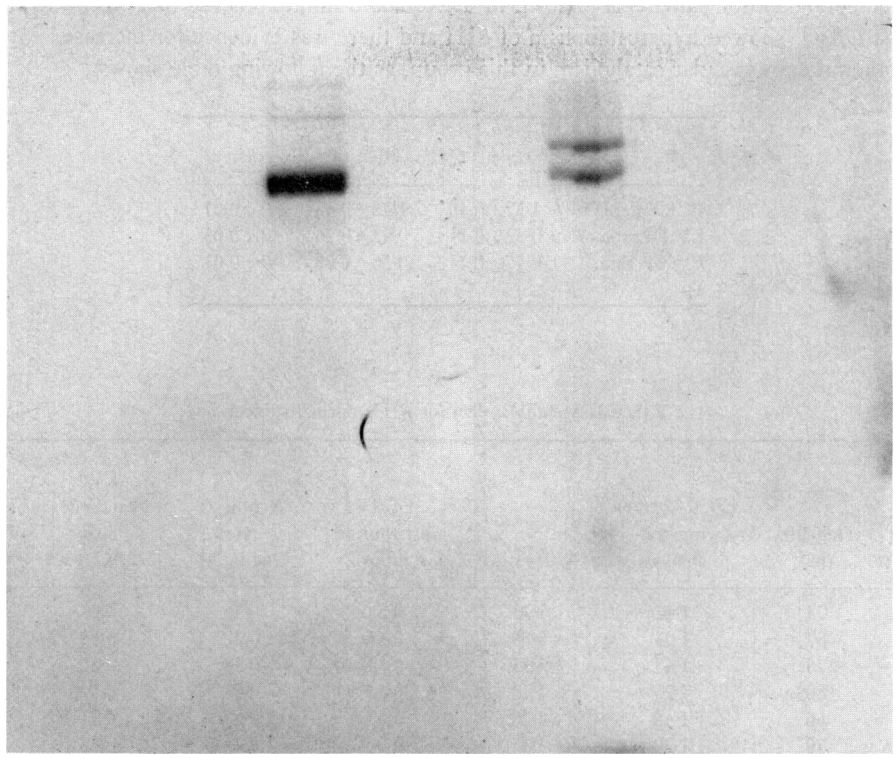

FIG. 1. Autoradiograph of PAGE-SDS of ^{125}I-labelled β1H showing the material before and after reduction with dithiothreitol (right).

preincubated with purified human C3b and C3b INA for 3 hr at 37°C. Serial blood samples and 12 hr catheter urine specimens were obtained throughout the study to give data on half-life ($T\frac{1}{2}$) and fractional catabolic rate (FRC). Serial measurements of serum C3 were made.

To examine the effect of NeF on β1H behaviour a rabbit was given 10 μCi ^{125}I-β1H preincubated with 1 ml of NHS containing 0·25 ml purified NeF and a control animal was given ^{125}I-β1H preincubated with NHS alone. Crossed immunoelectrophoresis showed total C3 conversion in the NHS incubated with NeF.

In all experiments the ^{125}I-β1H was subjected to SDS-PAGE analysis with radioautography.

Methods of turnover analysis. Only patients shown to be in a steady state were included in the study, i.e. those with less than 15% variation in β1H levels during the period of sampling.

Data were analysed by the following methods: (a) urine/plasma ratios (Berson & Yalow, 1957) and (b) integrated rate analysis (Nosslin, 1973); in cases with renal failure or in those where urine data was incomplete, exponential analysis (Matthews, 1957) was used.

Using the integrated rate analysis method values for the rate constant for extravascular catabolism, were calculated (Nosslin, 1973).

In the experimental animals urine and plasma data were analysed by urine/plasma ratios and Matthews exponential analysis.

RESULTS

Human subjects

The metabolic data for normal subjects are summarized in Table 1 and Fig. 2. In two subjects, samples taken more than 200 hr after injection showed the plasma disappearance curves to maintain linearity down to 5% residual protein-bound plasma radioactivity. Integral analysis of plasma and extravascular radioactivity showed a finite value for K4, the rate constant for extravascular catabolism (Nosslin, 1973) in all subjects. The normal range for serum concentration of β1H was 0·62–0·79 mg/ml (this was measured with two separate monospecific antibodies, one donated by Dr R. Harrison).

The metabolic data for patients are shown in Table 2 and immunological data in Table 3. The four patients with SLE showed hypercatabolism of β1H and there was evidence for increased extravascular distribution and extravascular catabolism in this group, as the following table shows.

	Normals (7)	SLE (4)	
FCR (%/hr)	1·32±0·19	1·76±0·43	$P<0·01$
EV/IV ratio	0·52±0·1	0·92±0·26	$P<0·05$
EVCat. ratio	0·77±0·11	0·56±0·08	$P<0·01$

TABLE 1. Metabolic data for β1H: normal subjects

Subject	Half-life (hr)	FCR (%/hr) integral analysis	U/P ratios	EV/IV distribution ratios	Synthesis rate (mg/kg/h)	EV* catabolic ratio K2/K2+K4	Plasma vol. (ml/kg)
GB	75	1·42	1·31	0·53	0·36	†	34
RR	87	1·04	0·89	0·54	0·22	0·58	36
AP	78	1·63	1·35	0·34	0·38	0·86	38
SB	80	1·25	1·13	0·53	0·37	0·72	39
JC	66	1·46	1·28	0·67	0·57	0·71	41
AR	70	1·20	1·15	0·6	0·41	0·88	42
NA	79	1·26	1·30	0·45	0·25	0·84	42
	76±6·9	1·32±0·19		0·52±0·10	0·37±0·11	0·77±0·11	

* Values approaching unity indicate an insignificant value for K4, the rate constant for extravascular catabolism. Values less than one indicate K4 is finite and therefore provide an estimate of extravascular catabolism. Values greater than one are occasionally found and this can only be explained by the presence of denatured material.

† Incomplete urine collection.

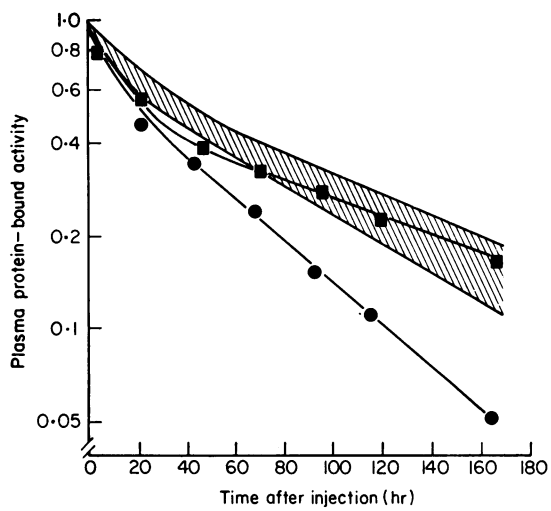

FIG. 2. β1H turnover in human subjects. Plasma disappearance curves are shown: normal subjects (hatched); (■) patient IM with PLD and (●) patient JR with SLE.

TABLE 2. Metabolic data for β1H: patients

Subject	Diagnosis	Half-life	(%/hr)	EV/IV distribution ratio	EV catabolic ratio K2/K2+K4	Synthesis rate (mg/kg/hr)
BB	SLE	76	1·42	0·83	0·50	0·51
CM	SLE	57	1·7	1·03	0·68	0·5
JR	SLE	46	2·38	0·6	0·51	0·82
ME	SLE	55	1·52	1·21	0·58	0·68
HH	RA	54	1·67	0·68	0·63	0·99
MH	RA	63	1·46	0·8	0·98	0·9
IM	PLD (+ve NeF)	97	1·27	0·83	0·73	0·39
MA	PLD (+ve NeF)	76	1·28	0·57	0·74	0·36
CD	PLD (+ve NeF)	80	1·32	0·36	0·74	0·39
SK	MCTD	89	1·23	0·67	0·87	0·41
KE	PAN	76	1·44	0·62	0·68	0·5
JV	WG	58	1·46	n.d.	n.d.	0·51
BW	Anti-GBM disease	65	1·29	n.d.	n.d.	0·66
DC	CGN	65	1·42	n.d.	n.d.	0·56
LW	CGN	71	1·49	n.d.	n.d.	0·67
RJ	Diabetic nephropathy	72	1·43	n.d.	n.d.	0·72
RC	Amyloid	51	1·97	n.d.	n.d.	0·72
Normal range		76±6·9	1·32±0·19	0·52±0·1	0·77±0·11	

n.d. = Not done.

TABLE 3. Immunological data: patients

Subject	Diagnosis	C1q	C2	C4	C3	Alt. path.	β1H	C1q-BA
BB	SLE	50	116	100	44	91	0·67	175
CM	SLE	51	36	40	79	159	0·97	−ve
JR	SLE	45	30	89	66	111	0·78	115
ME	SLE	70	68	217	93	125	1·09	−ve
HH	RA	60	70	58	70	90	1·16	−ve
MH	RA	50	63	100	100	34	1·16	190
IM	PLD	88	80	100	16	30	0·71	−ve
MA	PLD	100	101	140	17	84	0·72	−ve
CD	PLD	140	56	73	8	100	0·70	−ve
SK	MCTD	80	87	100	70	76	0·79	−ve
JV	WG	57	50	113	73	83	0·76	−ve
KE	PAN	100	105	180	160	101	0·76	50
BW	Anti GBM disease	117	65	75	90	98	0·97	−ve
JC	CGN	68	69	124	51	105	0·8	−ve
LW	CGN	95	85	100	75	34	0·78	70
RJ	Diabetic nephropathy	70	85	88	100	110	0·76	−ve
RC	Amyloid	100	198	217	93	98	1·09	−ve
Normal range		60–125*	40–200*	50–120*	60–135*	60–140*	0·62–0·80†	< 40‡

* % pool NHS.
† mg/ml.
‡ μg equiv. agg. IgG.

Calculation of FCR by integral analysis or urine/plasma ratios in fifteen subjects (including six controls) showed a mean difference of 7·7% (range: 0–17·2%) in the value obtained. Fig. 2 illustrates the behaviour of ^{125}I-β1H in two patients (a) PLD with NeF and profoundly reduced C3 and (b) clinically active SLE with low C1q, C2 and C3 and high levels of serum immune complexes on C1q-BA. No patient had reduced concentrations of β1H but high values were found in six cases. No red cell-bound ^{125}I-β1H was detected and urinary excretion of protein-bound radioactivity was negligible in all cases.

In a patient with PLD and NeF in whom β1H and C3 turnover were studied simultaneously normal catabolic value for β1H (1·26%/hr) was found despite gross hypercatabolism of C3 (6·01%/hr).

Rabbits

The half-life of β1H in four normal rabbits was 30–45 hr. The results of the animal experiments are summarized in Table 4. There was no significant difference between the behaviour of β1H preincubated with C3b and C3b INA (Group B) and the controls.

Preincubation of β1H with NHS containing NeF caused no significant change in the half-life (30 *vs* 33 hr in the control animal). On SDS-PAGE analysis there was no alteration of migration of the β1H caused by any of the preincubation procedures.

TABLE 4. Animal subjects

	β1H A			β1H B		
	1	2	3	4	5	6
$T\frac{1}{2}$ hr	35·5	29·6	45·0	32·5	24·0	29·6
FCR %/hr	0·71	1·45	0·39	0·53	2·94	1·79

β1H A – preincubated with buffer 37°C for 3 hr.
β1H B – preincubated with C3b+C3b INA 37°C for 3 hr.

Acute phase studies

Of nine patients studies, all showed an acute phase response as demonstrated by elevation of CRP during the early post-operative period. However in six patients no change in β1H was found and in three of them a small (mean = 20%) but significant increase occurred.

DISCUSSION

We have examined the turnover characteristics of a highly purified functionally active preparation of β1H. In normal subjects β1H proved to be a rapidly catabolized protein, at rates similar to other complement components, with a significant extravascular catabolic rate (see Table 5) similar to that found with Factor B of the alternative pathway.

The findings that in SLE β1H metabolism was accelerated, but was normal in NeF-associated PLD, are of considerable interest, and pose questions about the mechanism of hypercatabolism of β1H in disease. *In vitro* it is well established that β1H is not consumed as a result of its actions of C3b, or on $\overline{\text{C3b . Bb.}}$ In SLE C3 is broken down as a result of the generation of $\overline{\text{C42}}$ (Classical Pathway) and $\overline{\text{C3b . Bb}}$ (feedback activation), whereas NeF acts by binding to and stabilizing $\overline{\text{C3b . Bb}}$, preventing binding and inactivation by β1H and C3b INA. Therefore our findings in patients (and the studies in rabbits which showed that preincubation of β1H with C3, B and NeF similarly did not cause hypercatabolism) suggest that it is the binding of β1H to C3b which increases its catabolism, whereas in the presence of NeF its binding site is unavailable and so only the 'natural' catabolism of β1H is measured. Other factors which may be relevant concern the nature and site of complement activation in disease:

TABLE 5. Catabolism of complement proteins

	F.C.R.	EV/IV distribution ratio	EV catabolic ratio (K2/K2+K4)
B1H	1·13–1·51	0·42–0·62	0·66–0·88
C3*	1·36–1·95	0·19–0·55	0·83–1·67
B*	1·70–2·20	0·81–1·3	0·59–0·86
C5†	1·52–1·96	0·22–0·78	0·89–1·23

* Charlesworth *et al.*
† Sissons *et al.*

whereas in PLD complement activation is predominately fluid-phase, in SLE tissue bound convertases may be generated: in this connection K. Whaley (personal communication), has found β1H bound to kidney biopsies of patients with SLE and Wyatt *et al.* (1978) found β1H by immunofluorescence in the kidney biopsies of patients with membranoproliferative glomerulonephritis and that the distribution of the β1H was the same as C3.

In interpreting results of protein turnover studies it is important that chemical purity and functional biological activity of the protein is maintained. Although following reduction of our β1H preparation with dithiothreitol it produced two bands on SDS-PAGE we have demonstrated that *in vitro* functional activity was retained and its *in vivo* behaviour showed none of the characteristics of a denatured protein. Prior to reduction SDS-PAGE there was no evidence of significant contamination with other protein. The β1H preparation therefore satisfied our strict criteria for suitability for use in metabolic studies.

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