Proteins involved in the activation and control of the two pathways of human complement

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The human complement system is composed of about 20 serum proteins, the 13 proteins of the classical and alternative pathways (Fig. 1) and at least seven major control proteins by which they are regulated (for general reviews see Mayer et al., 1981; Reid & Porter, 1981; Müller-Eberhard & Schreiber, 1980; Lachmann, 1979; Porter & Reid, 1979; Fearon, 1979). A general feature of the early stages of complement activation is the generation of complex proteinases, each by the interaction of an enzymically active component with one, or more, of the non-enzymic components (Fig. 1). For example, the complex proteinases that are involved in splitting C5 have very different compositions, i.e. C4b,C2a,C3b in the classical pathway and C3b,Bb,C3b in the alternative pathway. However, the enzymic sites residing in the catalytic chains of these complexes (i.e. in C2a and Bb) appear to function in an identical manner by specifically splitting one bond in the α-chain of the C5 component, thus activating C5 and allowing the self-assembly of the C5b-9 complex, which is common to both pathways (Fig. 1). The enzymes, involved in the formation and control of these complex proteinases (Fig. 2), all show some homology with the serine proteinases as judged by amino acid-sequence studies, since each enzyme possesses the equivalent of an active-site serine residue at a position approximately 50 amino acid residues from its C-terminus. However, each of these enzymes show unexpected features: the 27000-mol.wt. catalytic chains of the Clr and Cls subcomponents of the C1 complex, despite showing very strong homology with the catalytic chains of other serine proteinases, lack the 'histidine-loop' disulphide bridge (Arlaud & Gagnon, 1981; Arlaud et al., 1982); factor D, which is involved in the formation of the initial enzyme complex of the alternative pathway, appears to have no proenzymic form and therefore to circulate in the blood in its active form; it also unexpectedly shows homology over its N-terminal section with the serine esterase known as 'group-specific proteinase' (Johnson et al., 1980; Reid et al., 1981); C2 and factor B appear to be novel types of serine proteinases in that their catalytic chains of mol.wt. approx. 60000 are unusually large, which suggests that the mechanism of activation of these two proenzymes will differ from that postulated for serine esterases, which have the more common size of catalytic chain of mol.wt. 24000 (Christie et al., 1980); the control enzyme I, which has a catalytic chain of mol.wt. approx. 38000 (Pangburn et al., 1977), shows strong sequence homology with the catalytic chains of other serine esterases, despite its resistance to inhibition by di-isopropyl phosphorofluoridate and other similar inhibitors (Davis, 1981; Hsiuing et al., 1982).

Both pathways of complement can be activated by antibody-dependent and antibody-independent means. The antibody-dependent activation of the classical pathway involves complexes formed from immunoglobulins of the IgG and IgM classes but apparently not those of the IgA, IgD or IgE classes. Antibody-independent activation of the classical pathway has been described for retrovirus membranes (Bartholomew & Esser, 1980), heart mitochondrial membranes (Giclas et al., 1979) and bacterial lipid A (Cooper & Morrison, 1978). Antibody-dependent activation of the alternative pathway has been described for immune aggregates formed from certain subclasses of IgG and IgA, and may also take place with aggregates containing IgD and IgE. However, antibody-independent activation of the alternative pathway may be relatively more important in certain situations, since a large number of efficient, non-immunoglobulin, activators have been described (Götte & Müller-Eberhard, 1976; Fearon, 1979). The alternative pathway can be efficiently activated via the C3b feedback loop (Lachmann, 1979) after initial activation of the complement system has taken place by means of the classical...
pathway, and this complicates any assessment of the exact contribution each pathway makes during the activation process. It is well established that, on activation of the complement system by immune aggregates, the components C1, C4 and C3 became tightly bound to the aggregates as a result of activation of both the classical pathway and the alternative pathway (Müller-Eberhard & Biro, 1963; Müller-Eberhard & Lepow, 1965; Takahashi et al., 1977; Goers & Porter, 1978; Campbell et al., 1980; Gadd & Reid, 1981b). The C1 complex, which is composed of the three subcomponents Clq, C1r and C1s, can be readily dissociated from the aggregates, by treatment with EDTA in the case of C1r and C1s, or by low pH and high salt concentration in the case of Clq. However, the activated forms of C4 and C3 appear to become covalently bound to the Fab
region of the IgG in the aggregates by means of an ester or amide bond (Law & Levine, 1977; Law et al., 1979; Campbell et al., 1980; Gadd & Reid, 1981).

The present Lecture is concerned with some aspects of the binding, activation and control of the C1 complex on its interaction with immune aggregates, in particular how the unusual structure of Clq may be involved in the process; the activation of the alternative pathway by immune aggregates containing IgG and the investigation of the possible role of the C3b, which becomes covalently bound to the Fab regions in these aggregates.

Structure of subcomponent Clq

Subcomponent Clq associates with the proenzymes C1r and C1s to give C1, the first component of complement. The C1r and C1s proenzymes both behave as single chains of mol.wt. 83000 in dissociating conditions, but under physiological conditions they form an Ca2+-dependent complex of the form C1r--Ca2--C1s, which interacts with Clq. Since Clq does not appear to contain an enzymic site, its function in the C1 complex is considered to involve the recognition and binding of activators of the classical pathway, thus allowing the activation of the C1r and C1s proenzymes to take place (Porter & Reid, 1979)—which in turn leads to the sequential activation of the later components in the pathway.

When viewed in the electron microscope, Clq can be seen to be composed of six peripheral globular regions, which are each joined by a collagen-like connecting stand to a fibril-like central portion (Knobell et al., 1975; Brodsky-Doyle et al., 1976). A knowledge of the amino acid sequences of the polypeptide chains of Clq, and how these chains are disulphide bonded to yield subunits, allowed a model to be proposed that is consistent with the electron-microscopic studies.

Subcomponent Clq is composed of 18 polypeptide chains (six A-, six B- and six C-chains), each of which has mol.wt. approx. 24000-28000 (Table 1). Disulphide bonds between the A- and B-chains and between pairs of C-chains yields nine dimers, i.e. six A-B dimers and three C-C dimers of mol.wts. 52750 and 47600 respectively (Table 1). These nine dimers associate to yield the intact molecule, which has an estimated mol.wt. 459300 (based on the completed amino acid sequences of the A- and B-chains, the composition and partial sequence of the C-chain and the total carbohydrate content of the subcomponent Clq molecule). This value of mol.wt. 459300 is significantly larger than the previously reported values of 410000 (Reid et al., 1977) and 400000 (Calcott & Müller-Eberhard, 1972).

Each of the three types of polypeptide chain present in Clq is approximately 225 amino acid residues long and contains a region of 78-81 amino acid residues of collagen-like (-Gly-Xaa-Yaa-) repeating sequence located close to the N-terminal residue (Reid, 1976). In the C-terminal portion of each chain there are approximately 136 amino acid residues of non-collagen-like amino acid sequence, which appear to form predominantly β-type structure (Reid et al., 1982). There is good evidence for the presence of triple-helical structure in Clq (Brodsky-Doyle et al., 1976; Porter & Reid, 1979), and therefore it was proposed that three chains (one A-, one B- and one C-chain) would form a triple helix via their collagen-like, N-terminal regions and that their 'globular' C-terminal portions would form one 'head' group (Table 1; Reid & Porter, 1976). Any model proposed for Clq also has to take account of the finding that the repeating nature of the collagen-like, (-Gly-Xaa-Yaa-), amino acid sequences of the A- and C-chains are interrupted approximately half-way along the length of each collagen-like region. The insertion of a threonine residue between two Gly-Xaa-Yaa triplets in the A-chain, and the finding of an alanine residue in the position expected to be occupied by glycine in the C-chain (Fig. 3), would cause a disruption in any triple helix formed between the A-, B- and C-chains in view of the absolute requirement for glycine as every third residue to form a true triple helix. This disruption to the triple-helix formation would therefore be expected approximately half-way along the proposed collagen-like triple helix (Table 1). These observations indicate that one Clq molecule is composed of six triple helices aligned in parallel throughout half their length and that then diverge for the remainder of their length and then merge into one of the globular 'head' regions composed of the 136 C-terminal amino acid residues of one A-, one B- and one C-chain (Table 1).

The predictions that can be made for the length of the collagen-like regions, and size of the globular regions, by using the proposed model, agree well with the measurements made by electron microscopy (Porter & Reid, 1979). Although the model proposed appears to account for some of the major features of the structure of Clq, it does not explain why six triple helices, in one molecule, should become aligned in parallel throughout half their length (Table 1), i.e. by means of non-covalent interaction of three proposed 'structural units'. The evidence for the presence of 'structural units' in the formation of subcomponent Clq is, at present, indirect: the positions of the interchain disulphide bonds indicate that such units would be formed; in electron micrographs the molecule often appears to be divided into three portions, each of which resembles a 'structural unit'. Direct evidence for the formation of 'structural units' may be provided by the study of a non-functional form of subcomponent Clq that is found in patients with immune-complex-related nephritis (Thompson et al., 1980). The sera of these patients are completely deficient in C1 haemolytic activity, but the addition of purified normal subcomponent Clq, to the sera at a concentration at which it is normally found in serum, completely restored the C1 haemolytic activity to normal values. A molecule that reacted with anti-subcomponent Clq antibody, but that was antigenically deficient with respect to normal Clq, was found in the patients' sera (Thompson et al., 1980). The non-functional form of Clq did not bind to immune aggregates, or to IgG covalently linked to Sepharose, which showed that the affinity for aggregated IgG had been lost, or greatly diminished.

The non-functional Clq shows many similarities to normal Clq: it has a similar glycanic content, which suggests approximately 40% of the weight is carbohydrate.
Table 1. Number and molecular weights of the polypeptide chains and non-covalently linked subunits present in human C1q and in the fragments of C1q produced by limited proteolysis

\[ \text{Type} \quad \text{Mol.wt.} \quad \text{Number in one molecule} \]

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* The exact molecular weight of the collagenase fragment depends on the amount of collagen-like sequence left at the N-terminal end of each chain (see the text).
the same amount of collagen-like structure in both molecules; also, the non-functional molecule contains the hydroxylated amino acids hydroxyproline and hydroxylysine, although the content is lower (approx. 60% of that found in normal Clq); it possesses subunits of apparent molecular weights that are very close to those of the normal Clq when examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis under non-reducing conditions (Skok et al., 1981); it shows preferential incorporation of $^{125}$I label, into only one of the types of polypeptide chain, in a manner similar to that found for normal Clq (Fig. 4). However, by using gel filtration, in non-dissociating conditions, Dr. R. A. Thompson (Birmingham, U.K.) has found that the apparent molecular weight of the non-functional Clq is almost one-third the size of the 460,000-mol.wt. normal Clq. These results suggest that the non-functional Clq may be similar in structure to the proposed "structural units" (Table 1) and that the defect in the molecule lies in the inability of these abnormal 'structural units' to form the large fragments such as the globular 'head' regions or the entire collagen-like regions (Table 1). The "head" regions were found to be the binding of intact Clq to antibody-coated erythrocytes or to human serum that was capable of precipitating soluble IgG with non-functional Clq, which was followed by the development of an immune-complex-related renal disease, illustrates the importance of the classical pathway in clearing such complexes (Thompson et al., 1980).

The 8.0% (w/w) carbohydrate present in Clq is of two distinct types (Yonematsu et al., 1971; Calcott & Miller-Eberhard, 1972); 69% of it being present as glucosylgalactosyl disaccharide units linked to certain hydroxylysine residues in the collagen-like regions (Shinkai & Yonematsu, 1979; Reid, 1979); the remaining 31% of the carbohydrate is composed of six asparagine-linked sugar chains, which are located in the C-terminal globular regions of Clq (Mizuochi et al., 1979). Amino acid-sequence studies indicate that there is probably one asparagine-linked sugar chain located at position 124 in each A-chain (Reid et al., 1982).

**Interaction of Clq with** (i) **immunoglobulin G, (ii) subcomponents C1r and C1s, (iii) cell receptors and (iv) fibroblastin**

The division of the Clq molecule into two very distinctive types of structure allows it to be split by limited proteolysis into large fragments such as the globular 'head' regions or the entire collagen-like regions (Table 1). These fragments can then be used in the study of the interaction of Clq with other proteins.

(i) **Immunoglobulin G-G1q interaction.** Subcomponent Clq was first described as an 11S heat-labile factor present in normal human serum that was capable of precipitating soluble IgG aggregates in the presence of EDTA (Müller-Eberhard & Kunkel, 1972; Jones & Gardener, 1972). Reid et al. (1979) showed that Clq was a subcomponent of the C1 complex and that it played a linking role between immunoglobulins and the complement system by means of its strong binding to aggregated IgG and its participation in the activation of C1r and C1s.

When Clq is digested with bacterial collagenase most of the collagen-like regions of the molecule are fragmented to small peptides, leaving the six globular 'head' regions intact (Reid et al., 1972; Knobel et al., 1974; Pâques et al., 1979; Hughes-Jones & Gardener, 1979). Each 'head' produced in this fashion is considered to be composed of the C-terminal 145 amino acid residues of one A-, one B- and one C-chain, i.e. a total of 435 residues of mol.wt. 48,000 (of which 1905 is composed of an asparagine-linked carbohydrate complex). The N-terminal ends of these portions of the A-, B- and C-chains in the 'head' regions may be ragged, with nine, 12 or 15 residues of collagen-like sequence (i.e. three, four or five collagen-like triplets) remaining after collagenase digestion, since this is what has been found in collagenase digests of the purified chains (Reid, 1974; Reid & Thompson, 1978). The 'head' regions were found to be haemolytically inactive when used in an attempt to reconstitute the Fab region complex along with Clr and C1s, but they did bind intact Clq to antibody-coated erythrocytes or to immune aggregates (Hughes-Jones & Gardener, 1979; Pâques et al., 1979).

Direct evidence that IgG binds to the 'heads' of Clq was obtained by Tschopp et al. (1980), by examining the interaction between Clq and cross-linked IgG dimmers, by electron microscopy. The binding of Clq to monomeric IgG is weak, and binding constants of $4 \times 10^{-10}$–$5 \times 10^{-10}$ M$^{-1}$ have been reported (Schumaker et al., 1976; Hughes-Jones & Gardener, 1978), but the binding of Clq to aggregated IgG is much stronger being in the range $10^{-5}$–$10^{-7}$ M$^{-1}$ (Hughes-Jones & Gardener, 1978). The isolated globular 'heads' show only a weak affinity $(10^{-9} \text{M}^{-1})$ for aggregated IgG (Hughes-Jones & Gardener, 1979; Pâques et al., 1982). The Fab region dimers have been shown to be composed of from one to three binding sites for IgG per globular 'head' of Clq (Schumaker et al., 1976), but Tschopp et al., (1980) have suggested that the number of cross-linked IgG dimers that could be accommodated on a single Clq molecule is three.

The evidence available at present appears to favour a model of Clq-IgG interaction in which the Clq becomes firmly attached to the multiple Fc regions, presented by aggregated IgG, without the requirement for a conformational change to have taken place in the Fc regions (i.e. mediated by the interaction of the Fab regions of IgG antibody with a large-molecular-weight antigen) (Mettler, 1978). The primary site on the IgG molecule that interacts with Clq is located in the C$_{6}$2 domain, as has been shown for human (Dorrington & Painter, 1974; Yasmeen et al., 1976), mouse (Kehoe & Fougerac, 1969) and rabbit (Colomb & Porter, 1975) IgG. It has not been generally agreed which residues in the C$_{6}$2 domain are directly involved in IgG-Clq interaction. Dweck and his colleagues (Burton et al., 1980; Emaneul et al., 1982) used a variety of approaches, such as: examination of conservation of amino acid sequence in the C$_{6}$2 domains of several species; use of inhibitors of Clq-IgG interaction; specific chemical modification and labelling of the residues in the Fc region of IgG that appear important in the
binding. They concluded, in agreement with earlier work (Hughes-Jones & Gardener, 1978), that formation of the Clq–IgG complex is largely the result of ionic interactions and that the charged residues Glu(138), Lys(159), Lys(162) and Glu(119) along with Thr(158) and Ser(159) in the 318–337 section of the heavy chain of IgG play an important role in the binding. Other studies (Prystowsky et al., 1981; Lukas et al., 1981), with the use of synthetic peptides, modelled on sequences of amino acid acid sequence found in the Cα2 domain, as inhibitors of Cl activity also suggest that charged residues are involved in Clq–IgG interaction. However, the site suggested by these workers lies in the 255–292 section of the heavy chain and involves the residues His(121), Lys(138), Lys(143) and Arg(161). Two other studies, one involving the use of fragments of the Cα2 domain of human IgG (Lee & Painter, 1980) and one comparing the Cα2 sequences of IgG from a variety of species (Brunhouse & Cebra, 1979), implicated residues 253–306 and 290–295 respectively as being involved in Clq binding. Thus, although the precise location on IgG that is involved in interaction with Clq is not agreed upon, most of the recent studies favour an ionic rather than a hydrophobic type of binding between the two molecules. The amino acid residues in the Clq molecule that are involved in Clq–IgG interaction would be expected to be primarily located in the globular ‘head’ regions. When the amino acid sequences of the C-terminal globular regions of the chains of Clq are aligned to give maximum homology it can be seen (Fig. 5) that there is a framework of conserved residues (approx. 27% of the total) that, apart from Lys(159) and Arg(161), are all hydrophobic in nature, or neutral in charge, and includes the intrachain disulphide bond. The residues may be important in conserving the structure of the ‘heads’ rather than being directly involved with binding. Over certain sections that are not highly conserved there are a preponderance of charged residues, e.g. sections 101–107, 154–159, 192–212 contain seven (three negative, four positive), nine (one negative, eight positive) and 16 (12 negative, four positive) charged groups respectively when the residues in these sections, in all three chains, are examined. Therefore any ionic interaction between IgG and Clq may possibly involve groups from one, or more, of these sections.

(ii) Interaction of subcomponents Clr and Cls with Clq. The collagen-like portions of the Clq molecule appear to be involved in the interaction with the Clr2-Ca2+-Clq complex during the formation of the C1q complex. The interaction presumably takes place primarily between the Clq and Clr subcomponents, since Clr can be bound to antigen–antibody–Clq complexes in the absence of Cls but the binding of Cls to the complexes requires the presence of Clr (Sim, 1981). The preparation of the collagen-like regions of Clq can be achieved by limited proteolysis of the native intact molecule with pepsin for 16 h at 37°C and at pH 4.5. Under these conditions most of the globular ‘head’ regions are digested to small peptides, leaving the large collagen-like peptic fragment of mol.wt. approx. 190000 (Table 1; Reid, 1976; Brodsky-Doyle et al., 1976). This fragment is an inhibitor of the reconstruction of C1 from Cl, Clr and Cls (Reid et al., 1977), which provides indirect evidence of Clr2-Ca2+-Clq, interaction with Clq. It has also been found that Clr2-Ca2+-Clq will block the binding of the Clq molecule to all receptors that recognize and bind the collagen-like peptic fragment of Clq (Tenner & Cooper, 1981). More direct evidence of the site of interaction of the Clr2-Ca2+-Clq complex with Clq was obtained by Strang et al. (1982), who found, on electron microscopy of the chemically cross-linked C1 complex, that in profile the Clr2-Ca2+-Clq tetramer appeared to be located in the region of the six connecting strands, i.e. between the Clq ‘heads’ and fibril-like central portion.

(iii) Cell receptors for Clq. Early studies (Dickler & Kunkel, 1972; Sobel & Bokisch, 1975) showed that Clq could bind to human lymphocytes, a finding that appeared to be consistent with the observation that the binding of aggregated IgG to

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lymphocytes or lymphoblastoid cell lines was enhanced by preincubation of the cells with Clq (Sundquist et al., 1974; Gupta et al., 1978). Only about 26% of human peripheral mononuclear cells bind Clq, and this population of cells is composed largely of monocytes and B-lymphocytes (Tenner & Cooper, 1981). A minor subpopulation of lymphocytes, which were negative for conventional B-cell and T-cell markers, as well as polymorphonuclear leucocytes were also found to bind Clq. The same authors (Tenner & Cooper, 1980) have also shown that the collagen-like regions of the Clq molecule are involved in the binding to the Clq receptor on peripheral-blood mononuclear cells.

Platelets also appear to have specific receptors for the collagen-like portions of Clq. Platelets adhere readily to collagen, and this binding causes the release of the contents of the platelet amine-storage granules, the released ADP then contributing to platelet aggregation. Intact native Clq, and the collagen-like peptic fragment of Clq, inhibit collagen-induced platelet aggregation (Cazenave et al., 1976; Wautier et al., 1977). The concentrations of Clq used to give strong inhibition in those experiments were less than those that occur in plasma, an observation that indicates that it may be of physiological significance that Clq can compete with collagen for specific sites on the platelet surface. The region in the Clq molecule that appears to be primarily responsible for platelet interaction is located in the collagen-like section of the A-chain (Wautier et al., 1980).

(ii) Fibronectin–Clq interaction Fibronectin is a 440,000-mol.wt. glycoprotein, present on cell surfaces and in plasma, that forms complexes with collagen, collagen-derived fragments and other proteins, such as asymmetric-form acetylcholinesterase, which contain collagen-like structures (Mosher et al., 1980; Emmerling et al., 1981). Both collagen and the asymmetric form of acetylcholinesterase can serve as substrates for transglutaminase to cross-like with fibronectin or, in the absence of transglutaminase, to bind fibronectin. The collagen-like peptic fragment of Clq, and the intact Clq molecule, have also been shown to interact with fibronectin, although the transglutaminase-mediated form of covalent cross-link has not yet been demonstrated (Menzel et al., 1981; Isliker et al., 1981). It is possible that the Clq-fibronectin interaction could be involved in reticuloendothelial clearance of Clq-coated immune complexes from the blood, with fibronectin acting as an opsonic protein that enhances ingestion by macrophages. In this connection it has been shown that plasma fibronectin is involved in the elimination of collagen-coated particles (Allan et al., 1973).

Activation and control of the complement system at the Cl step

As described above, the Clr→Ca2+→Cls complex appears to be located in a central position on the Clq molecule and to be associated with the collagen-like regions rather than the globular ‘head’ regions. In normal serum the collagen-like regions of Clq would therefore be expected to be masked by the Clr→Ca2+→Cls complex. However the activated form of the Cl complex is under the regulatory control of the glycoprotein Cl inhibitor, which binds rapidly and tightly to the activated forms of the Clr and Cls subcomponents, causing their rapid dissociation from antigen-antibody-C1 aggregates (Fig. 6) (Sim & Rebol, 1981). The Clq is left bound to the antigen-antibody complex, probably by means of the ‘heads’, which would leave the collagen-like regions available for interaction with suitable cell receptors, fibronectin (Fig. 6) or a recently described serum inhibitor of Clq (Ghebrehiwet, 1981).

Binding of the activated forms of C4 and C3 to immune aggregates

Component C4 is composed of three polypeptide chains, α, β, and γ, which have mol.wts. of 93,000, 75,000 and 33,000 respectively. Activation of C4, by the Cls enzyme of the Cl complex, results in the α-chain of C4 being split at one position.

Fig. 6. Activation and control via the Cl complex

Interaction of the Clq–Clr→Ca2+→Cls complex with antibody–antigen (Ag) brings about the activation of the Clr and Cls proenzymes. The bound Clr can continue the classical-pathway activation by splitting C4 into C4b and C4a. A small percentage of the freshly activated C4b becomes covalently bound to the Fd region of the antibody molecules (Campbell et al., 1980). Control of the Cl complex is mediated by the Cl inhibitor, which combines stoichiometrically with Clr and Cls. This removes the Clr and Cls from the Cl-antibody–antigen aggregate, leaving the collagen-like regions of Clq free to interact with suitable receptors.
group. These observations have lead to the proposal that a thioester bond is present in all three molecules (Fig. 7). The in all three that cannot be detected in the native molecule;'small covalent bonds after cleavage of a single peptide bond; they are to bind to receptive surfaces through hydroxy or amino groups

particles such as cell membranes, polysaccharides or immune aggregates. The bound fragments of C4 and C3 can participate in immune adherence and opsonization reactions by binding to specific receptors on certain types of cells and thus can enhance phagocytosis of foreign particles (Lachmann, 1979). Immune binding activity on activation (Law et al., 1979; Sim et al., 1981) was of interest to study the role of intact IgG, in the alternative-pathway activation of C3, conditions were employed that allowed only alternative-pathway activation, i.e. by the addition of EGTA and Mg2+ to the serum, which blocks the classical pathway at the C1 stage but allows alternative-pathway activation to proceed (Platts-Mills & Ishizaka, 1974). The use of EGTA therefore allowed the effects of IgG immune aggregates and F(ab)2, immune aggregates on the alternative pathway to be compared. It was found that both types of aggregate caused exactly the same extent of utilization of alternative-pathway components when incubated in serum under conditions allowing only alternative-pathway activation (Table 2). Further studies (Gadd & Reid, 1981b) showed that, under optimum conditions, approx. 200 molecules of C3b could be bound per 1000 molecules of either IgG or F(ab)2 when the ratio of C3 molecules to antibody molecules in the activation mixture was 6:1, i.e. approx. 3% of the C3 available was bound to the aggregates. The amount of C3b bound was found to correlate with the formation of C3-cleaving activity on the surface of the antibody–antigen aggregates (Table 3), which

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**Fig. 7. Amino acid sequence around the proposed thioester bond in C4**

The C4 amino acid-sequence data are from Campbell et al. (1981). The cysteine and glutamic acid residues considered to be involved in a thioester bond are marked with an asterisk (*). The amino acid sequences of the equivalent portions of C3 (Tack et al., 1980) and α2-macroglobulin (Swenson & Howard, 1980) are identical with the C4 sequence except that asparagine, rather than threonine, is found immediately after reactive glutamic acid residue.

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**Table 2. Utilization of complement under conditions allowing only alternative-pathway activation**

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<td>C1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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**Table 3. Binding of C3b and formation of an alternative-pathway C3-cleaving activity on the surface of antibody–antigen aggregates**

Ovulbin–anti-ovulbin aggregates, containing either IgG or F(ab)2, rabbit antibody (0.7nmol), were incubated with human serum (790μl) containing 10mM-EGTA, 5mM-MgCl2, and 125I-labelled C3 (10μg/ml) for various time periods. After a washing in ice-cold buffer, the amount of C3b bound was estimated by counting 125I radioactivity, and C3-cleaving activity was measured haemolytically (data taken from Gadd & Reid, 1981b).
indicated that the C3b,Bb-complex proteinase was being formed on the aggregates. Fujita et al. (1977) have also reported that C3-cleaving and C5-cleaving activity can be formed on the surface of immune aggregates on incubation of the aggregates in human serum.

The exact site on the rabbit IgG molecule to which the C3b becomes attached is not known, but by use of radiolabelled IgG and C3 preparations it has been established that there is probably a covalent bond formed between the α'-chain of C3b and the heavy chain of IgG (Gadd & Reid, 1981b) in a similar fashion to that observed of the binding of C4b to IgG (Figs. 6; Campbell et al., 1980). Aggregates containing P(ab')2 are as effective as those containing IgG at causing C3 activation and binding (Tables 2 and 3); therefore the site (or sites) at which C3b becomes bound must lie in the Fd region of the IgG molecule. It has been shown that mild reduction of the rabbit IgG molecule, which leads to the splitting of the α'-chain of C3b and the heavy chain of IgG under conditions allowing only alternative-pathway activation, it was found that immune aggregates containing mouse IgG were used to activate complement, under conditions allowing both classical-pathway and alternative-pathway activation, it was found that covalent bond formation took place between the α'-chain of mouse C3b and the heavy chain of mouse IgG (Pfaffenbach et al., 1982).

Antigen–antibody aggregates can be solubilized, under certain conditions, during complement activation by a process that involves the incorporation of C3b into the antigen–antibody lattice (Takahashi et al., 1976, 1980). This indicates that there is a second role for the binding of C3b to antibody. The solubilization, which is initiated after the binding of approx. 1 C3b molecule per rabbit IgG antibody molecule, is brought about primarily by the alternative pathway, since a mixture of the purified components C3, B, D, H, I and P will duplicate the effect mediated by EGTA Mg2+-treated human serum (Fujita et al., 1981). The molar ratio of C3b to rabbit IgG antibody in the solubilized complexes was 1:11, which indicates that there may be only one, or two, major binding sites for C3b on each antibody molecule.

Other studies in which IgG antibody appears to be required to initiate alternative-pathway activation and thus transform a non-activator of the alternative pathway into an activator include: the alternative-pathway lysis of sheep erythrocytes by the action of guinea-pig IgG, antibody, or its F(ab')2 fragments, and guinea-pig complement (Nicholson-Weller et al., 1981); the alternative-pathway activation by native capsular polysaccharide antigen of the type III group B Streptococcus, which requires the presence of a high ratio of antibody to the capsular.

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### Table 4. Effect of mild reduction of IgG on its ability to activate the alternative pathway under conditions allowing only alternative-pathway activation

The rabbit IgG antibody samples were reduced and alkylated to different degrees before aggregate formation. The number of disulphide bonds split were estimated by amino acid analysis, and the amount of intact remaining IgG was estimated by gel scan. The antibody–antigen aggregates were incubated with 50μl of human serum containing 10mm-EGTA and 5mm-Mg2+ for 60 min at 37°C. The data are taken from Gadd & Reid, (1981a).

<table>
<thead>
<tr>
<th>IgG immune aggregates (nmol)</th>
<th>No. of disulphide bonds split (nmol)</th>
<th>Intact IgG remaining (%)</th>
<th>Haemolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
<td>C3</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>80</td>
<td>B</td>
</tr>
<tr>
<td>10</td>
<td>1.5</td>
<td>50</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>0</td>
<td>None</td>
</tr>
</tbody>
</table>

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![Fig. 8 Possible role for the binding of C3b to antibody](image)

C3b* denotes the freshly activated metastable form of C3b. It is considered that any C3b that is not bound to a 'protected site' will readily undergo limited proteolysis at two positions in the α'-chain under the action of the enzyme factor I and its cofactor H to yield C3b (E. Sim et al., 1981; Harrison & Lachmann, 1980), which cannot be used in the formation of C3b,Bb complex.
antigen (Edwards et al., 1980). In these cases the initiating function of the antibody must be related to the formation of a site for the initial deposition of C3b, which affords the bound C3b protection from the action of factor H and the enzyme I. However, it is not clear whether the site is located on the antibody molecule or whether it perhaps becomes exposed on the surface of the cell, or particle, after antibody binding has taken place.

It has also been shown that IgG antibody to particulate and cellular activators of the alternative pathway will augment activation by increasing the rate of consumption of C3 and uptake of C3b. This has been found for the following alternative pathway activators: zymosan (Schenkein & Rudly, 1981a,b); rabbit and mouse erythrocytes (Polhill et al., 1978; Moore et al., 1981); cells infected with measles virus (Ehrnstr, 1978; Sissons et al., 1979, 1980). In each case the enhancing effect was still observed when the F(ab') region of the antibody molecule was used. Covalent binding of C3b to the enhancing antibody was not detected in the one study in which this point was examined (Schenkein & Rudly, 1981). Thus the possibility that antibody bound to the particle or cell surface exposes hidden sites that are capable of covalent interaction with freshly activated C3b has to be considered as a probably explanation of the enhancing effects observed on the alternative-pathway activation in these cases.

Several of the proteins involved in the initial activation steps of the classical and alternative pathways have, as has been discussed above, been found to show unusual structural features, i.e. the collagen-like regions in C1q, the covalent binding reaction of freshly activated C4 and C3, the circulation of factor D in only its activated form and the higher than expected molecular weights of the catalytic chains of the activated forms of C2 and factor B. Initial studies on the proteins, such as C1-inhibitor, C4-binding protein and properdin, indicate that these proteins may display some unusual properties. C1-inhibitor, for example, contains an exceptionally high carbohydrate content (69 mol of hexose, 47 mol of hexosamine and 51 mol of sialic acid per mol of protein of mol.wt. approx. 100000) and forms complexes, with the catalytic chains of the activated forms of C1r and C1s, that are resistant to reduction and boiling in sodium dodecyl sulphate/urea or treatment with other strong denaturants (for review see Sim & Reboul, 1981). The C4-binding protein is the largest of the complement proteins (having mol.wt. approx. 600000 in non-dissociating conditions); it interacts with surface bound and soluble forms of C4b, acting as a cofactor in the rapid cleavage of the a-chain of C4b at two positions by the enzyme I (Fujita et al., 1978; Giga et al., 1979; Nagaasawa et al., 1980; Press & Gagnon, 1981). In a system composed of purified components, it was found that the C4-binding protein may become covalently linked to freshly activated C4b, which property could be of physiological importance (Villiers et al., 1982). Despite its high molecular weight, C4-binding protein appears to be composed of only one type of polypeptide chain of mol.wt. approx. 70000; it is considered that there may be up to ten identical disulphide-linked chains per molecule (Villiers et al., 1982; Reid & Gagnon, 1982). Properdin, which stabilizes the complex proteinases C3b,Bb and C3b,Bb,C3b (Fearon & Austen, 1975; Medicus et al., 1980), appears to be present in serum in a native or precursor form that may be converted via a conformational change into an activated form on its binding to the C3b,Bb, C3b complex (Medicus et al., 1980). The native and activated forms can be distinguished only by functional assays, since chemically and antigenically they appear identical. Both forms of properdin are probably composed of three identical polypeptide chains of mol.wt. approx. 55000 (DiScipio, 1982). It has been reported that only four amino acids, namely proline, glycine, glutamic acid and half-cystine, account for over 46% of the total amino acid composition of properdin (Minta & Lepow, 1974; Reid & Gagnon, 1981). The high contents of proline and glycine could account for the rod-shaped structure that can be predicted for properdin from its physical parameters (DiScipio, 1982). However, amino acid sequence data, accounting for 50% of the molecule, indicate that the unusual amino acid composition is probably distributed throughout the molecule but there is no evidence for the presence of any repeating type of amino acid sequence (Reid & Gagnon, 1981).

Clearly further physical and chemical studies on the proteins of the complement system will allow a better understanding of its activation and control.

I thank the Wellcome Trust and the Biochemical Society for making this generous award, and also to acknowledge the help of the staff, students and visitors at the M.R.C. Immunology Unit who have been involved with various aspects of the work described in this Lecture. I especially acknowledge the advice and encouragement of Professor R. R. Porter, the contributions made by my co-workers Dr. D. M. Scott, Dr. R. B. Sim, Dr. D. M. A. Briggs and Dr. K. J. Wood, and the technical expertise of Mr. A. Dodds, Mr. T. Gascoyne, Mr. T. Willis and Ms. J. Parsons.

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