PROTEIN A FROM *STAPHYLOCOCCUS AUREUS** COMPLEMENT FIXATION BY AGGREGATES BETWEEN PROTEIN A AND IgG1 OR IgG2 FROM GUINEA PIG

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1. Introduction

Protein A from *Staphylococcus aureus* is known to react with the Fc fragment (**) of IgG1 and IgG2 from guinea pig [1]. It has also been shown that protein A is able to evoke anaphylactic reactions in normal guinea pigs [2] as well as the Arthus reaction in rabbits [3]. Since the Arthus reaction is known to depend on the activation of complement, we have investigated the interaction between protein A and complement (C). It was found that the protein A–IgG complex will fix guinea pig [4], human and rabbit [5] complement. Furthermore it was shown that protein A added to human serum resulted in conversion of complement factor C3 [6]. It was concluded that protein A–human IgG complexes act on human complement in the same way that ordinary antigen–antibody complexes act.

Different biological activities have been ascribed to the two subclasses of guinea pig IgG; IgG1 and IgG2 [7–9]. These differences are thought to depend on structural differences in the Fc fragments [10]. Recently it has been claimed that some biological activities of IgG1 and IgG2 can not be strictly differentiated [11, 12] because preformed aggregates between antigen and IgG2 as well as between antigen and IgG1 are able to fix complement.

The present communication confirms that protein A precipitates both IgG1 and IgG2 [13, 14]. Both IgG1 and IgG2, aggregated by protein A, are able to fix guinea pig complement. However, preformed complex between antigen (hen egg albumin, EA) and anti EA IgG1 was not found to fix complement in the system used.

2. Material and methods

Guinea pigs were immunized against hen egg albumin (EA, Worthington Biochemical Corp.) as described by Forsgren [13] to obtain antisera rich in IgG1 and IgG2. The guinea pig immunoglobulins were initially purified by two alternative procedures, one according to Forsgren [13] and one suggested by Oliveira et al. [11]. Since the first one is somewhat simpler and gave good results this was used throughout the investigation.

Tests for the purity of the IgG preparations were carried out by immunoelectrophoresis, immunodiffusion and polyacrylamide gel electrophoresis (fig. 1). The IgG preparations according to these criteria appeared to be pure. The IgG1 preparation showed trace contamination of IgG2 in immunoelectrophoresis as seen in fig. 1. In immunodiffusion with potent antiserum against guinea pig serum, the contamination of IgG2 was approximately 5%. Agarose 1% (l'Industrie Biologique Française, S.A.) in 0.01 M tris-acetic acid, pH 8.0 was used for immunoelectrophoresis which was run for 120 min at 50 V/7.7 cm. Polyacrylamide gel electrophoresis was performed in 7% gel, pH 8.9 as suggested by Maurer [15]. Protein A was produced as described elsewhere [5]. Antiserum against guinea pig serum was purchased from Hyland Laboratories,

* Part XIII of a series.

** Abbreviations for immunoglobulins, complement and complement factors conform with the rules suggested in [27] and [28].

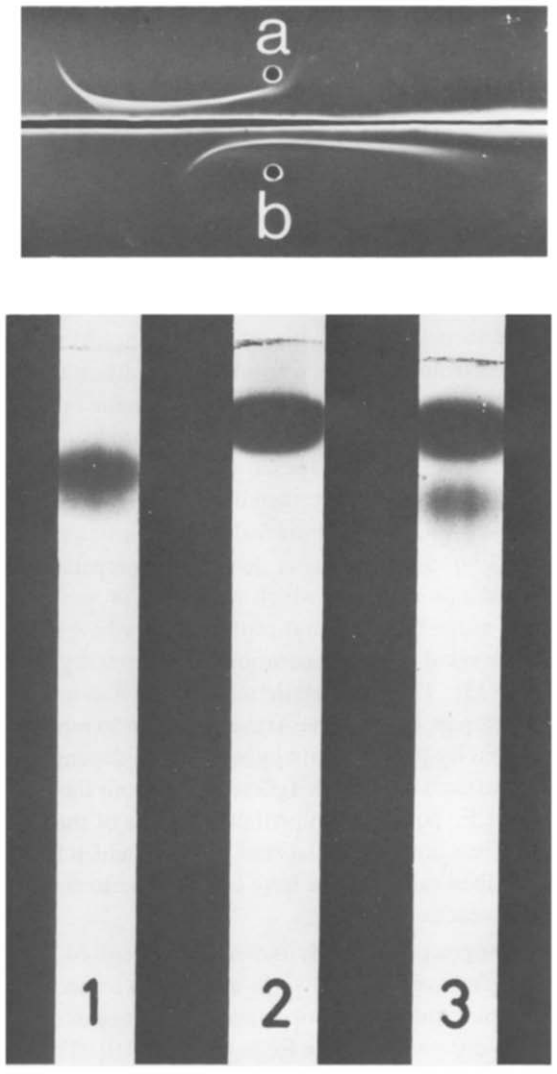

Fig. 1. (A) Immunoelectrophoresis developed with rabbit antiserum against guinea pig serum; a) IgG2 preparation; b) IgG1 preparation. Anode to the right. For details see text. (B) Polyacrylamide gel electrophoresis. (1) IgG1 preparation; (2) IgG2 preparation; (3) mixture of IgG1 and IgG2. Anode at the bottom. For details see text.

Los Angeles, California. Protein concentrations were determined by measuring the optical absorbancy at 280 nm [16].

Complement (C) determination and fixation were performed as described before using fresh frozen guinea pig serum as complement [4]. Protein A was quantitate by the hemagglutination method [4].

Precipitation curves were obtained by mixing equal volumes of 0.24% guinea pig IgG in veronal buffered saline (VBS, [17]) with protein A or EA in different amounts. The mixtures were incubated at 37° for 90 min and 4° overnight. The precipitates were spun down, washed twice in VBS and dissolved in 1.0 ml 0.1 N NaOH. The absorbancy at 280 nm was measured.

3. Results

The addition of 5–600 μg protein A or EA to 300 μl 0.24% immunoglobulin (IgG1 or IgG2) gave characteristic precipitation curves (fig. 2). Maximal precipitation occurred with both IgG1 and IgG2 when approximately 75 μl protein A was added. The amount of precipitate obtained between protein A and IgG2 was about 60% of that obtained with IgG1. Maximal precipitation with EA added to IgG1 or IgG2 was obtained with approximately 40 μg.

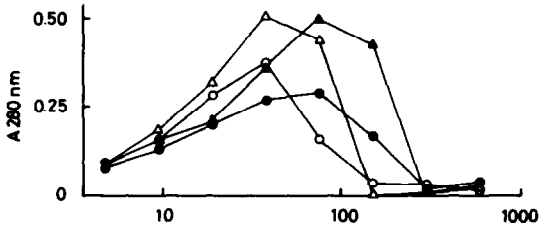

Fig. 2. Precipitation curves obtained with protein A and guinea pig IgG1 (▲—▲) with protein A and guinea pig IgG2 (●—●); with hen egg albumin (EA) and guinea pig IgG1 (△—△); and with EA and guinea pig IgG2 (○—○). The figures on the abscissa denote the amount in μg of protein A or EA added to 720 μg IgG. The reaction volume was 600 μl. The mixtures were incubated at 37° for 90 min and at 4° over night. Precipitate was collected, washed and dissolved in 1ml 0.1 NaOH and the absorbancy was measured at 280 nm.

Aggregates of IgG and EA or IgG and protein A formed in the presence of excess IgG were isolated. To a 0.5% solution of IgG an equal volume of 0.0125% EA or protein A was added. The mixtures were incubated at 37° for 90 min and at 4° over night. Precipitates were collected by centrifugation, washed and suspended in VBS. Aliquots of these suspensions

Table 1
Complement fixation by preformed aggregates between hen egg albumin (EA) or protein A and guinea pig IgG1 or IgG2.

	EA-		Protein A-	
	IgG1 aggregates	IgG2 aggregates	IgG1 aggregates	IgG2 aggregates
μg precipitated protein fixing 25 CH_{50}	> 1000	40	145	75

were added to guinea pig serum containing about 50 CH_{50}. VBS was added to 5.0 ml. Remaining complement activity was determined after incubation for 19 hr at 4°. The amount of precipitated protein fixing 25 CH_{50} units was calculated (table 1). Complexes between EA and IgG2 fixed complement well. At least 25 times the quantities of complexes of IgG1 and EA on the other hand were needed. Aggregates between IgG2 and protein A were somewhat less effective compared to complexes with EA. Precipitates of protein A and IgG1 gave a definite complement fixation in contrast to those of EA and IgG1. About twice the quantity of protein A–IgG1 complex as compared with protein A–IgG2 complex was needed for fixation of 25 CH_{50} units. The supernatants after centrifugation showed no complement fixation in the four assays performed. No free protein A was detected by the hemagglutination technique in any samples tested for C fixing activity. The technique allows determination of free protein A in concentrations above 80 ng/ml [4].

Mixtures of IgG and EA or IgG and protein A were precipitated with ammonium sulphate added to half saturation at room temperature. The supernatants showed no complement fixing activity. The ammonium sulphate precipitates of IgG1–protein A, IgG2–protein A and IgG2–EA manifest complement binding. Complement fixation by the ammonium sulphate precipitate of IgG1–EA was negligible. Free protein A is not precipitated by ammonium sulphate at the concentration used [4].

4. Discussion

It has been known for more than three decades that staphylococcal products are able to elicit reactions related to anaphylaxis without the presence of sera or tissues from immunized animals. In addition the tissue reactions could not be related to histamine or histamine like compounds in the challenging staphylococcal material used [18–23]. Jensen [24] and later Löfkvist [25] found that an extracted product from *S. aureus*, antigen A, contracted normal guinea pig ileum. Löfkvist and Sjöquist showed that this antigen preparation contained a protein [26] which was called protein A. Löfkvist suggested [25] that protein A may have been the active agent in the preparations of Dworetzky et al. [20–23]. The anaphylatic reactions in non-immunized guinea pigs [2] and the Arthus reaction in rabbits [3] elicited by protein A are believed to to depend on conformational changes of IgG resulting from the reaction of the Fc portion with protein A. Some of the anaphylactic reactions with *S. aureus* products which have been described earlier might have been expressions of the same reaction.

Guinea pig antibody molecules aggregated with antigen have been shown to differ with respect to complement fixing properties. These depend on structural differences in the Fc parts [7, 9, 10]. The ability of Fc to fix protein A is common to the two subclasses [13]. Oliveira et al. [11] and Sandberg et al. [12] have shown that the conditions under which antigen–antibody complexes are formed are relevant to C fixation. Thus, antigen–antibody complexes containing IgG1 formed in the complement source were not able to fix complement, but the preformed aggregates were able to do so. The loss in lytic potency manifested by preformed IgG1 aggregates was explained as an activation of the complement system starting with C3 rather than C1 [12].

We have not been able to confirm the results of Sandberg et al. using hen egg albumin as antigen. Preformed aggregates of antigen and IgG1 did not fix

significant amounts of complement. The present test system is slightly different from that used by Sandberg et al., which might account for the discrepancies. Preformed aggregates of protein A and IgG1 fixed complement. However, the amount of aggregate needed to fix 25 CH_{50} was greater with IgG1 than with IgG2 in the aggregate. Contamination of the IgG1 preparation with IgG2 is not the likely cause of the complement fixation because the amount of contaminating protein appears not larger than 5%. Furthermore, if contamination of IgG1 with IgG2 was responsible for the C fixation, one might also expect to find complement fixation by the EA–IgG1 preparation.

Free protein A present in samples tested for C fixation would cause C binding by reaction with IgG2 present in the complement source. Since free protein A was not demonstrated by the hemagglutination technique this explanation could be ruled out.

Analyses for C fixation by protein A–IgG1 complexes formed in the complement source, cannot be performed since protein A added to guinea pig serum would also react with IgG2 of that serum.

The relative capacities of IgG1 and IgG2 to bind complement, are presumably related to the specific conformational changes in the immunoglobulin induced by protein A or homologous antigen. The C fixing differences observed may also be related to some serum inhibitor as Sandberg et al. suggested [12]. Although some of our results do not agree with those of Sandberg et al. [12] they nevertheless confirm that IgG1 may fix complement. Whether protein A–IgG1 complexes react with C1 or some other complement factor is not known.

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