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The kinetics and distribution of C9 and SC5b-9 *in vivo*: effects of complement activation

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SUMMARY

Many diseases associated with complement activation are characterized by tissue deposition of components of the terminal complement complex (TCC). The ninth component of complement (C9) plays an important role in the cytolytic effects, and may contribute to the non-lethal cell-regulating functions of the TCC [1]. In this study we examined the behaviour of radiolabelled human C9 and its soluble complexed form SC5b-9 *in vivo* in order to determine the effects of complement activation on its turnover, distribution and molecular size. In normal rabbits the metabolic parameters of ^{125}I-C9 (median and range) were: plasma half-life ($t_{1/2}$) 25·9 (20·6–29·5) h, fractional catabolic rate (FCR) 5·7 (5·3–7·0)%/h, and extravascular/intravascular ratio (EV/IV) 0·7 (0·6–1·1). The distribution of radiolabelled C9 amongst body tissues was similar to that observed for rabbit serum albumin (RSA). Activation of the complement cascade with i.v. injection of cobra venom factor (CVF) resulted in rapid disappearance of C9 from the plasma and accumulation of protein-bound radiolabel in the spleen (exceeding the plasma concentration) and the liver. RSA metabolism and distribution were unaffected by CVF. Fine performance liquid chromatography (FPLC) gel filtration of plasma samples suggested that monomeric C9 was the only major radiolabelled protein present during normal turnovers, whereas CVF administration was accompanied by the prompt appearance of a high mol. wt species consistent in size with SC5b-9. When injected directly, ^{125}I-SC5b-9 disappeared rapidly from the plasma, falling by 50% in 0·7 (0·6–0·8) h, and less than 15% remaining after 4 h with accumulation of protein-bound label in the spleen and liver. These results demonstrate the complexity of C9 metabolism during complement activation.

Keywords C9 terminal complement complex metabolic turnover tissue distribution SC5b-9

INTRODUCTION

The ninth component of complement (C9) is a 73-kD glycoprotein which, upon activation of the complement system, may become polymerized and incorporated into the terminal complement complex (TCC) [2–4]. Complement activation occurring on or adjacent to a cell surface results in generation of the pore-forming membrane attack complex (MAC or C5b-9(m)) [4,5]. Fluid-phase complement activity typically produces a soluble TCC (SC5b-9) which, by the inclusion of vitronectin (Vn) and clusterin, is rendered unable to disrupt cell membranes [6,7].

Several studies have documented the presence of C9 neoantigens (expressed on polymerized C9 and TCC) in the tissues of patients with a variety of inflammatory diseases, including IgA nephropathy, membranous nephropathy and systemic lupus erythematosus (SLE). Vn and clusterin are often found in a similar distribution [8–10]. A pathogenic role for TCC components has been established in the Heymann model of membranous nephropathy [11,12] and may similarly contribute to tissue damage or dysfunction in many diseases associated with complement activation.

The assembly of C9 molecules within the TCC has been studied extensively from a biochemical and morphological perspective. However, little is known of its kinetics *in vivo*. Specifically, changes in polymerization and regional distribution in response to complement activation have not been examined. These parameters may be important determinants of phlogistic complement reactivity in disorders where maintenance of C5b-9 solubilization is required to prevent tissue damage. In the current study, we have investigated the metabolism of haemolytically active human C9 and SC5b-9 (produced from rabbit serum supplemented with human C9) in rabbits. The specific aims were to examine: (i) the influence of complement activation with cobra venom factor (CVF) on C9 turnover and distribution; and (ii) the kinetics and distribution of the soluble complex SC5b-9.

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MATERIALS AND METHODS

Purification of human C9

C9 was purified from normal human plasma by a modification to the method described by Biesecker & Muller-Eberhard [13]. Briefly, 100 ml plasma containing di-sodiumethylenediaminetetraacetic acid (Na_2EDTA, 4·4 mM), benzamidine (25 mM) and PMSF (0·6 mM), were fractionated in 7–21% polyethylene glycol 4000. The final precipitate was redissolved in K_2HPO_4 buffer (100 mM) containing Na_2EDTA (5 mM), NaCl (150 mM), benzamidine (25 mM) and chloramphenicol (25 mg/*l*), pH 7·0, and passed down a lysine Sepharose column (AMRAD Pharmacia, Melbourne, Australia). Unbound protein was loaded onto a DEAE-A50 Sephadex column (AMRAD Pharmacia) and eluted with a NaCl gradient from 100 mM to 400 mM, pH 7·0. The C9-containing fractions were identified by radial immunodiffusion, dialysed against K_2HPO_4 buffer (25 mM) containing KCl (100 mM), pH 7·5, and loaded onto a hydroxylapatite column (BioRad, Sydney, Australia). Following elution with a phosphate gradient from 80 mM to 400 mM, the C9-containing fractions were concentrated using a Centricon microconcentrator (Amicon, Beverly, MA) and stored frozen in aliquots at −70°C.

Radiolabelling

C9 was labelled with either ^{125}I or ^{131}I immediately before use by the lactoperoxidase technique [14] to a specific activity of approx. 5000 ct/min per ng. Rabbit serum albumin (RSA; Sigma, Sydney, Australia) was labelled similarly with ^{131}I.

C9-dependent haemolytic assay

Haemolysin-sensitized sheep erythrocytes (EA) were incubated with C9-deficient human serum (Sigma) for 15 min at 37°C to produce EA_{C1-8}. After washing in PBS (Oxoid, Melbourne, Australia), aliquots of EA_{C1-8} cells were incubated for 30 min at 37°C with various dilutions of either pooled normal human serum (NHS), purified C9 or radiolabelled C9. The C9 content of each solution, as a proportion of NHS, was established by radial immunodiffusion. The haemolytic activity was calculated from the absorbance of the supernatant at 415 nm and expressed as per cent of maximal haemolysis with respect to NHS (after adjustment for C9 concentration). Background haemolysis (approx. 15% of maximal) was subtracted from the assay results.

Production of radiolabelled SC5b-9

Fifty microlitres of purified ^{125}I-human C9 (1 μg) were incubated with 50 μl of either fresh human serum or rabbit serum, 100 μl of complement fixation diluent (Oxoid) and 8 μl (2 μg) of purified CVF (or PBS as a control) for 3 h at 37°C. The reaction mixture was analysed on a fine performance liquid chromatography (FPLC) gel filtration column (Superose-12; AMRAD Pharmacia), to separate the high molecular weight, SC5b-9-containing fractions (approx. 1000 kD) from monomeric C9 and free iodide. Fresh rabbit serum supplemented with ^{125}I-C9 was used to produce SC5b-9 for metabolic study, while activated human serum containing ^{125}I-C9 provided SC5b-9 for radioimmunoassay. Radiolabelled proteins were stored at 4°C for less than 18 h before use.

Radioimmunoassay for SC5b-9 components

Polystyrene tubes (Nunc, Roskilde, Denmark) were coated with either (i) polyclonal anti-human C5 globulin (Dako, Glostrup, Denmark); (ii) monoclonal antibody against human Vn (HV2 [15]); or (iii) polyclonal goat anti-mouse IgG-Fc (Jackson Immunosearch) by incubation with the antibody diluted to 25 μg/ml in 200 mM carbonate/bicarbonate buffer pH 9·6 for 2 h at room temperature. Tubes were pre-incubated with PBS containing 0·05% Tween-20 (BioRad), 1% bovine serum albumin (BSA; Sigma) and 5% Lactose (Sigma) to block non-specific binding. The tubes coated with goat anti-mouse IgG-Fc were subsequently incubated with mouse MoAb against human C9 neoantigen (aE11; Sigma) at 5 μg/ml for a further 30 min at room temperature. Tubes treated only with the blocking solution were used as controls. Monomeric ^{125}I-C9 and the high molecular weight fraction of activated ^{125}I-C9-supplemented human serum were incubated in the coated tubes for 3 h at room temperature with gentle shaking. The tubes were then washed in PBS/0·05% Tween 20 (× 4) and the bound radioactivity counted and expressed as a percent of total counts added.

Studies in experimental animals

Adult, male New Zealand white rabbits were used in all studies. For 3 days prior to, and continuing throughout the experimental period, rabbits were given oral potassium iodide (100 mg/*l* of drinking water) to block thyroid iodide uptake. Protein solutions were passed through a 0·2-μm filter (Gelman, Sydney, Australia) before administration. The animals received an injection of radiolabelled protein via a marginal ear vein and blood samples were drawn from a marginal vein on the contralateral ear at 10 min, 3, 6 and 12 h, then two to three times daily until $< 8\%$ of the initial protein-bound radioactivity remained in the plasma. Blood sampling was more frequent following injection of ^{125}I-SC5b-9. Urine was collected throughout the study period and samples were tested for protein-bound radioactivity by trichloroacetic acid (TCA) precipitation using BSA as a carrier. At completion of the study the animals received a lethal i.v. dose of pentobarbitone. The experimental protocols are: (i) five normal rabbits received 5–10 μCi of ^{125}I-C9; (ii) three rabbits received 5–10 μCi ^{125}I-C9 followed by 50 μg/kg of purified CVF at 10 min ($n = 2$) and 24 h ($n = 1$). Control animals received an equal volume of isotonic saline (approx. 1·5 ml) instead of CVF; (iii) two rabbits received approx. 10 μCi ^{125}I-C9 concurrently with approx. 5 μCi ^{131}I-RSA followed by 200 μg/kg CVF at 10 min. Control rabbits received isotonic saline instead of CVF. Animals were killed at 6 h and samples of their plasma, liver, spleen, kidneys, lungs, heart and skeletal muscle obtained for counting of radioactivity; (iv) three rabbits received 0·5–1 μCi ^{125}I-SC5b-9, and two of these animals were given a concurrent dose of 0·5–1 μCi ^{131}I-C9 which had been pre-incubated in a similar fashion to the SC5b-9 except for the omission of CVF. The same two animals were killed at 4 h and their tissues obtained as above.

Collection and analysis of samples

Plasma samples. Blood samples were collected into plastic tubes containing EDTA and promptly separated by centrifugation. The following plasma aliquots were analysed: (i) 200 μl for FPLC gel filtration (Superose 12; AMRAD Pharmacia); (ii) 0·5 ml for counting total radioactivity; (iii) a further 0·5 ml for precipitation with 1·5 ml of 24% TCA; this was then centrifuged at 1400 *g* for 20 min, the supernatant filtered through

nylon wool and counted to assess free iodide; and (iv) 10 μl for the measurement of C3 concentration by radial immunodiffusion using a polyclonal anti-rabbit C3 antibody (Organon Teknika, Turnhout, Belgium).

Blood cells. Following separation of plasma, the packed blood cells were washed seven times in PBS and 0·5 ml taken from the centre of the pellet for counting. Cells were then lysed and precipitated in 24% TCA to assess protein-bound radioactivity.

Tissues. Immediately following a lethal dose of pentobarbitone, liver, spleen, kidneys, lungs, heart and samples of skeletal muscle were removed, washed in saline, cleared of surrounding fat, and a portion of each tissue weighed and placed into polystyrene tubes for counting of total radioactivity. A further sample of tissue was homogenized in PBS containing 25 mM benzamidine, 0·5 mM PMSF, and 4·4 mM Na_2EDTA. A 0·5-ml aliquot of organ homogenate was added to 1·5 ml of 24% TCA, centrifuged at 1400 *g* for 20 min, then filtered through packed nylon wool and the supernatant (containing free iodide) counted.

Statistical analysis

Analysis of metabolic data has been previously described [16]. Briefly, plasma protein-bound radioactivity was plotted against time, assuming that the radioactivity at 10 min represented 97% of the injected dose (i.e. at time 0 or t_0). Fractional catabolic rates (FCR) were determined both by the metabolic clearance method of Berson & Yalow (i.e. urine/plasma ratios) [17] and also by analysis of the plasma disappearance curves [18], while the half-life ($t_{1/2}$) was derived from the final exponential of the plasma curve. Extravascular/intravascular (EV/IV) protein distribution was calculated by the method described by Mathews [18]. Plasma volume was determined by dilution of radiolabelled C9. FCR, $t_{1/2}$, EV/IV and plasma volumes are expressed in the text as median and range.

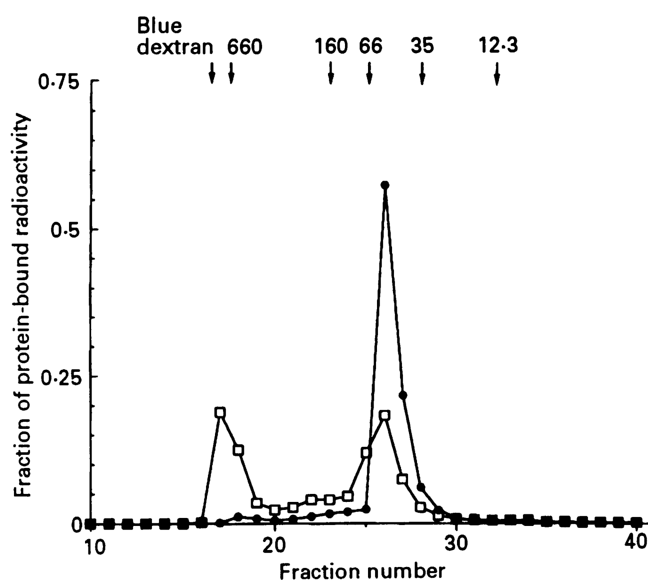

Fig. 1. FPLC gel filtration of normal rabbit serum incubated with ^{125}I-human C9 for 3 h at 37°C in the presence (□) and absence (●) of cobra venom factor (CVF). Solid arrows indicate molecular weight markers.

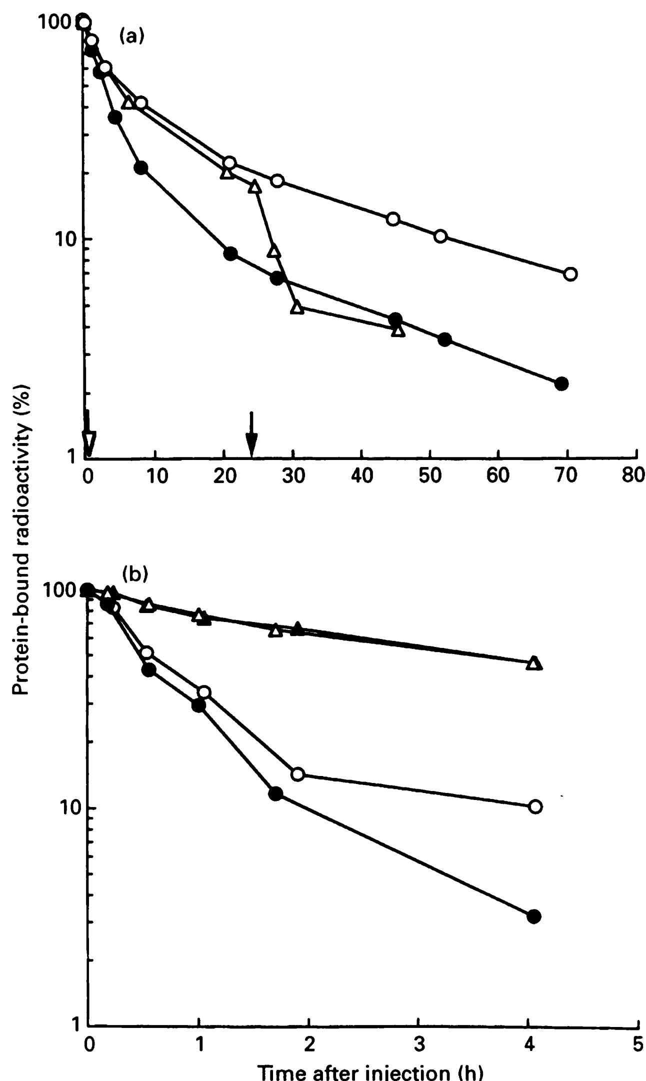

Fig. 2. Plasma radioactivity disappearance curves for radio-labelled C9 and SC5b-9. (a) ^{125}I-C9 in a normal rabbit (○), ^{125}I-C9 followed by cobra venom factor (CVF) (open arrow) at 10 min (●), and ^{125}I-C9 followed by CVF (solid arrow) at 24 h (△). (b) ^{131}I-C9 and ^{125}I-SC5b-9 administered concurrently to two rabbits: rabbit 1, ^{131}I-C9 (▲) and ^{125}I-SC5b-9 (●); rabbit 2, ^{131}I-C9 (△) and ^{125}I-SC5b-9 (○).

RESULTS

C9 purity and functional activity

Purified C9 migrated as a single band of approx. 73 kD on coomassie blue-stained, reduced SDS–PAGE (10%) and, after iodination, produced a single band at approx. 73 kD on autoradiography. Analysis of ^{125}I-C9 by FPLC gel filtration showed in excess of 90% of radioactivity to reside in a single peak corresponding to monomolecular C9, with small amounts of radioactivity in two other regions (mol. wt 200–300 kD and approx. 1000 kD). C9 maintained 100% haemolytic activity, with respect to NHS, for up to 2 months when stored in frozen aliquots at −70°C. Lactoperoxidase radiolabelling did not alter the haemolytic activity.

Activation of serum supplemented with ^{125}I-C9

Following a 3-h incubation at 37°C with NHS and CVF, 90–

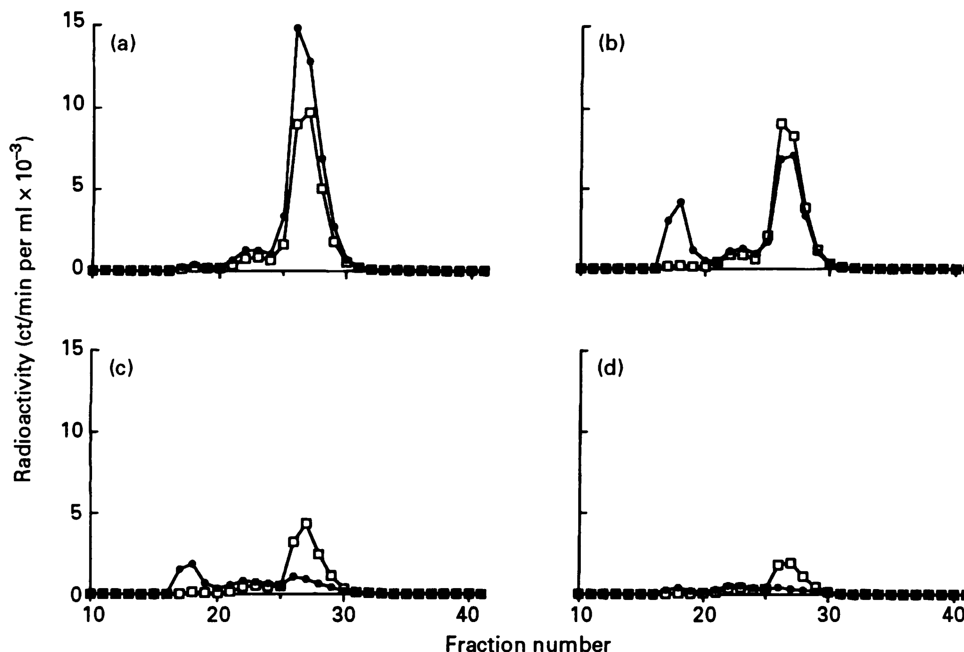

Fig. 3. FPLC gel filtration of plasma samples from two rabbits given ^{125}I-C9 followed at 10 min by cobra venom factor (CVF) (●) or equivalent volume of isotonic saline (□). Samples were taken just before injection of CVF or saline (a), 1 h (b), 8 h (c), and 21 h (d) after injection.

95% of labelled C9 became incorporated into a high mol. wt fraction (approx. 1000 kD). CVF-activated rabbit serum, incubated under similar conditions, incorporated about 30% of labelled human C9 into this peak (Fig. 1). When CVF was omitted, approximately 10% of labelled C9 was converted to the high mol. wt fraction in human serum and less than 5% in rabbit serum.

Radioimmunoassay of C9-containing fractions

The labelled high mol. wt fraction of CVF-activated human serum bound to solid-phase antibodies against human Vn, human C5 and C9 neoantigen (10–50% of counts retained), while purified monomeric ^{125}I-C9 showed little affinity for any of these antibodies (< 0·5% of counts retained). Neither protein fraction showed significant reactivity with the blocking solution alone.

Metabolic studies in normal rabbits

In five normal rabbits the $t_{1/2}$ of human C9 was 25·9 (20·6–29·5) h (Fig. 2). Fractional catabolic rate (FCR) was 5·7 (5·3–7·0)% of the plasma pool per hour derived by exponential analysis and 5·4 (4·6–5·8)%/h ($n = 4$) calculated from urine/plasma ratios. The EV/IV distribution ratio was 0·7 (0·6–1·1). Free iodide represented less than 5% of total plasma radioactivity throughout the turnovers. Plasma volume was 37 (30–47) ml/kg ($n = 13$).

FPLC gel filtration analysis of plasma samples collected during C9 turnovers in normal rabbits showed the major portion of the plasma radioactivity to remain in the monomeric C9 region throughout the study. In particular, no small or high mol. wt molecules appeared (Fig. 3)

Washed, packed blood cells retained no significant radioactivity (i.e. < 0·01% of plasma counts).

Complement activation with CVF in vivo

After the administration of CVF there was a marked increase in the rate of disappearance of plasma protein-bound radioactivity, which was most evident 2–12 h following injection (Fig. 2). During this period plasma C3 concentration fell to < 15% of the initial level, in contrast to normal turnovers in which it was unchanged.

FPLC gel filtration of plasma demonstrated the early appearance of a high mol. wt (approx. 1000 kD) C9-containing complex associated with a reduction in the proportion of total protein-bound radioactivity present as monomeric C9 (Fig. 3). As with the normal turnover, there was no evidence of fragmented ^{125}I-C9 in the plasma. An estimate of the absolute contribution of each protein moiety separated by gel filtration to the total plasma protein-bound radioactivity was made by calculating the area under each peak, expressing this as a proportion of the total area under the profile, and multiplying by the measured protein-bound radioactivity. Figure 4 shows the calculated plasma concentrations of monomeric C9 and the high mol. wt (SC5b-9-containing) fraction in a rabbit given CVF at 10 min following ^{125}I-C9. This demonstrates an absolute rise in the quantity of high mol. wt radioactivity and rapid fall in monomeric C9 apparent from 1 h post-CVF. Plasma-free iodide concentration rose significantly in the first 12 h following CVF, approaching 20% of total counts.

Packed blood cells showed a small but consistent increase in radioactivity (i.e. approaching 10% of plasma protein-bound radioactivity), maximal at approx. 8 h following injection of CVF (Fig. 4), and of this radioactivity 98% was precipitable with TCA.

SC5b-9 turnover

^{125}I-SC5b-9 was cleared rapidly from the plasma, falling by

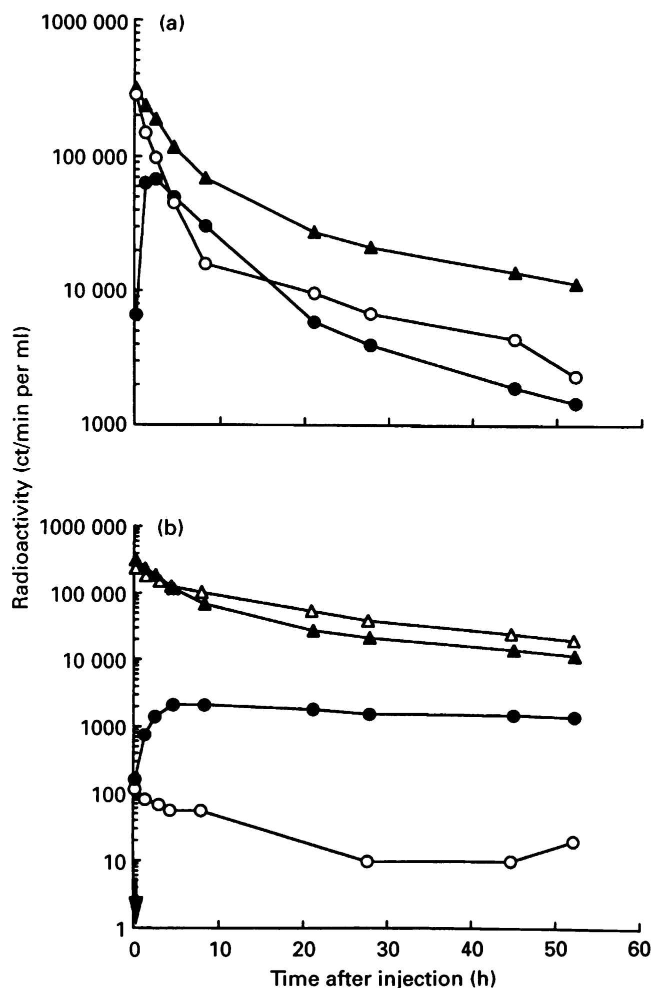

Fig. 4. (a) Plasma radioactivity disappearance curves for a rabbit given ^{125}I-C9 followed by cobra venom factor (CVF) at 10 min. Total protein-bound radioactivity (▲), C9 monomer (○), and SC5b-9-containing fraction (●). (b) Protein-bound radioactivity associated with packed blood cells and plasma for two rabbits given ^{125}I-C9 followed at 10 min (arrow) either by CVF (blood cells (●), plasma (▲)), or isotonic saline (blood cells (○), plasma (△)).

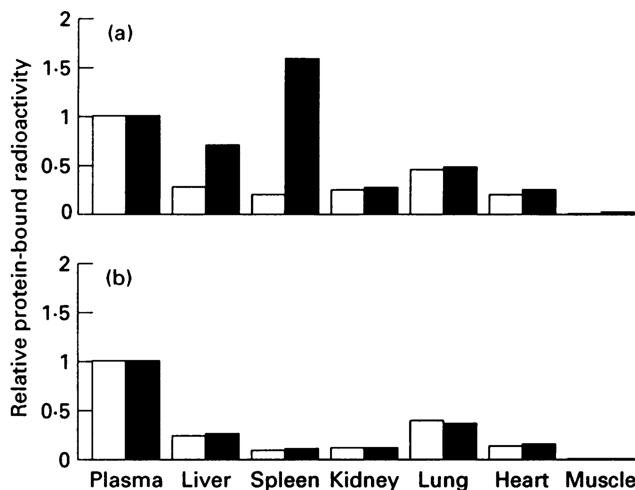

Fig. 5. Tissue protein-bound ^{125}I (a) and ^{131}I (b), expressed as (ct/min per g of fresh tissue)/(ct/min per ml plasma), in two rabbits given ^{125}I-C9 and ^{131}I-rabbit serum albumin (RSA) and killed at 6 h. One rabbit received cobra venom factor (CVF) at 10 min (■) while the other was given an equivalent volume of isotonic saline (□).

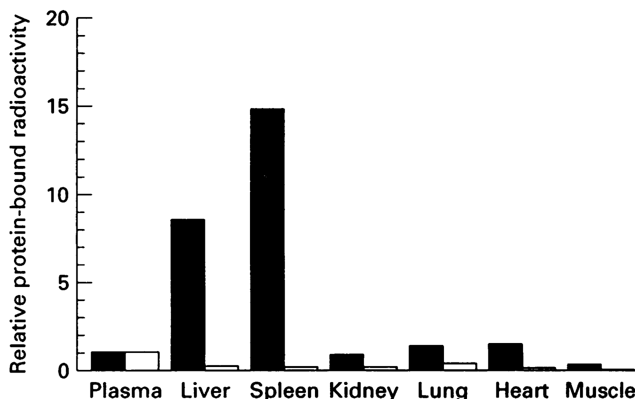

Fig. 6. Tissue protein-bound ^{125}I (■) and ^{131}I (□), expressed as (ct/min per g fresh tissue)/(ct/min per ml plasma), in a rabbit given ^{125}I-SC5b-9 and ^{131}I-C9 and killed at 4 h.

50% in 0·7 (0·6–0·8) h (n = 3), with < 15% of the injected dose remaining after 4 h (Fig. 2). The rate of disappearance of ^{131}I-C9 (pre-incubated at 37°C for 3 h without CVF) given to two of these rabbits was similar to non-incubated C9. Packed blood cell-associated radioactivity was not detected.

Tissue distribution

In normal rabbits given ^{125}I-C9 and ^{131}I-RSA (as a marker of the plasma space), protein-bound ^{125}I concentration (ct/min per g) in the organs tested (i.e. liver, spleen, kidneys, lungs, heart, and skeletal muscle) varied between 5% and 50% of the plasma concentration (ct/min per ml) at 6 h following injection (Fig. 5). ^{131}I-RSA distribution amongst organs was similar in magnitude and profile to C9. Two rabbits given CVF following ^{125}I-C9 and ^{131}I-RSA showed accumulation of protein-bound ^{125}I in the spleen (up to 1·6 × plasma concentration) and the liver, with the other organs remaining similar to or slightly higher than the control rabbits. CVF had no significant effect on either the rate of disappearance of ^{131}I-RSA from the plasma or uptake by the organs (Fig. 5).

Two rabbits injected with ^{125}I-SC5b-9 and ^{131}I-C9 and killed at 4 h showed marked uptake of protein-bound ^{125}I by the spleen and liver (up to 15 and nine times, respectively, the concentration found in the plasma). The other organs had protein-bound ^{125}I concentrations similar to, or slightly greater than plasma (Fig. 6). The concentration of protein-bound ^{131}I was < 50% of the plasma level for all organs tested. For all studies, < 10% of total urinary radioactivity was precipitable with 24% TCA.

DISCUSSION

The current study has investigated the metabolism of C9 and SC5b-9 in rabbits. Purified ^{125}I-C9 retained full haemolytic activity, and FPLC gel filtration revealed that > 90% of the

protein was present as C9 monomer. Small amounts of radioactivity in the higher mol. wt regions may have been due to spontaneous aggregation of C9 or minor impurities. Activation of both rabbit and human sera in the presence of ^{125}I-human C9 by CVF *in vitro* showed that the C9 was readily incorporated into a high mol. wt species of approx. 1000 kD (consistent with the published mol. wt of SC5b-9 [4]), and radioimmunoassay of this fraction of human serum demonstrated components of SC5b-9. Studies by Bhakdi and Tranum-Jensen have previously demonstrated similarity between the subunit composition of rabbit and human SC5b-9 [19]. Although in our study the reaction of human C9 with rabbit serum was slower than with human serum, these results further indicate that the purified ^{125}I-human C9 was functional and compatible with both the human and rabbit complement cascades.

The disappearance of ^{125}I-C9 from the plasma in control rabbits occurred in two phases. There was an initial rapid fall, without significant release of free iodide, which was consistent with redistribution rather than hypercatabolism or protein denaturation. Subsequently, C9 was catabolized with a final $t_{1/2}$ of approx. 26 h. In comparison with results obtained previously for other human complement proteins in rabbits such as C3 ($t_{1/2}$ 35–40 h [16]) and factor H ($t_{1/2}$ 30–45 h [20]), the plasma disappearance of C9 was more rapid. The EV/IV ratio of approx. 0·7 indicated a significant extravascular pool, and sequestration of C9 monomer onto circulating or fixed cells may occur in addition to true extravasation. However, we were unable to identify specific sites of C9 uptake in the absence of complement activation. FPLC gel filtration of plasma showed the C9 monomer to be the only major radioprotein present throughout the turnover period. C9 metabolism was not associated with the generation of low mol. wt, slowly eliminated breakdown products.

CVF administration accelerated C9 disappearance from the plasma, and increased release and excretion of free iodide. This was associated with the generation of a high mol. wt species consistent with SC5b-9. Estimation of the absolute contributions of ^{125}I-SC5b-9 and monomeric ^{125}I-C9 to total protein-bound plasma radioactivity showed that the ^{125}I-SC5b-9 concentration reached a peak at 2–3 h following CVF, i.e. at a time when the rate of disappearance of C9 monomer was maximal.

Washed blood cells from rabbits treated with CVF consistently bound a small but significant amount of TCA-precipitable ^{125}I, not seen in control rabbits. Although blood cell-associated radioactivity amounted to < 10% of plasma counts, it accompanied the relatively rapid fall in total plasma protein-bound radioactivity induced by CVF. Formation of MAC on cell surfaces rather than uptake of SC5b-9 is a likely explanation for this observation, as it was not seen in animals injected directly with ^{125}I-SC5b-9. While the vast majority of circulating cells counted were erythrocytes, we did not address the issue of TCC deposition on specific types of cells.

Rabbits treated with CVF following injections of ^{125}I-C9 and ^{131}I-RSA showed accumulation of protein-bound ^{125}I in the spleen and the liver, in comparison with control animals. Uptake of ^{125}I-C9 by the kidneys, lungs, heart and skeletal muscle was not altered by complement activation, and in general reflected the distribution of ^{131}I-RSA. As no effect of CVF on the EV/IV ratio of ^{131}I-RSA was observed, these results probably represent specific organ uptake of SC5b-9. Similarly, the spleen and liver were sites of sequestration of protein-bound ^{125}I in rabbits injected with ^{125}I-SC5b-9 directly, while levels in the kidneys, lungs, heart and skeletal muscle were similar to plasma. ^{125}I-SC5b-9 was eliminated rapidly from the plasma (time to 50% 0·6–0·8 h) with < 15% of the injected dose remaining after 4 h. This result is consistent with previous measurements of SC5b-9 clearance in man and rabbits [21–23]. We were unable, however, to calculate the true plasma $t_{1/2}$, FCR and EV/IV distribution for SC5b-9 due to its rapid clearance and lack of equilibration across body compartments. Rabbits given ^{125}I-SC5b-9 were also injected with ^{131}I-C9 which had been pre-incubated in fresh rabbit serum for 3 h at 37°C as a control for the production of SC5b-9. The rate of disappearance of ^{131}I-C9 from the plasma in these animals over the 4 h of study was similar to non-incubated C9, indicating that our method of SC5b-9 generation *in vitro* was unlikely to have induced denaturation.

The level of complement activation in disease states is generally much lower than that achieved in this study. Rapid activation by CVF allowed the generation of sufficient quantities of the short-lived complex SC5b-9 for its behaviour, and that of C9 during complement activation, to be examined reliably *in vivo*. It remains unclear whether SC5b-9, formed in the circulation, contributes to tissue deposits of Vn and C9 neoantigen. Vn, through its cell binding capacity and affinity for a variety of interstitial matrix molecules (e.g. sulphated polysaccharides) may guide SC5b-9 to areas of pre-existing tissue damage or sites of elimination [4,24]. Disturbed metabolism of the fluid-phase TCC and its components may lead to inappropriate tissue distribution. The potential for ongoing tissue damage requires further study.

These results suggest that several mechanisms may be involved in C9 metabolism. Complement activation by CVF leads to a change in the ratio of monomeric C9: SC5b-9 in the plasma. These changes occur without free iodide release, and presumably reflect incorporation of C9 monomer into the TCC. Once formed, the SC5b-9 is rapidly catabolized with concurrent free iodide release. We did not specifically investigate the fate of SC5b-9 sequestered by organs, but it remains probable that the spleen and liver, or the reticuloendothelial elements therein, are sites of its metabolism.

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