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The preparation and characterization of monoclonal antibodies to human complement component C8 and their use in purification of C8 and C8 subunits

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1. Ten mouse monoclonal antibodies to human complement component C8 were prepared. It was found that six of these antibodies reacted with the α-subunit, two with the β-subunit and two with the γ-subunit, when assessed by immunoblotting after separation of C8 subunits by SDS/polyacrylamide-gel electrophoresis. 2. Epitope analysis of the ten monoclonal antibodies in a competitive binding assay showed that the six antibodies to the α-subunit could be classified in four overlapping epitope groups. The antibodies to the β- and γ-subunits bound to a single antigenic site on each, but also cross-reacted with the antigenic sites on the α-subunit. 3. Monoclonal anti-C8 immunoaffinity columns were used to purify C8 from fresh human plasma and to prepare C8-depleted serum. Immunoaffinity purified C8 was biologically active when assessed by using haemolysis assays of sheep and rabbit erythrocytes. 4. Salt elution was used to purify either αγ- or β-subunits when C8 was respectively bound to an anti-β or anti-α immunoaffinity column. The purified subunits reconstituted C8-depleted serum when added together in a haemolysis assay.

INTRODUCTION

The formation of the complement membrane-attack complex (MAC, consisting of five proteins: C5b, C6, C7, C8, C9) and insertion of the terminal complement component C9 into appropriate target-cell membranes provides the principal mechanism of complement-mediated cell killing (Bhakdi & Tranum-Jensen, 1984; Podack & Tschopp, 1984; Podack, 1986), plays a role in defence against bacterial infection (Joiner *et al.*, 1984; Taylor & Kroll, 1985; Dankert & Esser, 1986, 1987) and may contribute to sub-lytic damage of mammalian cells in autoimmune disease (Campbell & Luzio, 1981; Morgan *et al.*, 1986). Membrane insertion of C9 requires that the target membranes contain membrane-bound complement components C5b–8 acting as a receptor for C9 and catalysing its polymerization (Tschopp *et al.*, 1982, 1985), though polymerization may not be necessary for C9 function (Dankert & Esser, 1985). C8, which is a glycoprotein present in human serum at 50–80 μg/ml (Mueller-Eberhard, 1975; Podack, 1986; Reid, 1986), is essential for membrane insertion and polymerization of C9 (Monahan *et al.*, 1983). It has an apparent M_r of 151000 and is composed of three non-identical polypeptide chains: α (M_r 64000), β (M_r 64000) and γ (M_r 22000). These exist as a covalently linked αγ dimer non-covalently associated with the β-subunit (Steckel *et al.*, 1980).

The molecular biology of C9 and its mechanism of insertion into the target membrane is now well understood (DiScipio *et al.*, 1984; DiScipio & Hugli, 1985; Stanley *et al.*, 1985, 1986; Shiver *et al.*, 1986; Stanley & Herz, 1987). Monoclonal antibodies to C9 (Morgan *et al.*, 1983) have been shown to be important reagents for its investigation, providing information on the mechanism of insertion (Morgan *et al.*, 1984*a*; Stanley *et al.*, 1986), surface features (Stanley *et al.*, 1985; Stanley & Herz, 1987), function (Morgan *et al.*, 1986; Stanley *et al.*, 1986), subcellular localization (Morgan *et al.*, 1984*b*) and fate (Campbell & Morgan, 1985; Morgan *et al.*, 1987) of this molecule. In the present study anti-(human C8) monoclonal antibodies were prepared and characterized to use for investigating the role of C8 within the MAC.

EXPERIMENTAL

Materials

Human C8 used for primary immunization in the preparation of monoclonal antibodies was prepared by the method of Steckel *et al.* (1980). This yielded less than 1 mg of C8 from 1 litre of out-dated plasma from the Blood Transfusion Service, Cambridge, U.K. C8 used for booster injections and screening assays was a gift from Dr. A. Esser (Department of Comparative and Experimental Pathology, University of Florida, Gainesville, FL, U.S.A.).

Veronal buffer contained 50 mM-sodium barbitone, 86 mM-NaCl, 0.1% NaN_3 and 0.5% bovine serum albumin, adjusted to pH 8.0 with HCl. Borate buffer contained 200 mM-boric acid and 200 mM-KCl adjusted to pH 8.2 with NaOH. PBS contained 0.15 M-NaCl, 2 mM-NaH_2PO_4 and 16 mM-Na_2HPO_4, pH 7.4. VBS contained 0.145 M-NaCl, 3 mM-barbitone, 1 mM-sodium barbitone, 0.8 mM-$MgCl_2$ and 0.2 mM-$CaCl_2$, adjusted to

Abbreviations used: C8 etc., complement component C8 etc.; MAC, membrane-attack complex; PBS, VBS and TBS, phosphate-, veronal- and Tris-buffered saline respectively (full compositions and pH values are given in the text); NHS, normal human serum; E, fresh washed rabbit erythrocytes.

pH 7.2 with HCl. TBS contained 0.15 M-NaCl and 0.025 M-Tris, adjusted to pH 7.4 with HCl.

Sheep anti-mouse immunoglobulin–cellulose immunoadsorbents containing approx. 300 μg of protein/mg of cellulose were prepared as previously described (Soos & Siddle, 1982).

C8 was iodinated as previously described for C9 (Morgan *et al.*, 1983), with incorporation of 0.5–2.6 atoms of ^{125}I per molecule of C8.

Protein was measured as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Preparation of monoclonal antibodies

Monoclonal antibodies to C8 were prepared by standard methods (Galfrè & Milstein, 1981; Soos & Siddle, 1982) as previously described for antibodies to C9 (Morgan *et al.*, 1983). BALB/C mice were immunized with 5 μg of C8 in Freund's complete adjuvant, receiving 200 μl of emulsion subcutaneously. After 18 weeks the mice were boosted intraperitoneally with 5 μg of C8 in 0.2 ml of PBS and test-bled 10 days later. Positive mice received a further boost intraperitoneally with 5 μg in 0.2 ml of PBS 10 days later.

On day 4 after this boost, spleen cells were fused with P3-NSI/1-Ag 4-1 mouse myeloma cells and the products of fusion cultured in microtitre trays. Culture supernatants were tested for antibody after 10–14 days. The assay for antibody consisted of incubating 10–100 μl of culture supernatant, or suitable dilutions of other antibody-containing fluids, with ^{125}I-C8 (2×10^4 c.p.m.) in 100 μl of VBS overnight at 4 °C. Sheep anti-mouse immunoglobulin immunoadsorbent (250 μg of cellulose in 50 μl of VBS) was then added for 1 h. Incubations were terminated by addition of 2 ml of VBS, followed by centrifugation (5 min, 2000 *g*) and washing (1×2 ml of VBS) of the pellet. Radioactivity in the pellet was then counted. Cells producing antibody were cloned twice at limiting dilution before growth as ascites tumours in mice. Antibody was recovered from ascitic fluid by two precipitations with 50%-satd. $(NH_4)_2SO_4$.

Measurement of site specificity

Epitope mapping of the monoclonal antibodies was conducted by using antibodies in solution phase to interfere with the binding of ^{125}I-labelled C8 to antibodies on solid phases prepared by incubating each monoclonal antibody with sheep anti-mouse immunoglobulin adsorbents for 48 h at 4 °C (Soos & Siddle, 1982; Morgan *et al.*, 1983).

Reaction of antibodies with individual C8 subunits was demonstrated by immunoblotting. SDS/polyacrylamide-gel electrophoresis was by the method of Laemmli (1970); immunoblotting was performed as described by Burnette (1981), with development as described by Stanley (1983).

Immunoaffinity purification of C8 and C8 subunits

Columns (12 ml) of Sepharose 4B-linked monoclonal anti-C8 antibodies (C8-B1, C8-L6 or C8-M1) were prepared as described for anti-C9 antibodies, giving 2 mg of antibody/ml of Sepharose (Morgan *et al.*, 1983), and equilibrated with TBS.

Fresh normal human plasma (65 ml) was adjusted to 10 mM-EDTA and 1 mM-phenylmethanesulphonyl fluoride. C8 was precipitated by addition of concentrated $(NH_4)_2SO_4$ to 30% saturation followed by a further addition of concentrated $(NH_4)_2SO_4$ to the supernatant to 50% saturation at 4 °C; the 50%-satd.-$(NH_4)_2SO_4$ precipitate was dissolved in PBS (65 ml) and dialysed for 18 h at 4 °C against 5 litres of PBS. The solution was applied to a monoclonal-antibody-C8-B1 or -C8-L6 column (20 cm × 1 cm) at 1 ml/min at 4 °C, followed by washing with 1 litre of TBS, 4 °C. Bound C8 was then eluted with 20 ml of fresh 50 mM-diethylamine, pH 11.4, at 4 °C, and 5 ml fractions were collected into 1 ml aliquots of 1 M-Tris/HCl, pH 6.8, to neutralize (Bailyes *et al.*, 1982). The third fraction collected contained approx. 90% of the C8 eluted.

Purification of C8 subunits was essentially as described above, except that elution was with a gradient (100 ml) of 0.15–1 M-NaCl, pH 7.4. The $\alpha\gamma$ subunit of C8 was prepared by using the C8-B1 column and the β-subunit by using the C8-L6 column. Samples of subunits for SDS/polyacrylamide-gel electrophoresis were precipitated with 10% (w/v) trichloroacetic acid at 0 °C. Samples for haemolysis assays were dialysed against VBS.

Identification of fractions containing C8 or its subunits, and assay of C8 recovery during purification, was by rocket immunoelectrophoresis (Laurell, 1972; Morgan *et al.*, 1983) or dot-blot immunoassay. The latter simply involved filtration on to nitrocellulose, incubation with antibodies and development as described by Stanley (1983), with quantitative assessment by scanning on a Joyce–Loebl Chromoscan 3. For rocket immunoelectrophoresis, rabbit anti-(human C8) antiserum (Hallett *et al.*, 1981) was used, and for dot-blots, monoclonal antibodies C8-B1 and C8-L6 (each at 1:100 dilution of ascites fluid) together or separately.

Haemolytic assays

The functional activity of C8 fractions in the classical complement pathway was assessed by using antibody-sensitized aged sheep erythrocytes as described by Whaley (1985).

In order to assess components in alternative-pathway assays, fresh washed rabbit erythrocytes (E) were resuspended at 10% (v/v) in VBS. Aliquots (10 μl) were added to 1 ml VBS in plastic spectrophotometer cuvettes at 37 °C with subsequent addition of 25 μl of NHS or NHS depleted of C8 (NHS-C8) with or without addition of C8 or C8 subunits. Alternatively EC5b-7 intermediates were prepared by incubation of E (300 μl of the 10% suspension) with 125 μl of NHS-C8, 575 μl of VBS for 10 min at 37 °C, then washing and resuspending in 300 μl of VBS. Aliquots (10 μl) were incubated with C8 subunits and purified C9 in a total volume of 1 ml of VBS. Lysis was continuously monitored at A_{600} in a Varian DMS 90 spectrophotometer. NHS-C8 was prepared by passage of 15 ml of fresh NHS through a 12 ml C8-B1–Sepharose 4B column, the first 10 ml of eluate being discarded.

RESULTS

Monoclonal antibodies to human C8

Four mice with positive serum titres for C8 were obtained from ten mice immunized with C8. The spleens of three of the serum-positive mice were fused with myeloma cells, resulting in 150 positive culture supernatants in the binding assay with ^{125}I-labelled C8. Of

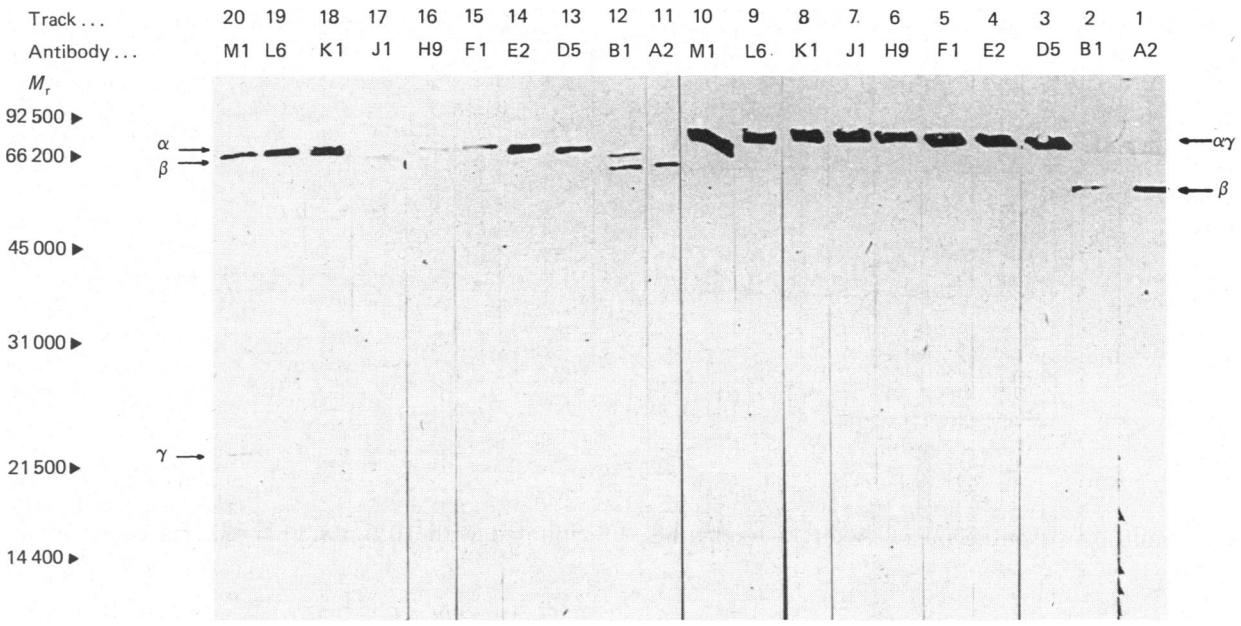

Fig. 1. Immunoblotting of C8 with monoclonal antibodies

C8 (1 μg/track) was treated with SDS in the absence (tracks 1–10) or presence (tracks 11–20) of dithiothreitol and subjected to SDS/polyacrylamide (15% total monomer)-gel electrophoresis. After electrophoretic transfer on to nitrocellulose, tracks were allowed to react with individual monoclonal antibodies as designated (i.e. track 1, C8-A2; track 2, C8-B1 etc.) each as diluted ascites fluid (tracks 1–10, 2 ml of 1:5000-diluted ascites fluid; tracks 11–20, 2 ml of 1:100-diluted ascites fluid), washed and developed after reaction with peroxidase-labelled goat anti-mouse immunoglobulin. The positions of C8 polypeptide chains (arrows) running in adjacent tracks and M_r standards (Bio-Rad; arrowheads) are indicated.

these, 29 cell lines were initially cloned and ten were successfully cloned twice more and injected intraperitoneally into mice to produce ascites fluid. Seven of the ten resulting monoclonal antibodies, when assayed in hybridoma culture supernatants using class- and subclass-specific rabbit anti-mouse antisera (Miles, Stoke Poges, Slough, Berks., U.K.) were found to be IgG_1, one IgG_{2a} (coded C8-J1), one IgG_{2b} (C8-M1) and one IgM (C8-H9) using a dot immunoblotting assay (Beyer, 1984).

Interaction of the monoclonal antibodies with the subunits of C8 was assessed by immunoblotting after SDS/polyacrylamide-gel electrophoresis of C8 (1 μg/gel track) with or without reduction to separate the subunits (Fig. 1). After gel electrophoresis in non-reducing conditions two antibodies (C8-A2 and C8-B1) reacted with the β-subunit and the remainder with the αγ-subunit. A low level of cross-reaction of anti-β antibodies with the αγ-subunit was observed at high concentrations (Fig. 2). However, diluting the antibodies to 1:500 resulted in no detectable cross-reactivity. After gel electrophoresis under reducing conditions, 50-fold higher concentrations of antibody were required to observe a reaction with C8 by immunoblotting. Six antibodies reacted only with α-chain. Two antibodies (C8-J1 and C8-M1) were found to react with the γ-chain, but these also showed a strong cross-reaction with α (Fig. 1). The anti-β antibody C8-B1 still cross-reacted with the α-subunit after gel electrophoresis under reducing conditions (Fig. 1, lane 12). None of the antibodies immunoblotted C9 under the conditions described, even with 30 μg of C9/gel track.

Epitope mapping of the monoclonal antibodies was conducted by using each antibody in solution phase to interfere with the binding of ^{125}I-labelled C8 to each

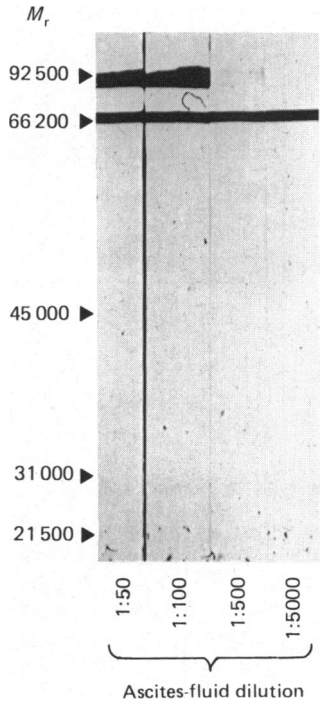

Fig. 2. Cross-reaction of anti-β monoclonal antibody C8-B1 with the C9 α-subunit

C8 (1 μg/track) was subjected to SDS/polyacrylamide-gel electrophoresis (under non-reducing conditions) and immunoblotting as described in Fig. 1. Each track was allowed to react with a different dilution of C8-B1 ascites fluid as indicated, and then with peroxidase-labelled goat anti-mouse immunoglobulin. The positions of M_r standards (Bio-Rad) in adjacent tracks are indicated (arrowheads).

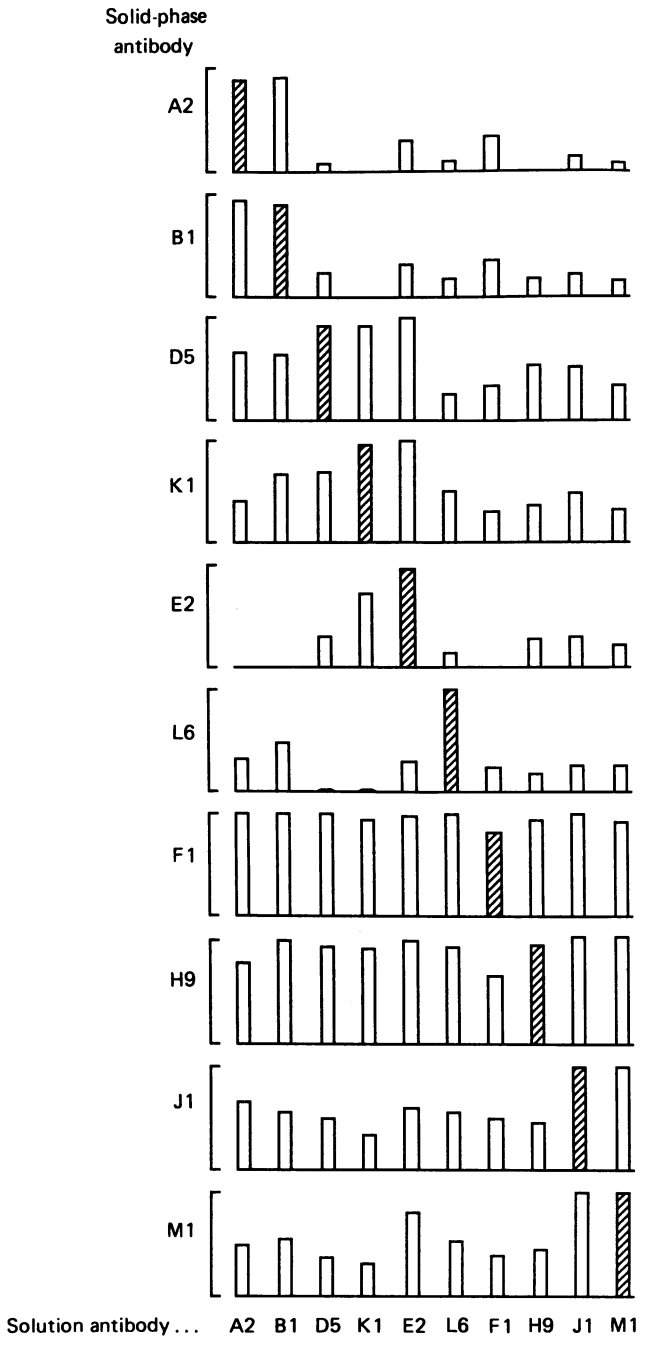

Fig. 3. Epitope analysis by binding competition

Results are expressed as the percentage inhibition of binding of ^{125}I-C8 to representative immobilized antibodies after preincubation of ^{125}I-C8 with the same (▨) or different (□) antibodies in solution. Where no bars are shown, no inhibition was observed. The scales at the side indicate 0% (bottom) and 100% (top) inhibition for each antibody.

antibody on a solid phase (Fig. 3). The observed inhibition was not always 'all or none' as predicted by simple models of steric competition, and certain antibody pairs used in solution or solid phase did not show reciprocal effects. Nevertheless, the binding studies suggested that, whereas the antibodies to β (C8-A2, C8-B1) and γ (C8-J1, C8-M1) bound to a single epitope on

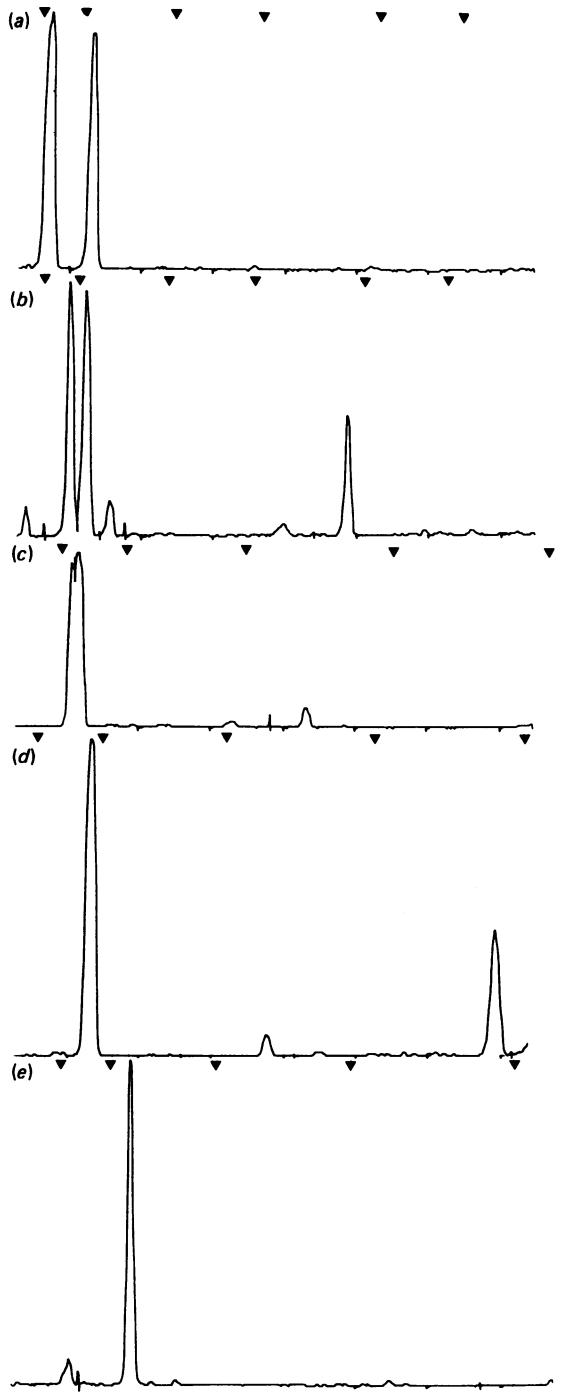

Fig. 4. SDS/polyacrylamide-gel electrophoresis of immunoaffinity-purified C8 and C8 subunits

Densitometric scans of Coomassie Blue-stained gels (15% total monomer). (*a*) and (*b*), Whole C8 purified by diethylamine elution from a C8-M1 column; (*c*) and (*d*), $\alpha\gamma$-subunit purified by NaCl-gradient (0.15–1 M) elution from a C8-B1 column (Fig. 5); (*e*), β-subunit purified by NaCl gradient (0.15–1 M) elution from a C8-L6 column (Fig. 5). The protein load per track was approx. 5 μg (reduced with dithiothreitol for tracks *b* and *d*). The positions of M_r standards (Bio-Rad) for each gel are indicated (arrowheads) and are, from left to right, M_r 92500, 66200, 45000, 31000, 21500 and (*a* and *b* only) 14400.

Table 1. Immunoaffinity purification of C8

A two-step procedure was used as described in the Experimental section with a monoclonal-antibody-C8-M1 immunoaffinity column. Activity of C8 was assayed by rocket immunoelectrophoresis using 1–8 μl samples. Activity was measured in rocket units (R.U.); arbitrary units of area of the rocket.

Fraction	Volume (ml)	Total protein (mg)	Activity (R.U.)	Specific activity (R.U./mg)	Purification (fold)	Yield (%)
Plasma	65	5655	293	0.052	1	100
$(NH_4)_2SO_4$ (30–50%-satd.) precipitate	65	780	195	0.25	4.8	67
Immunoaffinity chromatography	5	2.7	105	38.8	746	36

each subunit, the monoclonal antibodies to α could be divided into four epitope groups. Thus C8-F1 and C8-H9 bind to one epitope, C8-L6 to a second epitope, C8-D5 and C8-K1 to a third and C8-E2 to a closely related fourth. When cross-reacting with the α-subunit the monoclonal antibodies to β interfered particularly with the binding sites for C8-D5, C8-F1 and C8-H9, and the antibodies to γ particularly with the binding sites for C8-F1 and C8-H9 respectively. The complex binding patterns without simple reciprocal effects are in agreement with data obtained with panels of monoclonal antibodies to other proteins [insulin receptor (Soos *et al.*, 1986); alkaline phosphatase (Bailyes *et al.*, 1987)].

Immunoaffinity purification of C8 and its subunits

Purification of C8 was achieved by a simple two-step procedure involving $(NH_4)_2SO_4$ precipitation, followed by binding to a monoclonal-antibody immunoaffinity column and elution with 50 mM-diethylamine. This method gave a yield of 36% of C8 from fresh normal plasma using either a C8-M1, a C8-B1 or a C8-L6 column. Purification of 746-fold was obtained after measuring C8 by rocket immunoelectrophoresis and expressing the C8 specific activity as rocket units per mg of protein (Table 1). The purity and subunit stoichiometry of immunoaffinity-isolated C8 was further assessed by densitometric scanning and integration of Coomassie Blue-stained C8 after SDS/polyacrylamide-gel electrophoresis (Figs. 4*a* and 4*b*). After electrophoresis under non-reducing conditions, 51% of stain was associated with the $\alpha\gamma$ subunit and 49% with the β-subunit (Fig. 4*a*); under reducing conditions stain was associated with 33% (α), 35% (β) and 17% (γ) (Fig. 4*b*).

In preliminary experiments to establish washing and elution conditions for immunoaffinity purification of C8, it was found that salt washes preferentially removed the β-subunit from anti-$\alpha\gamma$ (C8-L6) columns and the $\alpha\gamma$-subunit from anti-β (C8-B1) columns. By using a gradient of NaCl it was found that, after binding of C8 to a monoclonal-antibody affinity column, elution of the unbound subunit commenced at 0.24 M-NaCl; there was 50% elution at 0.45 M-NaCl, and > 90% was eluted by

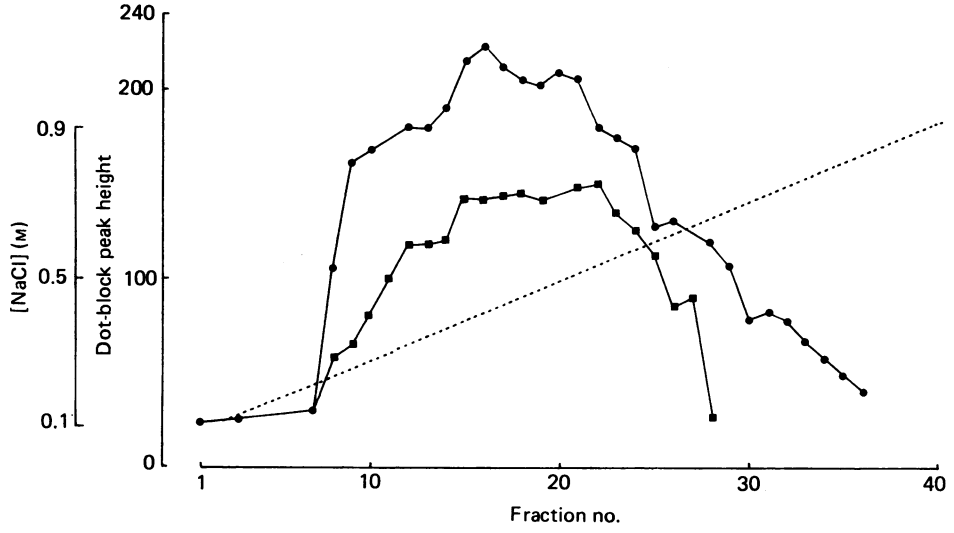

Fig. 5. Salt-gradient elution of C8 subunits from monoclonal-antibody immunoaffinity columns

After allowing C8 to bind to an anti-α column (C8-L6) and an anti-β column (C8-B1) respectively, the β-subunit (●) and $\alpha\gamma$-subunit (■) were eluted with a gradient of NaCl (----); 2.5 ml fractions were collected, and 50 μl aliquots were assessed by dot-blot immunoassay using C8-B1 and C8-L6 as subunit-identifying antibodies. After densitometric scanning of the dot blots, peak height was measured and plotted in arbitrary units (●, ■).

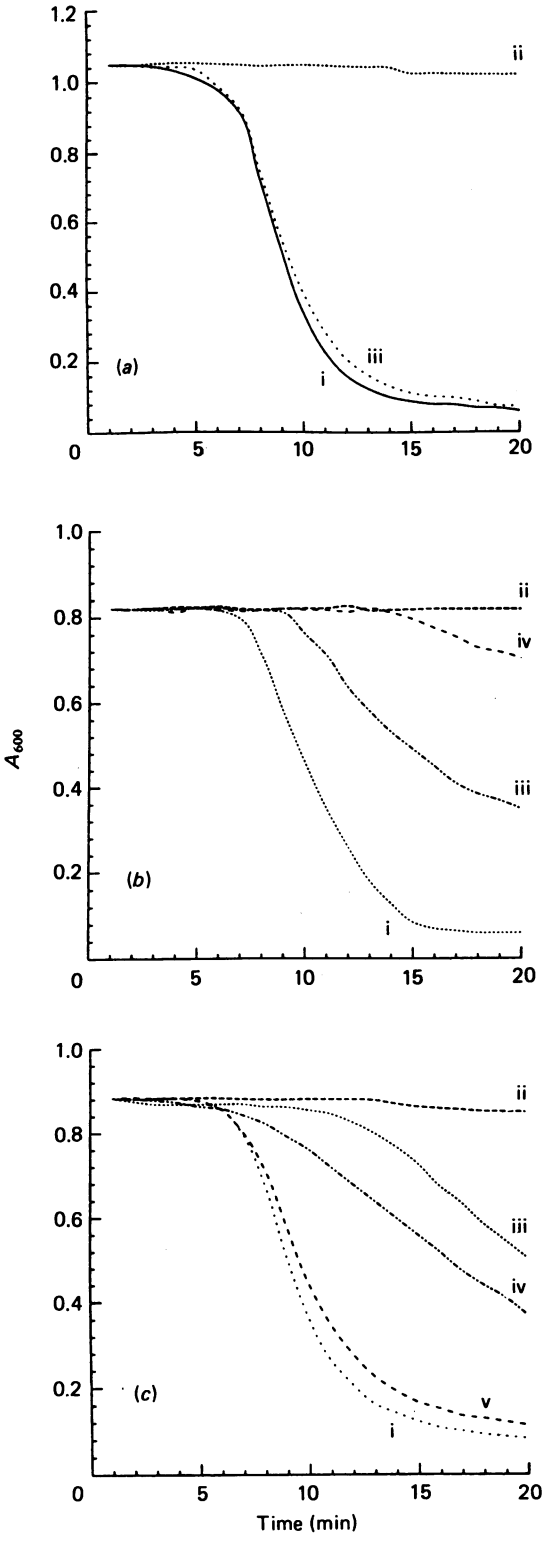

Fig. 6. Lysis of rabbit erythrocytes by C8-depleted serum reconstituted with immunoaffinity-purified C8

Assays were performed as described in the Experimental section. (*a*) E+NHS (i), NHS-C8 (ii) or NHS-C8+5 μg of C8 (iii) (NHS-C8+2.5 μg of C8 gave an identical trace); (*b*) E+NHS-C8+VBS (ii), 2 μg of C8 $\alpha\gamma$+2 μg of C8 β (i), 2 μg of C8 $\alpha\gamma$ (iv) or 2 μg of C8 β (iii); (*c*) EC5b-7+0.4 μg of C9+2 μg of C8 $\alpha\gamma$+2 μg of C8 β (i), VBS (ii), 2 μg of C8 β (iii), 2 μg of C8 $\alpha\gamma$+0.2 μg of C8 β (iv) or 0.2 μg of C8 $\alpha\gamma$+2 μg of C8 β (v).

0.65 M-NaCl (Fig. 5). The purity of C8 subunits prepared by such salt-gradient elution was further assessed by SDS/polyacrylamide-gel electrophoresis, when > 85% of the Coomassie Blue stain was observed associated with C8 subunits (Figs. 4*d* and 4*e*), and no major impurities were detected.

Haemolytic activity of immunoaffinity-purified C8 and its subunits

Immunoaffinity-purified C8 retained full haemolytic activity when assessed by addition to NHS-C8 (prepared by passage of NHS through a monoclonal-anti-C8-antibody column) and either antibody-sensitized sheep erythrocytes or rabbit erythrocytes (Fig. 6*a*). By using serial dilutions of purified C8 in the antibody-sensitized-sheep-erythrocyte assay, it was found that the C8 had 104% of the biological activity of C8 in serum, assuming a serum concentration of 50 μg/ml. The rate of lysis assessed with rabbit erythrocytes was the same with NHS and with C8 added to NHS-C8 (Fig. 6*a*). Rabbit erythrocytes have little surface sialic acid and activate serum complement by the alternative pathway (Platts-Mills & Ishizaka, 1974; Fearon, 1978), thus no anti-cell antibody is required to initiate MAC formation. Purified $\alpha\gamma$- and β-subunits failed to reconstitute the haemolytic activity of NHS-C8 when added alone, but were fully active when added together (Fig. 6*b*). Purified β-subunit apparently showed some haemolytic activity, but addition of different concentrations of the $\alpha\gamma$ subunit (Fig. 6*c*) showed that this could be accounted for by the presence of approx. 1% contamination with $\alpha\gamma$-subunit, since full haemolytic activity could be reconstituted by 2 μg of β plus 0.2 μg of $\alpha\gamma$ under the conditions of this experiment. Contamination of the β-subunit with $\alpha\gamma$ was not detectable on SDS/polyacrylamide gels stained with either Coomassie Blue (Fig. 4*e*) or silver stain (Ansorge, 1982; results not shown).

DISCUSSION

In the present experiments monoclonal antibodies were prepared to all three polypeptide chains of human complement component C8. The identity of the C8 subunit with which each of the ten monoclonal antibodies reacted was established by immunoblotting after SDS/polyacrylamide-gel electrophoresis. Epitope mapping by competition of ^{125}I-C8 binding to pairs of antibodies in solution and on solid phase showed the presence of four closely related epitopes for the six antibodies to the α-subunit and one each for the pairs of antibodies reacting with the β- and γ-subunits.

Cross-reactions of anti-β and anti-γ monoclonal antibodies with the α-subunit were observed, but none of the anti-C8 antibodies reacted with C9. Polyclonal-antibody cross-reactions with different components of the MAC have been observed in previous studies (Tschopp & Mollnes, 1986; Tschopp *et al.*, 1986). Recently cDNA cloning has demonstrated homologies betwen C8 α- and β-subunits and between these and C9 (Haefliger *et al.*, 1987; Howard *et al.*, 1987; Rao *et al.*, 1987). In the case of C9, fine epitope mapping using expression cloning (Stanley & Luzio, 1984) to generate fragments of the molecule has indicated the likelihood of necessary flanking regions for at least one monoclonal-antibody site (Stanley & Herz, 1987). The presence of similar flanking regions could help explain epitope

overlaps observed in the present experiments with C8, though simple steric hindrance is also possible.

The use of monoclonal-antibody immunoaffinity columns for the purification of C8 and C8 subunits provides a simple method for the preparation of biologically active components in higher yield than those obtained with previous methods (Steckel *et al.*, 1980; Monahan *et al.*, 1983; Rao & Sodetz, 1984). The effect of salt in disrupting the non-covalent bonds between the subunits of C8 when bound to monoclonal-antibody immunoadsorbents (Fig. 6) extends previous observations using 1.5 M-NaCl (Rao & Sodetz, 1984) on the effect of salt on the interaction between α- and β-subunits, and showed that dissociation of C8 occurred even at 0.24 M-NaCl.

The requirements for both the C8 α- and β-subunits in formation of the MAC has been established by study of individuals deficient in $\alpha\gamma$- and β-subnits (Tedesco *et al.*, 1983), by binding studies of radiolabelled C8 subunits to membrane-bound C5b-7 (Monahan & Sodetz, 1981) and by solution binding studies using purified complement components (Stewart & Sodetz, 1985). These have suggested that the β-subunit mediates the binding of C8 to C5b–7 and that the α-subunit interacts with C9. Although there is evidence that C8 α-subunit is incorporated into the tubular poly(C9) complex formed in a target membrane (Podack, 1984), cross-linking studies have shown that most of the C9 in the MAC is not in close physical proximity to C8 (Monahan *et al.*, 1983). The exact role played by the C8 subunits in the functional insertion and polymerization of C9 in target membranes therefore remains to be established. The monoclonal anti-C8 antibodies prepared in the present study should prove important tools in such investigations. Preliminary experiments have shown that they can be used to detect C8 in target membranes attacked by the complement system, and that immunoglobulin-enriched $(NH_4)_2SO_4$ fractions of both anti-α and anti-β ascites fluids can interfere with the functional insertion of C9 (Abraha *et al.*, 1986, 1987; Luzio *et al.*, 1987).

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