Clin. exp. Immunol. (1970) **7**, 211–220.

ACTIVATION AND INHIBITION OF IgG MEDIATED COMPLEMENT FIXATION BY STAPHYLOCOCCAL PROTEIN A

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(*Received* 10 *February* 1970)

SUMMARY

Staphylococcal protein A, when added to fresh human, guinea-pig, dog or pig serum, caused a marked depletion of complement. This complement consumption, further studied in the human system, was noted only in γ-globulin excess. The consumption of individual complement components indicated an activation mechanism similar to the one induced by aggregated human γ-globulin; i.e. a marked depletion of early-acting components. The activation was time- and temperature-dependent. Almost no complement activation was seen using fresh serum from a patient with agammaglobulinaemia. Inhibition of complement activation was noted when protein A was added at equivalence of precipitation or in excess. The dual effect of protein A might be explained by (I) its ability to arrange γ-globulin molecules in a way initiating the complement cascade and (II) inhibition of Fc mediated complement activation by steric hindrance when added in excess. Possible roles of protein A in the pathogenesis of staphylococcal infections are discussed.

INTRODUCTION

The primary step in complement activation mediated by antibody IgG or aggregated IgG involves a reaction with Clq (Müller-Eberhard, 1968). This complement component combines with the Fc part of human immunoglobulin G, which also carries other biological activities such as the cytophilic site (LoBuglio, Cotran & Jandl, 1967), opsonic site (Quie, Messner & Williams, 1968), and skin sensitizing activity (Terry, 1965). The antibody combining activity for antigens, on the other hand, resides in the Fab parts of the γ-globulin molecules. The role of the antigen in IgG induced complement fixation seems to be that of bringing two antibody molecules together, thereby initiating the reaction (Borsos & Rapp, 1965; Cohen, 1968). Native human γG globulin combines with Clq without activating Cl esterase (Müller-Eberhard, 1968). The precise molecular events relating the Fc piece of γ-globulin to the activation of Cl esterase are not yet clear.

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A striking exception to the rule that antigens combine with sites located on the Fab parts of γ-globulin molecules has been discovered by Forsgren & Sjöquist (1966). Most *Staphylococcus aureus* strains contain a cell wall substance, protein A, which gives a precipitin reaction with all human sera tested (Jensen, 1959). This protein A, however, combines with the Fc portion of human γG globulin (Forsgren & Sjöquist, 1966). It has later been shown that the reactivity is only present in IgG subgroups 1, 2 and 4, whereas IgG-3 molecules lack combining structures for protein A (Kronvall & Williams, 1969). When tested in phagocytosis systems utilizing IgG opsonins, protein A completely blocked the uptake and killing of bacteria (Dossett *et al.*, 1969). Both protein A reacting structures and opsonic sites are located on the Fc part of the molecules. As complement fixation is also induced via structures on the Fc part of IgG antibodies, a blocking effect might be expected by protein A in a manner parallel to blocking of the opsonic site. However, injection of protein A in rabbits, passively sensitized with human γ-globulin, produces an Arthus-like reaction (Gustafson, Sjöquist & Stålenheim, 1967). Since this phenomenon is mediated via the complement system (Cochrane, 1967), this seems to indicate that protein A in fact can induce complement fixation. Such a function might be of considerable importance in staphylococcal disease states. These contradictory findings prompted us to study the effect of staphylococcal protein A on complement reactivity.

Recently, Sjöquist & Stålenheim (1969) reported that protein A–IgG complexes are capable of activating guinea-pig complement. It was also shown that this activation took place only in antibody excess. The present investigation confirms these results. We were also able to show a similar activation of human complement and further to characterize the specific type of complement activation by kinetic data and complement component profiles. An apparent complement inhibiting capacity of protein A was also demonstrated under certain circumstances.

MATERIALS AND METHODS

Protein A

Staphylococcal protein A was partially purified from *Staphylococcus aureus*, Cowan I, according to Jensen (1959) as described previously (Kronvall & Williams, 1969). Protein A was further purified using disk electrophoresis. Protein A material, as defined by its reactivity with the Fc part of γG globulin, was localized after disk electrophoretic separation by its precipitation in agar gel with a myeloma globulin (Fig. 1b). The apparent two-zone migration was confirmed by cutting gels after disk electrophoresis in slices and determining the protein A content of the eluates of these slices (Fig. 1a). Protein A from eluates of both the cathodal and the anodal zone were used in studies on complement fixation in normal human serum.

Quantitative precipitin curves

To 0·1 ml aliquots of normal human serum, increasing amounts of protein A were added. The total volume of the mixtures was adjusted to 0·3 ml with phosphate buffered saline (PBS, 0·15 M NaCl, 0·01 M phosphate, pH 7·3). The tubes were kept at 4°C for 1 week and the precipitates formed spun down at 9000 rev/min for 30 min. The precipitates were then washed with cold PBS and dissolved in 0·1 ml 0·1 N NaOH. The protein content was measured with a modification of the Folin method (Lowry *et al.*, 1951).

Aggregated human γ-globulin

For studies on IgG mediated complement fixation, human γ-globulin preparations (Cohn Fraction II, Hyland Laboratories) were heated to 63°C for 20 min at a concentration of 1·6 mg/ml.

Complement sources

Fresh frozen normal human, guinea-pig, dog and pig sera were stored at −70°C until

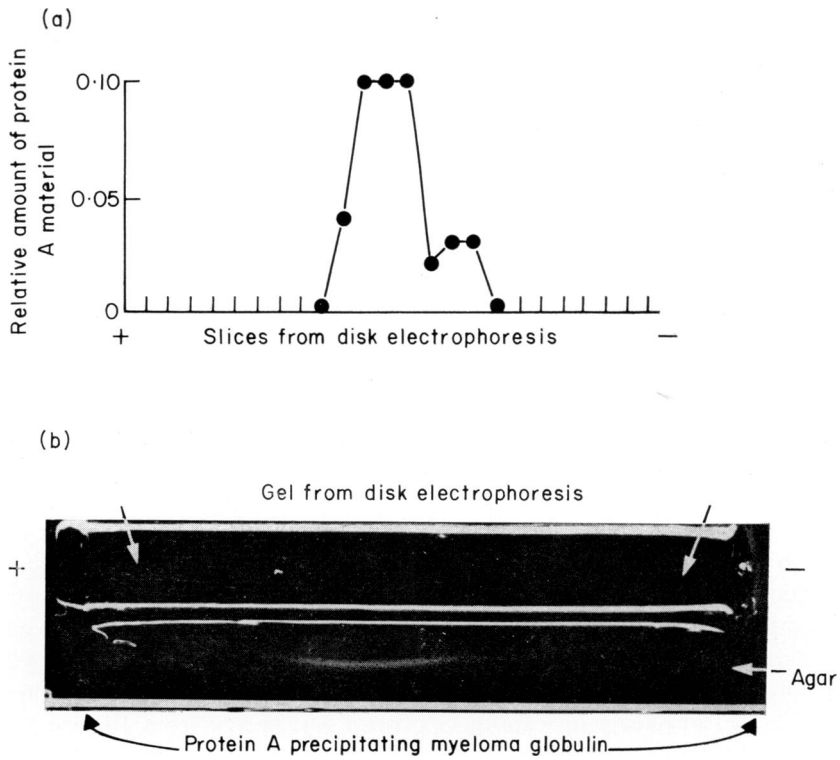

FIG. 1. Further purification of staphylococcal protein A by disk electrophoresis. Fig. 1(a) shows the protein A concentration in fractions obtained by cutting and eluting the gel after electrophoretic separation. Fig. 1(a) confirms the two-zone migration of protein A: a protein A precipitating myeloma globulin (1 mg/ml) was allowed to diffuse in agar towards the disk electrophoresis gel giving a double-arched precipitation line.

used. Both human and guinea-pig serum contain γ-globulin molecules capable of reacting with protein A via structures on their Fc parts (Jensen, 1959; Löfkvist, 1966; Forsgren, 1968). Previous phylogenetic studies have indicated that dog and pig sera also contain similar protein A reacting γ-globulin molecules (Kronvall *et al.*, 1970b). Serum from a patient with agammaglobulinemia, *KE*, was used as a complement control with low γ-globulin. Using monospecific antisera in the Oudin technique, the IgG concentration of this serum was estimated to 83 mg%, IgA to <20 mg% and IgM to 40 mg%. The complement level of this serum was 40 CH_{50} units/ml by the method of Osler, Strauss & Meyer (1952) and 60 CH_{50} units/ml in the assay described below.

Complement assay

Twenty μl of test reactants were added to 100 μl of test sera and incubated at 37°C for 60 min. Veronal buffered saline with optimal CA^{++}, Mg^{++} and gelatin concentrations (GVB^{++}) was then added to a final volume of 1 ml. For dose–response curves protein A was added to 0·1 ml of fresh serum and the volume adjusted to 0·3 ml with GVB^{++} before incubation. Serial dilutions were performed with 0·5 ml of GVB^{++}. 1·5 ml of GVB^{++} and 0·5 ml of optimally sensitized sheep erythrocytes (1×10^8 per ml) were added and incubation continued for 60 min at 37°C. Five ml of saline was added, the mixture centrifuged and the optical density of the supernatants determined at 412 mμ. The CH_{50} value per ml was determined by interpolation.

Cobra venom factor (CVF)

A complement activating fraction of Egyptian cobra (*Naja haja*) venom was separated by chromatography on DEAE cellulose (Nelson, 1966).

Endotoxin lipopolysaccharide

Endotoxin lipopolysaccharide (LPS), prepared from *Escherichia coli* by trichloracetic extraction, was generously provided by Dr W. W. Spink, Minneapolis, Minnesota.

RESULTS

When partially purified protein A was added to fresh normal human serum and incubated for 60 min at 37°C, an increasing consumption of complement was noted with increasing amounts of protein A (Figs. 2 and 3). For maximal consumption of the complement in 0·1 ml of human serum only 50 μg of a protein A preparation was necessary. In most antigen–antibody systems the degree of complement fixation usually parallels that of precipitation. A quantitative precipitin curve using the same reagents revealed, however, that maximal complement fixation occurred at a protein A concentration which was only about 1/10 of that giving maximal precipitation (Fig. 1). The question was therefore raised whether this complement activation really was the result of the interaction between protein A and γ-globulin. Further purified preparations of protein A obtained from disk electrophoresis including both the anodal as well as the cathodal peaks (Fig. 1) were therefore tested and also shown to induce complement fixation. The degree of activation paralleled the amount of protein A added. When protein A was added to serum from a patient with agammaglobulinaemia (KE, 83 mg% γG), the resulting drop in complement was much less when compared to normal human serum (Fig. 3).

Protein A preparations, when added to fresh guinea-pig serum, depleted complement in a way similar to the human system. Protein A was also added to fresh dog and pig sera with consequent drop in complement. γ-globulin from both these animal species reacts with protein A in a similar fashion to human and guinea-pig γ-globulin (Kronvall *et al.*, 1970b).

The protein A induced complement fixation in fresh normal human serum was further studied in several ways. Time–temperature dependence curves showed that the activation varied inversely with the temperature (Fig. 4). No complement consumption was noted when tests samples were incubated at 0°C. An analysis of the activation of individual complement components was also performed (Fig. 5). With an 80% depletion of total complement by protein A the C2 component showed the most marked decrease. As much as 94%

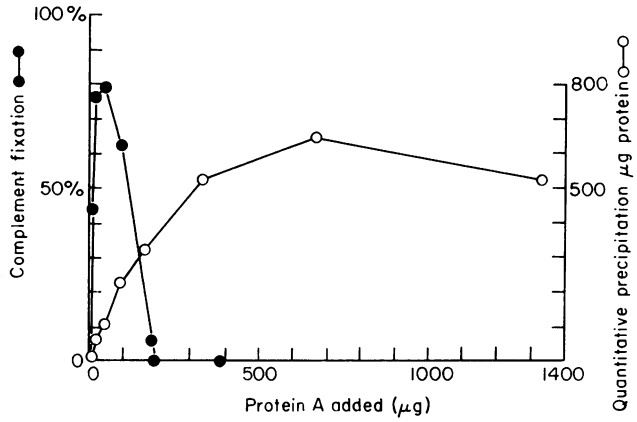

FIG. 2. Comparison between complement fixation and quantitative precipitation by staphylococcal protein A when added to 0·1 ml of fresh human serum in a total volume of 0·3 ml. Complement fixation was tested after incubation at 37° C for 1 hr. Precipitates were recovered after 7-day incubation at 4° C and the protein contents determined.

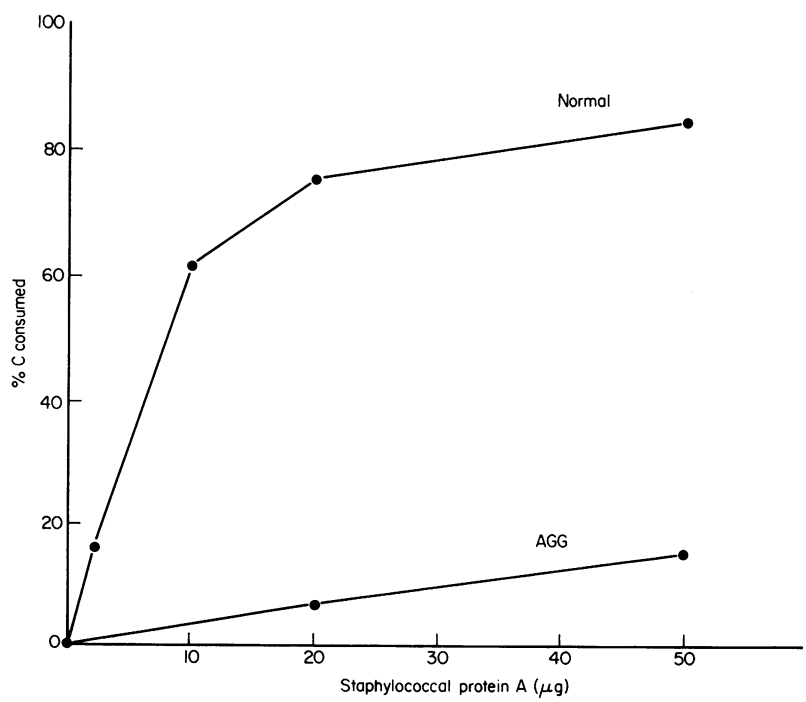

FIG. 3. Complement fixation induced by protein A in fresh normal human serum (curve marked Normal) and in fresh serum from a patient with agammaglobulinaemia (curve marked AGG).

of the original C2 concentration was consumed. The other components tested, C1, C4 and C3 (including later components), showed depletions between 63% and 75%. As a comparison

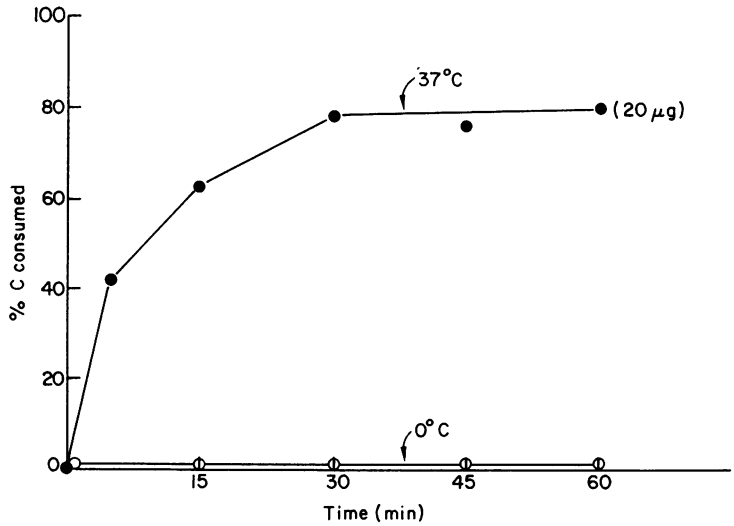

FIG. 4. Complement fixation induced by protein A (20 μg) in fresh normal human serum after various periods of incubation at two different temperatures (37°C and 0°C).

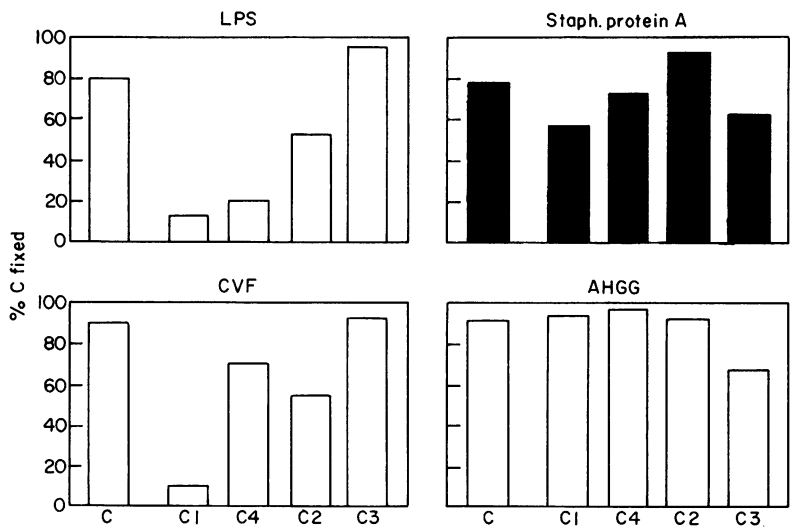

FIG. 5. Consumption of individual components of complement by staphylococcal protein A, with the complement profiles induced by aggregated human γ-globulin (AHGG), cobra venom factor (CVF) and purified endotoxic lipopolysaccharide (LPS) as a comparison.

the profiles induced by heat aggregated human γ-globulin (AHGG) as well as purified gram-negative lipopolysaccharide (LPS) and cobra venom factor (CVF) were determined (Fig. 5). LPS and CVF are known to primarily deplete the later components in the complement cascade (Gewurz, Shin & Mergenhagen, 1966) and their profiles are different from the

one induced by protein A. The AHGG-profile, a primarily early component response, shows a close similarity to the activation of complement by protein A. It is thus possible that similar mechanisms in immunoglobulin orientation are present in these two types of complement fixation.

Since staphylococcal protein A combines with Fc structures of human γG globulin, complement fixation might theoretically be impeded by this substance. This could in fact be the case as no complement activation was noted in the region of precipitation at equivalence. To study this hypothesis, AHGG was added to normal human serum or serum from a patient with agammaglobulinaemia. In both cases, AHGG caused an almost complete drop in measurable haemolytic complement (Table 1). When protein A was included, a complete

TABLE 1. The effect on human complement of heat aggregated human γ-globulin (AHGG) and staphylococcal protein A (pA). Fresh sera from an agammaglobulinaemic patient as well as a normal individual were used as complement sources.

	Agammaglobulinaemic serum (% depletion of complement)	Normal human serum (% depletion of complement)
AHGG (32 μg)	> 90	> 90
pA (200 μg)	< 10	35
AHGG + pA	< 10	50

block of the complement fixing activity of AHGG in agammaglobulinaemic serum was evident. Protein A could thus be shown to act as an effective blocking agent for complement fixation induced by aggregated IgG.

DISCUSSION

Previous experiments by Gustafson *et al.* (1967) showed that the reaction between human γG globulin and staphylococcal protein A gives rise to an Arthus-like reaction in the rabbit. The results indicated that protein A, although reacting with the same portion of γ-globulin as the first complement component, in fact can initiate complement activation. This has in fact recently been shown to occur using guinea-pig complement (Sjöquist & Stålenheim, 1969). Activation of complement was noted when protein A was added directly to fresh guinea-pig serum and also upon addition of performed aggregates of protein A and human and guinea-pig γ-globulin. Under the experimental conditions used, maximal complement fixation occurred at marked antibody-excess. There was still complement fixation noted at the equivalence point of precipitation. Our data presented here confirm the occurrence of complement activation by protein A when added to fresh guinea-pig serum. The same effect on complement could also be shown using fresh human serum and dog and pig serum as well as the complement source. When correlated to quantitative precipitation between protein A and human serum (Fig. 2), we found maximal complement activation when adding about 1/10 the amount of protein A giving precipitation at equivalence. In contrast to the results obtained by Sjöquist & Stålenheim (1969) using a slightly different technique with guinea-pig complement we did not see any fixation of human complement at the point of maximal precipitation.

The complement component profile shown by protein A-induced complement fixation with a marked depletion of early components is characteristic of IgG induced complement fixation via Clq. Activation of this sequence requires a close arrangement of γ-globulin molecules, either by antigen (Borsos & Rapp, 1965; Cohen, 1968) or induced artificially by various methods of aggregation (Ishizaka *et al.*, 1967). Protein A, therefore, seems to represent another substance capable of bringing γ-globulin molecules into sufficient proximity necessary for the activation of Cl-esterase.

The precise configuration of protein A–IgG complexes inducing complement fixation is not yet known. The valency of γ-globulin molecules for protein A seems to be 2 with an equilibrium constant in the range of 4×10^7 litres/mole (Kronvall, Quie & Williams, 1970a). The valency of the protein A molecules, however, is not known. By ultracentrifugational separation 30–40S human γG globulin aggregates were shown to be twenty times more effective in complement activation that 10S dimers (Isliker *et al.*, 1968). Therefore, it seems possible that both dimerization of IgG by protein A and larger structures due to secondary aggregation might give rise to complement activating complexes.

The question was raised whether protein A might in some circumstances act as a blocking agent of IgG induced complement fixation. Results obtained when protein A was added to a complement activating system utilizing heat aggregated human γ-globulin indicated that such blocking indeed can take place (Table 1). An inhibiting effect by staphylococcal protein A is in line with the blocking of γG dependent phagocytosis reported previously (Dossett *et al.*, 1969).

The lack of parallelism between ability to fix complement and precipitation of human γG can be explained on the basis of the two phenomena described above: (I) ability to arrange γG molecules in a way necessary for complement activation and (II) inhibition of complement activation by competing for closely located Fc-structures. At marked γ-globulin excess the former mechanism is prevalent, and at a higher concentration of protein A the latter inhibiting activity becomes apparent. In their study Sjöquist & Stålenheim (1969) interpret the fact that complement fixation can be induced by protein A as an indication that the combining sites for protein A and Clq are not identical. Our results, emphasizing the inhibiting capacity of protein A, would indicate that the sites in fact may be the same. The IgG subgroup patterns for these two reactivities, however, are different, IgG-3 molecules being capable of combining with Clq (Kohler & Müller-Eberhard, 1969) but not with protein A (Kronvall & Williams, 1969); the reverse being true for IgG-4 molecules. From these subgroup data it is evident that the primary structures really are different. The inhibition noted may therefore be due to steric hindrance.

It has been reported that complement fixation can take place via a pathway other than the Fc-induced Cl esterase activation. $F(ab')_2$-antigen precipitates were found to be capable of activating complement, showing that the presence of the Fc part is not always necessary (Amiraian & Leikhim, 1961; Shur & Becker, 1963; Isliker *et al.*, 1968). About 40% of the total complement can be consumed by these $F(ab')_2$ fragments. It is not known if this pathway is induced by intact antibodies and if both are at hand normally. Protein A might provide a suitable reagent for the extension of such studies, blocking γG activation of the common Clq induced pathway for complement fixation.

A salient feature of staphylococcal infection, pus formation, indicates the production of chemotactic factors. These proteins are known to be produced from complement components (Ward, 1967; Ward, Cochrane & Müller-Eberhard, 1966; Snyderman *et al.*, 1969).

Present data on the activation of human complement by protein A naturally raise the question as to the importance of this bacterial substance for pus formation *in vivo*. The fact that in one strain of *Staphylococcus aureus* as many as 80,000 protein A residues are available per bacterial cell (Kronvall *et al.*, 1970a) and also, that as much as 92% of human γ-globulin can combine with protein A (Kronvall & Williams, 1969) makes such a possibility highly probable. Immune complex lesions sometimes seen in the kidneys of patients with staphylococcal endocarditis (Tu, Shearn & Lee, 1969) might also in fact be due to protein A. When this material is released from bacteria into the bloodstream the excess γ-globulin present should favour the formation of protein A–γG complexes capable of activating complement. Studies to elucidate these possible mechanisms must now be pursued.

ACKNOWLEDGMENTS

This investigation was supported by grants from UDPHS (#AM AI 13824-01), the Minnesota Arthritis Foundation, the American Heart Association and the Graduate School, University of Minnesota.

ABBREVIATIONS

AHGG, aggregated human gammaglobulin
CVF, cobra venom factor
LPS, lipopolysaccharide

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