Two populations of complement factor H differ in their ability to bind to cell surfaces

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Using hydrophobic affinity chromatography on phenyl-Sepharose, human complement factor H can be separated into two subpopulations, ϕ_1 and ϕ_2 . Although ϕ_1 and ϕ_2 are known to differ in their aggregation properties under non-physiological low ionic strength conditions, no difference in aggregation state was detected under the conditions used for cell-binding experiments. We have investigated these two subpopulations further to determine whether functional differences exist between them. The subpopulation ϕ_2 was found to bind specifically and saturably to the surface of Raji cells. The binding of the other subpopulation, ϕ_1 , was low, and essentially non-specific. A monoclonal anti-factor H antibody, BGH-1, was raised which recognizes preferentially the ϕ_2 subpopulation and inhibits the binding of factor H to cell surfaces.

INTRODUCTION

Factor H is an abundant (200–500 $\mu\text{g/ml}$) plasma protein which plays a key role in the regulation of the activation of the complement system (for review see Reid, 1983). This protein is a single-chain polypeptide of about 155000 M_r , the primary structure of which is made up of repeating units of 60 amino acids, having a framework of highly conserved cysteine, proline and tryptophan residues (Kristensen *et al.*, 1986; Ripoché *et al.*, 1986; Sim *et al.*, 1986; Ripoché *et al.*, 1988). The same repeating structure is also found in the other regulatory proteins of the complement system, C4 binding protein (Chung *et al.*, 1985) and complement receptor type 1 (Klickstein *et al.*, 1985; Wong *et al.*, 1985). Factor H is thought to have an unusual secondary structure and an elongated shape on the basis of a high frictional ratio, a high content of proline (Gardner *et al.*, 1980; Sim & DiScipio, 1982), an unusual c.d. spectrum (DiScipio & Hugli, 1982) and electron microscopy studies (Smith *et al.*, 1983).

Factor H controls the activation of the alternative pathway of the complement system by two basic mechanisms, namely the acceleration of the dissociation of the alternative pathway convertase C3bBb (Whaley & Ruddy, 1976), and by acting as a non-enzymic cofactor for the proteolytic cleavage of C3b to iC3b by the diisopropylfluorophosphate-insensitive plasma serine protease factor I (Whaley & Ruddy, 1976; Pangburn *et al.*, 1977; Harrison & Lachmann, 1980; Sim *et al.*, 1981). In both cases, factor H performs its function by binding to its physiological ligand, fluid-phase or surface-bound C3b. The C3b binding site of factor H has been located on the 36000–38000 M_r tryptic fragment (Alsenz *et al.*,

1984). Other recent studies have suggested that factor H may play some physiological role other than regulation of the alternative pathway C3 convertase, by interacting with a specific receptor on leukocytes. For example, factor H has been suggested to promote the release of factor I from B lymphocytes (Lambris *et al.*, 1980), although this is disputed (Sim & Sim, 1983), the release of prostaglandin from macrophages (Hartung *et al.*, 1984), or to have more general effects such as the triggering of blastogenesis of murine splenocytes (Hammann *et al.*, 1981), enhancement of oxidative metabolism in monocytes (Schopf *et al.*, 1982), and inhibition of the differentiation of peripheral B lymphocytes (Tsokos *et al.*, 1985). These results strongly suggest the presence of a factor H receptor at the surface of these cells. The nature of this receptor is not yet fully determined. Lambris & Ross (1982) demonstrated, using an antibody directed against antifactor H idiotopes, that a candidate for this receptor on B lymphoblastoid cell lines, Raji and Bf, consisted of three 50000 M_r subunits linked together by disulphide bridges. In a more recent study, Erdei & Sim (1987) have identified a single polypeptide chain protein of 140000–150000 M_r , present on the surface of Raji and tonsil B cells. This protein may be the same as that reported by Lambris & Ross (1982), if it is assumed that the protein isolated in the earlier study was degraded.

In studies of the binding of factor H to its cellular receptor, it has been suggested that an oligomeric form of the proteins serves as the ligand, but this has not been adequately investigated (Lambris *et al.*, 1980; Lambris & Ross, 1982; Tsokos *et al.*, 1985). The use of hydrophobic affinity chromatography on phenyl-Sepharose has allowed us to separate factor H into two subpopulations,

Abbreviations used: e.l.i.s.a., enzyme-linked immunosorbent assay; VBS, veronal-buffered saline (150 mM-NaCl/5 mM-sodium 5,5-diethylbarbiturate, pH 7.2); VBS-BSA, VBS containing 5% (w/v) bovine serum albumin; PBS, phosphate-buffered saline (8.2 mM-disodium hydrogen phosphate/1.5 mM-potassium dihydrogen phosphate/139 mM-NaCl/3 mM-KCl, pH 7.5); DAB, Dulbecco's A and B (PBS containing 1 mM-CaCl₂ and 1 mM-MgCl₂); DAB-BSA, Dulbecco's A and B containing 5% (w/v) bovine serum albumin.

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factor H (ϕ_1) and factor H (ϕ_2). These two populations are of identical apparent polypeptide chain size, and interact equally well with C3b, but they differ in their hydrophobic properties. They also differ in their aggregation properties, in that ϕ_2 more readily forms oligomers at low ionic strength, as judged by gel-filtration and agglutination studies (Ripoché *et al.*, 1984).

MATERIALS AND METHODS

All tissue culture materials were from Flow Laboratories (Puteaux, France) unless otherwise stated. Trypsin, soya-bean trypsin inhibitor, ethylene glycol, 6-amino-n-hexanoic acid, ethylene diamine tetra-acetic acid were from Sigma (Eurobio, Paris, France). Chromatographic media, Sephacryl S-300 and phenyl-Sepharose, were from Pharmacia (Bois d'Arcy, France). Micro-titration plates were from Falcon Laboratories (Becton Dickinson, Grenoble, France), horse serum from Boehringer-Mannheim (Meylan, France), peroxidase-conjugated antibodies against mouse immunoglobulins from Nordic Lab (TEBU, Le Perray en Yvelines, France) and 2,2-azino-di-(3-ethylbenzthiazoline sulphonic acid) from Boehringer-Mannheim. The semi-automated e.l.i.s.a. reader was from Biotech Instruments (OSI, Paris, France).

Complement components

Factor H was purified as described previously (Ripoché *et al.*, 1984). The two subpopulations of factor H, ϕ_1 and ϕ_2 , were separated by phenyl-Sepharose chromatography (Ripoché *et al.*, 1984). Briefly, purified factor H (5–10 mg in 0.15 M-NaCl/0.02 M-EDTA/0.05 M-6-aminohexanoic acid, pH 7.2) was loaded onto a phenyl-Sepharose chromatography column (30 cm $\times$ 2.6 cm) equilibrated in 0.15 M-NaCl/0.02 M-EDTA/0.05 M-6-aminohexanoic acid, pH 7.2, at 20 °C. The resin was washed with the equilibrating buffer and this wash eluted a fraction (approx. 40 %) of the input factor H which we designate as factor H (ϕ_1). This material was slightly retarded on the column. Approx. 60 % of the input factor H bound to the resin and could only be eluted with a low ionic strength buffer (50 % ethylene glycol in distilled water). We designate this material as factor H (ϕ_2). Both fractions were dialysed against VBS and concentrated by ultrafiltration to 1–2 mg/ml. Protein concentrations were measured using a colorimetric assay (Bradford, 1976) (Bio-Rad protein assay, Bio-Rad Lab., Touzart et Matignon, Paris, France) using human IgG as standard.

Highly purified C3 was prepared as described by Al Salihi *et al.* (1982). C3b was prepared by limited trypsin digestion of C3. C3 (1 mg/ml) in PBS was incubated for 1 min at 37 °C with trypsin (1 %, w/w; Worthington Biochemical, Millipore, Velisy, France). The reaction was stopped with a 2-fold molar excess over trypsin of soya-bean trypsin inhibitor.

Radioactive labelling procedures

C3b and the two factor H subpopulations were labelled with ^{125}I using the Bolton-Hunter reagent (Bolton & Hunter, 1973). Specific activities were of the order of 10^6 c.p.m./ μg . F(ab')₂ fragments of affinity-purified rabbit anti-mouse IgG, kindly donated by Dr A. F. Williams (MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, University of Oxford,

Oxford, U.K.), were labelled with ^{125}I using Iodogen (Pierce Chemical Co., Rockford, IL, U.S.A.) by the method described by Markwell & Fox (1978). Specific activities were of the order of 2×10^7 c.p.m./ μg . The two factor H subpopulations were also iodinated by the latter method for use only in sucrose density gradient centrifugation experiments.

Cell culture and binding of factor H to Raji cells

Raji, a human B lymphoblastoid cell line, was grown in RPMI 1640 medium containing 10 % fetal-calf serum, penicillin (100 i.u./ml), streptomycin (100 i.u./ml) in an atmosphere of 1:19 CO₂/air at 95 % humidity.

Direct binding assays. For direct binding assays, Raji cells were washed thoroughly in VBS-BSA, prewarmed at 37 °C. Cells (10^6) were incubated with increasing amounts (0–20 μg) of either ^{125}I -labelled factor H (ϕ_2) or factor H (ϕ_1) in a final volume of 200 μl of VBS-BSA. Incubation was carried out at 37 °C for 1 h. Cells were then pelleted by centrifugation for 10 min at 2000 rev./min and washed 5 times with 1 ml of VBS-BSA. Before the fifth wash, cell suspensions were transferred to a clean tube to eliminate radioactivity non-specifically bound to the tube. In some experiments, binding assays were performed in a 100-fold molar excess of unlabelled factor H. Radioactivity associated with the pellet was measured in a gamma-radiation counter (NE 1600, Nuclear Enterprises).

Indirect binding assays. Binding of factor H (the total unseparated population), factor H (ϕ_1) and factor H (ϕ_2) to Raji cells was detected in an indirect binding assay as described by Mason & Williams (1986). The assays were performed in duplicate. Factor H, factor H (ϕ_1) and factor H (ϕ_2) were serially diluted in DAB-BSA, and 20 μl of each dilution were added to 20 μl of the Raji cell suspension (10^8 cells/ml) in the same buffer. After incubation for 1 h at 4 °C, cells were washed with 1 ml of DAB-BSA. Then 2×10^6 sheep erythrocytes were added to each sample together with a 1/100 dilution of the factor H-specific monoclonal antibody, MRC OX 23 (Sim *et al.*, 1983), or the same dilution of the control monoclonal antibody, MRC OX 1, a rat anti-mouse leukocyte common antigen (Sunderland *et al.*, 1979) generously supplied by Dr A. F. Williams.

Both monoclonal antibodies were of IgG1 subclass and were used as ascites fluids. After incubation for 1 h at 4 °C, the cells were washed again and further incubated with 25 μl of ^{125}I -labelled rabbit anti-mouse F(ab')₂ (300 000 c.p.m. at 2×10^7 c.p.m./ μg) at 4 °C for 1 h. Finally, the cells were washed twice with 1 ml of DAB-BSA, transferred to clean tubes, and the radioactivity measured using an LKB-1270 rack-gamma counter.

Development of monoclonal antibodies against factor H

Mice (*Balb/c*, female, 8 weeks old) were immunized with purified factor H by administering 100 μg of unfractionated factor H in Freund's complete adjuvant in each of three sub-cutaneous injections over 1 month. A final injection (100 μg), without adjuvant, was made intraperitoneally 3 days before fusion. Spleen cells were fused with the SP2/O-Ag14 myeloma cell line as described by Galfre & Milstein (1981). The culture supernatants were tested for the presence of antibodies against factor H using e.l.i.s.a. Hybridoma cultures

producing antifactor H antibodies were cloned by limiting dilution. Positive clones were then grown in tissue culture flasks. For large-scale production of monoclonal antibodies in ascites fluid, 4×10^6 hybridoma cells were injected intraperitoneally into *Balb/c* mice previously primed with pristane. Where indicated, monoclonal antibodies were further purified by standard ammonium sulphate precipitation.

E.i.s.a.

E.i.s.a. assays were performed in 96-well microtitration plates. Wells were coated with 50 μ l of factor H (10 μ g/ml in PBS) by incubation for 2 h at 4 °C. Excess fluid was removed by aspiration and plates were saturated with 10 % horse serum in PBS for 30 min at 4 °C. The saturating solution was removed by aspiration, then 100 μ l of the sample to be tested was added to the wells and the plates were left incubating overnight at 4 °C. After six washes with PBS containing 10 % horse serum, bound antibodies were detected by incubation with 50 μ l of a 1/500 dilution (in PBS containing 10 % horse serum) of the peroxidase-conjugated rabbit anti-mouse immunoglobulins for 2 h at 4 °C. Plates were then washed six times in PBS and the peroxidase substrate, 2,2-azino-di-(3-ethylbenzthiazoline sulphonic acid), added. Assignment of the monoclonal antibody class and subclass was done by double radial immunodiffusion against sheep antisera specific for different mouse heavy-chain class and subclass (Nordic Lab.).

Effect of monoclonal antibodies on the biological activities of factor H

To determine the effects of monoclonal antibodies on the binding of factor H to cell surfaces, factor H (3 μ g) was incubated overnight at 4 °C in VBS containing 10 % fetal-calf serum and with increasing amounts (0–10 μ g) of monoclonal antibody (purified from ascites fluids by ammonium sulphate precipitation) and then assayed for direct binding to cells as described above.

SDS/polyacrylamide-gel electrophoresis and Western blotting

SDS/polyacrylamide-gel electrophoresis was performed as described by Laemmli (1970) using 7 % (w/v) polyacrylamide slab gels. The gels were stained and destained as described by Fairbanks *et al.* (1971). Electrophoretic transfer of protein to nitrocellulose membranes (Bio-Rad) was carried out as described by Towbin *et al.* (1979). The blots were stained by the immunoperoxidase technique (Towbin *et al.*, 1979) using antifactor H monoclonal antibodies as primary antibodies.

Sucrose density gradient centrifugation

The two factor H subpopulations were analysed for the presence of aggregated material by sucrose density gradient centrifugation. Protein samples were layered on linear gradients of 10–40 % sucrose in VBS, and centrifuged in a Beckman SW40Ti rotor for 16 h at 4 °C and 195000 g (r_{av} 10.93 cm). Approximate sedimentation coefficients were calculated (Martin & Ames, 1961) relative to a single standard marker protein, bovine liver catalase (Sigma). Factor H (50 μ g of ϕ_1 or ϕ_2) was mixed with 500000 c.p.m. (0.1 μ g) of 125 I-labelled ϕ_1 or ϕ_2 , and the volume made up to 200 μ l with VBS. Gradients were fractionated into 25 fractions (approx. 570 μ l each) and

Fig. 1. Detection of antifactor H monoclonal antibodies using an e.i.s.a. system

Factor H (ϕ_2) (■), factor H (ϕ_1) (●), or unfractionated factor H (□), was coated onto the microtitration plate as described in Materials and methods section. Serial dilutions of culture supernatants of the hybridomas were then assayed with each of these factor H preparations as described in the Materials and methods section. (a) BGH1; (b) BGH2.

125 I-labelled factor H was detected by counting the radioactivity. Unlabelled factor H was detected by analysing gradient fractions by SDS/polyacrylamide-gel electrophoresis, followed by Coomassie Blue staining.

In this work, factor H subpopulations were examined at a concentration similar to the range used for the assays of direct binding to cells, and in the same buffer. The initial concentration of factor H loaded on the gradient was 250 μ g/ml. The final concentration in the peak gradient fraction was 35 μ g/ml.

RESULTS

Monoclonal antibodies against factor H

The screening of the hybridoma clones using the e.i.s.a. technique described in the Materials and methods section allowed us to select 15 clones producing a constant high titre of antibodies against factor H. When each of the two subpopulations of factor H, factor H (ϕ_1) and factor H (ϕ_2) were bound to the e.i.s.a. plate,

Fig. 2. BGH1 and BGH2 both recognize the 38 kDa tryptic fragment of factor H

Pure factor H was digested mildly with trypsin [1 % (w/w), 1 min at 37 °C] and then run on SDS/polyacrylamide gel under reducing conditions. The gel was electroblotted onto nitrocellulose filters as described under Materials and methods. Lane 1, Control track showing the SDS/polyacrylamide gel stained with Coomassie Blue. Top band represents undigested factor H. Lane 2, Immunoblotting analysis with BGH1 as primary antibody. The antibody BGH2 gave the same result.

12 clones appeared to produce a monoclonal antibody which recognized, with higher avidity than factor H (ϕ_1), the subpopulation factor H (ϕ_2), and 3 clones appeared, to produce a monoclonal antibody recognizing equally both populations. One of the monoclonal antibodies recognizing preferentially factor H (ϕ_2), (designated BGH1) and one recognizing equally both subpopulations (designated BGH2) (Fig. 1), were kept for further use. BGH1 was of the IgG1 subclass and BGH2 was an IgM. BGH1 was further purified from ascitic fluid by affinity chromatography on Protein A-Sepharose (Pharmacia). The antibody eluted from this column at pH 6.0, thus behaving typically as an IgG1 subclass antibody (Ey *et al.*, 1978). BGH1 was tested for binding to factor H fragments by a Western blot analysis and was shown to recognize the 38000 M_r N-terminal tryptic fragment of factor H (Alsenz *et al.*, 1984) (Fig. 2). Intact factor H appeared to be very badly transferred and was only faintly revealed in this analysis.

Differential binding of factor H (ϕ_1) and factor H (ϕ_2) to Raji cells

Binding of the two subpopulations of factor H to Raji cells was assayed by both direct and indirect binding experiments. Direct binding experiments showed that factor H (ϕ_2) binds significantly more to the cell surface than factor H (ϕ_1) (Fig. 3). Assuming factor H (ϕ_2) was monomeric, an average of 60000 binding sites for factor

Fig. 3. Binding of factor H to Raji cell surface by (a) direct binding assay, or (b) indirect binding assay

Increasing amounts of factor H (ϕ_1) or factor H (ϕ_2) were incubated with 10^6 Raji cells and binding was assayed as described under Materials and methods. (○) Factor H (ϕ_1); (□) factor H (ϕ_2); (●) factor H (ϕ_1) or factor H (ϕ_2) with a 100-fold molar excess of factor H (ϕ_1) or factor H (ϕ_2) respectively. Indirect binding assay for factor H was performed as described by Mason & Williams (1986). Factor H (unfractionated), factor H (ϕ_1) and factor H (ϕ_2), diluted to within the range 50–400 $\mu\text{g/ml}$, were incubated together with Raji cells, then with MRC OX23 anti-(factor H) monoclonal antibody and ^{125}I -labelled rabbit anti-mouse F(ab')₂, as described under Materials and methods. Data shown are not corrected for non-specific binding. Assays were performed in duplicate. (○) Factor H (ϕ_1); (●) factor H (ϕ_2); (□) factor H (unfractionated). The concentrations of factor H are final concentrations and are given on a logarithmic scale.

H (ϕ_2) can be calculated per Raji cell. Binding of factor H (ϕ_2) was inhibited by a 100-fold molar excess of unlabelled factor H (ϕ_2). BGH1 monoclonal antibody inhibited the binding of factor H (ϕ_2) in a dose-dependent fashion (Fig. 4). BGH2 had no effect on the binding of factor H (ϕ_2). The binding of factor H (ϕ_1) to Raji cells

Fig. 4. Inhibition of the binding of factor H (ϕ_2) on Raji cells by BGH1 and BGH2 monoclonal antibodies

Factor H (ϕ_2) (3 μ g), was incubated with increasing amounts (0–10 μ g) of either BGH1 (□) or BGH2 (○) and then assayed for binding to Raji cell surface as described under Materials and methods. The broken line indicates the level of non-specific binding when 125 I-labelled factor H (ϕ_2) was incubated in the presence of 100-fold molar excess of cold factor H (ϕ_2).

was essentially non-specific, as the binding curves for labelled factor H (ϕ_1), in the absence or presence of a 100-fold molar excess of cold factor H (ϕ_1), were not distinguishable (Fig. 3). These results were confirmed by using an indirect binding assay as described in the Materials and methods section. Using this indirect binding assay, the binding of factor H (ϕ_2) was found to be significantly higher than the binding of factor H which has not been fractionated on phenyl-Sepharose, and again the binding of factor H (ϕ_1) appeared to be essentially non-specific (Fig. 4).

Aggregation state of factor H (ϕ_1) and factor H (ϕ_2)

Sucrose density gradient centrifugation of factor H (ϕ_1) and factor H (ϕ_2) in conditions similar to those used for the direct cell binding assay, showed that the subpopulations of factor H behaved identically. Both migrated as single symmetrical peaks, with an approximate $s_{20,w}$ value (calculated relative to catalase, 10 S) of 5.0–5.4. This corresponds to the factor H monomer (Sim & DiScipio, 1982). There was no evidence for significant oligomer formation in either subpopulation under these conditions. 125 I-labelled factor H and unlabelled factor H of both subpopulations behaved identically when assessed as described in the Materials and methods section.

DISCUSSION

Previous work (Ripoche *et al.*, 1984) showed that factor H could be separated into two subpopulations by phenyl-Sepharose chromatography. The population which binds to phenyl-Sepharose [factor H (ϕ_2)] appears to present a stable conformational change making it more hydrophobic and more susceptible to oligomer formation at low ionic strength (Ripoche *et al.*, 1984). This conformational change could result from exposure

to phenyl-Sepharose but results indicated the possibility that a similar form of factor H existed *in vivo*. It has been suggested (Lambris *et al.*, 1980; Tsokos *et al.*, 1985) that oligomeric forms of factor H are the active forms of the molecule with respect to binding to factor H receptors but no experimental evidence was presented to support these suggestions.

In this respect we have analysed further the difference between the two factor H populations which can be obtained after chromatography on phenyl-Sepharose. The main finding of this study is that factor H (ϕ_2) binds specifically to its receptor on Raji cells whereas the other population, factor H (ϕ_1), does not appear to bind specifically. This was assessed by direct and indirect binding experiments. The binding of factor H to Raji cells (Fig. 3) has been observed in other studies (Lambris *et al.*, 1980; Schmitt *et al.*, 1981; Erdei & Sim, 1987). Our results show that only factor H which has undergone the conformational change characterizing factor H (ϕ_2) can bind specifically to Raji cells, suggesting the presence of ϕ_2 -like factor H species in standard factor H preparations. Indeed, as shown in Fig. 3, the control factor H which has not been fractionated on phenyl-Sepharose, binds specifically to Raji cells, but to a lesser extent than the pure factor H (ϕ_2) species. Previous results (Ripoche *et al.*, 1984) demonstrate that factor H (ϕ_2) does differ markedly from factor H (ϕ_1) in that it readily forms aggregates (oligomers). This was, however, demonstrable only under conditions of low ionic strength. Analysis of the two factor H subpopulations by sucrose density gradient centrifugation in the same buffer, and over similar concentrations to those used in the direct cell-binding assay, showed that both subpopulations were monomeric. Thus, at physiological ionic strength, factor H (ϕ_2) is not significantly aggregated in solution. It therefore appears that factor H does not need to be presented to cells in an oligomeric form for binding to the cell-surface receptor to occur. Aggregation of ϕ_2 after initial binding to the cell may occur, but we have not investigated this point. We suggest therefore that factor H (ϕ_2) behaves differently from factor H (ϕ_1) in binding to cells because of a conformational change in the monomer, and not because of aggregation.

The findings of an antigenic difference between the two populations (Fig. 1) supports the conclusion that there is a difference in conformation between the two populations. The site recognized by antibody BGH-1 is the N-terminal 38000 M_r tryptic fragment of factor H (Fig. 2). Interestingly, all of the mouse anti-factor H monoclonal antibodies described in the literature have their binding sites within this region of factor H (Sim *et al.*, 1983; Alsenz *et al.*, 1984, 1985; and the present work).

The existence of an activated form of factor H occurring in plasma has often been suggested (Lambris *et al.*, 1980; Hartung *et al.*, 1984; Tsokos *et al.*, 1985). In standard purified factor H preparations, such an activated form of the protein could result from the purification procedures, as has been shown for properdin (Farries *et al.*, 1985). Therefore the existence *in vivo* of a ϕ_2 -like factor H molecule, functionally characterized by specific fixation on factor H receptor, remains to be tested.

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REFERENCES

- Al Salihi, A., Ripoche, J., Pruvost, L. & Fontaine, M. (1982) *FEBS Lett.* **150**, 238–242
- Alsenz, J., Lambris, J. D., Schulz, T. F. & Dierich, M. P. (1984) *Biochem. J.* **224**, 389–398
- Alsenz, J., Schulz, T. F., Lambris, J. D., Sim, R. B. & Dierich, M. P. (1985) *Biochem. J.* **232**, 841–850
- Bolton, A. E. & Hunter, N. M. (1973) *Biochem. J.* **133**, 529–538
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- Chung, L. P., Bentley, D. R. & Reid, K. B. M. (1985) *Biochem. J.* **230**, 133–141
- DiScipio, R. G. & Hugli, T. E. (1982) *Biochim. Biophys. Acta* **709**, 58–64
- Erdei, A. & Sim, R. B. (1987) *Biochem. J.* **246**, 149–156
- Ey, P. L., Prowse, S. J. & Jenkin, C. R. (1978) *Immunochemistry* **15**, 429–436
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Farries, T. C., Finch, J. T., Lachmann, P. & Harrison, R. A. (1985) *Complement* **2**, 24 (abstr.)
- Galfre, G. & Milstein, C. (1981) Preparation of Monoclonal Antibodies: Strategies and Procedures, Proceedings of a Symposium held at the National University of Singapore, UNDP/World Bank/WHO
- Gardner, W. D., White, P. J. & Hoch, S. O. (1980) *Biochem. Biophys. Res. Commun.* **94**, 61–64
- Hammann, K. P., Raile, A., Schmitt, M., Mussel, H. H., Peters, H. & Dierich, M. P. (1981) *Immunobiology* **160**, 289–301
- Harrison, R. A. & Lachmann, P. J. (1980) *Mol. Immunol.* **17**, 9–20
- Hartung, H. P., Hadding, U., Bitter-Suermann, D. & Gemsa, J. (1984) *Clin. Exp. Immunol.* **56**, 453–458
- Klickstein, L. B., Wong, W. W., Smith, J. A., Morton, C., Fearon, D. T. & Weis, J. H. (1985) *Complement* **2**, 44–45
- Kristensen, T., Wetsel, R. A. & Tack, B. F. (1986) *J. Immunol.* **136**, 3407–3411
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Lambris, J. D. & Ross, G. D. (1982) *J. Exp. Med.* **155**, 1400–1411
- Lambris, J. D., Dobson, N. J. & Ross, G. D. (1980) *J. Exp. Med.* **152**, 1625–1644
- Markwell, M. A. R. & Fox, C. F. (1978) *Biochemistry* **17**, 4807–4817
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- Mason, D. W. & Williams, A. F. (1986) in *Handbook of Experimental Immunology* (Weir, D. M., ed.), 4th edn., chapter 38, Blackwell, Oxford
- Pangburn, M. K., Schreiber, R. D. & Müller-Eberhard, H. J. (1977) *J. Exp. Med.* **146**, 257–270
- Reid, K. B. M. (1983) *Biochem. Soc. Trans.* **11**, 1–12
- Ripoche, J., Al Salihi, A., Rousseaux, J. & Fontaine, M. (1984) *Biochem. J.* **221**, 80–96
- Ripoche, J., Day, A. J., Willis, A. C., Belt, K. T., Campbell, R. D. & Sim, R. B. (1986) *Biosci. Rep.* **6**, 65–72
- Ripoche, J., Day, A. J., Harris, T. J. R. & Sim, R. B. (1988) *Biochem. J.* **249**, 593–602
- Schmitt, M., Mussel, H. H., Hamman, K. P., Scheiner, O. & Dierich, M. P. (1981) *Eur. J. Immunol.* **11**, 739–745
- Schopf, R. E., Hamman, K. P., Scheiner, O., Lemmel, E. M. & Dierich, M. P. (1982) *Immunobiology* **46**, 307–312
- Sim, E. & Sim, R. B. (1983) *Biochem. J.* **210**, 567–576
- Sim, E., Wood, A. B., Hsiung, L. M. & Sim, R. B. (1981) *FEBS Lett.* **132**, 55–60
- Sim, E., Palmer, M. S., Puklavec, M. & Sim, R. B. (1983) *Biosci. Rep.* **3**, 1119–1131
- Sim, R. B. & DiScipio, R. G. (1982) *Biochem. J.* **205**, 285–293
- Sim, R. B., Malhotra, V., Ripoche, J., Day A. J., Micklem, K. J. & Sim, E. (1986) *Biochem. Soc. Symp.* **51**, 83–96
- Smith, C. A., Pangburn, M. K., Vogel, C. W. & Müller-Eberhard, H. J. (1983) *Immunobiology* **164**, 298 (abstr.)
- Sunderland, C. A., McMaster, W. R. & Williams, A. F. (1979) *Eur. J. Immunol.* **9**, 155–162
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Tsokos, G. C., Inghirami, G., Tsoukas, C. D., Barlow, J. E. & Lambris, J. D. (1985) *Immunology* **55**, 419–426
- Whaley, K. & Ruddy, S. (1976) *J. Exp. Med.* **144**, 1147–1162
- Wong, W. W., Klickstein, L. B., Smith, J. A., Weis, J. H. & Fearon, D. T. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7711–7715

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