JCI The Journal of Clinical Investigation

Staphylococci-induced Human Platelet Injury Mediated by Protein A and Immunoglobulin G Fc Fragment Receptor

Jack Hawiger, Sylvia Steckley, Dianne Hammond, Charles Cheng, Sheila Timmons, Alan D. Glick, Roger M. Des Prez

J Clin Invest. 1979;64(4):931-937. https://doi.org/10.1172/JCI109559.

Research Article

Bloodstream infections with staphylococci are accompanied by thromboembolic complications. We have studied the mechanism of the interaction of staphylococci with human blood platelets.

Staphylococci that possess protein A, a bacterial receptor for the Fc fragment of immunoglobulin G (IgG), caused aggregation of human platelets in whole plasma accompanied by release of [^{3}H]serotonin. These reactions were time and concentration dependent, requiring two or more staphylococci per platelet to give maximal response within 5 min. The interaction between staphylococci and platelets required the presence of cell wall-bound protein A and of IgG with an intact Fc fragment. It did not require an intact complement system. Cell wall-bound protein A (solid phase) was capable of aggregating human platelets in whole plasma. In contrast, free, solubilized protein A (fluid phase) did not cause measurable aggregation, and release of [^{3}H]serotonin was reduced. An excess of free, solubilized protein A blocked aggregation of human platelets induced by staphylococci in whole plasma.

The role of the Fc fragment of IgG in the staphylococci-human platelet interaction was demonstrated by an experiment in which free, isolated Fc fragment blocked aggregation of platelets in whole plasma induced by staphylococci. Furthermore, binding of ^{125}I-protein A to human platelets was demonstrated in the presence of complete IgG with intact Fc fragment but not in the presence of the F(ab)$_2$ fragment. Binding of […]

Staphylococci-induced Human Platelet Injury Mediated by Protein A and Immunoglobulin G Fc Fragment Receptor

JACK HAWIGER, SYLVIA STECKLEY, DIANNE HAMMOND, CHARLES CHENG, SHEILA TIMMONS, ALAN D. GLICK, and ROGER M. DES PREZ, *Departments of Pathology and Medicine, Vanderbilt University School of Medicine, and Veterans Administration Hospital, Nashville, Tennessee 37232*

ABSTRACT Bloodstream infections with staphylococci are accompanied by thromboembolic complications. We have studied the mechanism of the interaction of staphylococci with human blood platelets.

Staphylococci that possess protein A, a bacterial receptor for the Fc fragment of immunoglobulin G (IgG), caused aggregation of human platelets in whole plasma accompanied by release of [^{3}H]serotonin. These reactions were time and concentration dependent, requiring two or more staphylococci per platelet to give maximal response within 5 min. The interaction between staphylococci and platelets required the presence of cell wall-bound protein A and of IgG with an intact Fc fragment. It did not require an intact complement system. Cell wall-bound protein A (solid phase) was capable of aggregating human platelets in whole plasma. In contrast, free, solubilized protein A (fluid phase) did not cause measurable aggregation, and release of [^{3}H]serotonin was reduced. An excess of free, solubilized protein A blocked aggregation of human platelets induced by staphylococci in whole plasma.

The role of the Fc fragment of IgG in the staphylococci-human platelet interaction was demonstrated by an experiment in which free, isolated Fc fragment blocked aggregation of platelets in whole plasma induced by staphylococci. Furthermore, binding of ^{125}I-protein A to human platelets was demonstrated in the presence of complete IgG with intact Fc fragment but not in the presence of the $F(ab)_2$ fragment. Binding of the protein A-IgG complex to the human platelet Fc receptor was paralleled by the release of [^{3}H]serotonin.

These results represent a novel example of the interaction of two phylogenetically different Fc receptors, one on prokaryotic staphylococci and the other on human platelets. Their common ligand, IgG, is amplified by one Fc receptor (protein A) to react with another Fc receptor present on human platelets, which results in membrane-mediated aggregation and release reaction occurring in whole plasma. This mechanism can be of significance in the pathomechanism of thromboembolic complications at the site(s) of intravascular staphylococcal infection.

INTRODUCTION

Staphylococcal bacteremia, endocarditis, and infected vascular prostheses and grafts are frequently accompanied by thromboembolic complications (2–7). Blood platelets are intimately involved in the mechanism of thrombosis (8), and staphylococci are among the microorganisms known to interact with platelets (9). The mode of human platelet interaction with staphylococci, however, remains obscure. Unlike gram-negative bacteria, which produce a complex lipopolysaccharide endotoxin able to activate platelets (10, 11), pathogenic staphylococci possess a cell wall component termed protein A (12). It is covalently bound to the cell wall of 40% of the isolates of coagulase positive staphylococci (13). Staphylococcal protein A constitutes a unique example of a bacterial Fc fragment receptor which interacts with all human immunoglobulin G (IgG) subclasses except IgG_3 (14, 15).

We have now obtained evidence indicating that the interaction of human platelets with staphylococci depends on the interaction of protein A and the Fc fragment of IgG. Using protein A-bearing staphylococci and protein A isolated therefrom, we found that platelet aggregation in whole plasma also depends on the phase in which this bacterial Fc receptor was present,

This work was presented in part at the 69th Annual Meeting of The American Society for Clinical Investigation, Washington, D. C., May 2, 1977 (1).

Received for publication 20 October 1978 and in revised form 29 May 1979.

i.e., cell wall-bound (solid phase) vs. free, isolated (fluid phase). Furthermore, the essential role of the Fc fragment of IgG has been demonstrated because the Fc fragment blocked the interaction of staphylococci with human platelets in whole plasma and was needed for binding of the ^{125}I-protein A-IgG complex to the human platelet Fc fragment receptor.

METHODS

Preparation of protein A-bearing staphylococci. A culture of *Staphylococcus aureus* Cowan I was grown in nutrient broth (16) at 37°C for 16 h in a rotating incubator at 150 rpm. Samples were taken, diluted logarithmically, and pour plates made for bacterial counting. The bacteria were washed twice with saline, once with distilled water, and frozen for isolation of protein A or lyophilized for use in experiments with whole bacteria.

Stripping of protein A from staphylococci by trypsinization. A culture of Cowan I was washed once with saline and once with distilled water. Bacteria from 1 liter of culture were suspended in 200 ml of 0.01% trypsin (Worthington Biochemical Corp., Freehold, N. J.), pH 6.8, and incubated at 37°C for 2 h (17). After incubation, the mixture was centrifuged at 3,000 rpm for 15 min. The bacteria were washed twice with saline, once with distilled water, and lyophilized. A parallel culture was run without trypsin treatment as a control. When both preparations of bacteria were examined for the presence of protein A, trypsinized staphylococci showed approximately a 10-fold reduction in the protein A content.

Affinity chromatographic purification of protein A. Human IgG was prepared as detailed below and was coupled to 50 ml cyanogen bromide activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.) (18). The preparation of lysostaphin-treated *S. aureus* Cowan I and affinity isolation of protein A were performed according to the method of Sjoquist et al. (19) and Hjelm et al. (20). Solubilized purified protein A was homogenous in sodium dodecyl sulfate (SDS)[1] polyacrylamide gel electrophoresis and had an apparent molecular weight of 42,000 in both nonreduced and reduced systems.

Isolation of human IgG. Human IgG was isolated from fresh serum on a DEAE-cellulose column (Whatman Inc., Clifton, N. J.) which had been equilibrated with 0.01 M phosphate buffer, pH 8.0 (21). Material from the first peak of four separations was pooled, concentrated to 40 ml in an Amicon ultrafiltration unit (Amicon Corp., Lexington, Mass.) using a PM 30 membrane, and rechromatographed on a second DEAE-cellulose column equilibrated with the same buffer. The IgG was then dialyzed against 0.01 M phosphate in saline, pH 7.4, and centrifuged at 150,000 *g* for 30 min before use in an experiment.

Preparation of F(ab)$_2$ fragment of IgG. Isolated human IgG was dialyzed against 0.1 M sodium acetate buffer, pH 4.5, and after dialysis, IgG (200 mg in 7.6 ml buffer) was treated with 2 mg crystallized pepsin (Nutritional Biochemical Co., Cleveland, Ohio). After 20 h at 37°C, the pH of the incubation mixture was adjusted to 8.0 with 1 N NaOH, and 25% Na_2SO_4 (wt/vol) was added dropwise, with stirring, to a final concentration of 0.18 g/ml (22). The heavy precipitate was separated by centrifugation, the pellet dissolved in water, and dialyzed against 0.05 M Tris in 0.1 M NaCl, pH 7.4.

Preparation of Fc fragment of IgG. Fragments of human IgG were obtained by enzymatic digestion according to Porter (23), using 1 mg twice recrystallized papain (Worthington Biochemical Corp., Freehold, N. J.) per 100 mg IgG. The resulting Fab and Fc fragments were isolated by chromatography on carboxymethyl cellulose and DEAE-cellulose according to the procedure of Franklin and Prelli (24). Isolated fragments were examined by SDS polyacrylamide gel electrophoresis and stored at −70°C.

Preparation of [^{3}H]serotonin-labeled platelets in whole plasma and platelets separated from plasma proteins. Blood was drawn from normal, healthy volunteers into syringes containing 0.11 M citrate buffer, pH 5.0, to have a final blood to buffer ratio of 9:1. It was centrifuged at 160 *g* for 15 min, the platelet-rich plasma was collected, and platelets counted in a Coulter model ZBI Particle Counter (Coulter Electronics, Inc., Hialeah, Fla.). The platelet count was adjusted to 2×10^8/ml. For release experiments, the platelet-rich plasma was incubated at room temperature with 0.05 μCi/ml of [^{3}H]serotonin (5-hydroxy[G-^{3}H]tryptamine creatinine sulfate, Amersham Corp., Arlington Heights, Ill.) for 4 min. Incorporation of [^{3}H]serotonin into the platelets ranged from 84 to 90%. In experiments using platelets separated from plasma proteins, platelet-rich plasma labeled with [^{3}H]serotonin was applied to the top of a stepwise albumin gradient (25) and, after centrifugation, the platelet layer was filtered through a Sepharose 2B column (Pharmacia Fine Chemicals) equilibrated with Tyrode buffer, pH 7.4. The peak fractions were collected, sampled for platelet count and radioactivity counts, and the gel-filtered platelets used immediately in the experimental system in a concentration of 2×10^8/ml.

Aggregation of human platelets. Aggregation studies were done according to the method of Born (26) using a Payton Dual Channel Aggregometer (Payton Assoc., Buffalo, N. Y.). The aggregometer base line (10%) was set using platelet-rich plasma with staphylococci and buffer added in a concentration equivalent to the test system. Full transmission (100%) was set using an aggregated sample without stirring.

Platelets suspended in whole plasma were challenged with staphylococci using calculated concentrations of bacteria and with isolated protein A in concentrations determined by quantitation on an IgG immunodiffusion plate (27, 28). When platelets separated from plasma proteins were used, the aggregometer was adjusted in a manner similar to that described for platelets in plasma. Aggregation was measured using percent maximum transmission and slope (29) which represented the change along a tangent line to the sharpest increase in light transmission.

Release of [^{3}H]serotonin from platelets. Platelets suspended in whole plasma and labeled with [^{3}H]serotonin were incubated at 37°C with staphylococci and with isolated protein A at different concentrations for 10 min with stirring. Samples were taken for total radioactivity counts, the incubation mixture centrifuged at 18,000 *g* for 2 min, and the supernates assayed for released radioactivity. Platelets, labeled with [^{3}H]serotonin and separated from plasma proteins, were incubated as described above. Imipramine (Ciba-Geigy Corp., Summit, N. J.), at a concentration of 0.15 μg/ml, was used in all release reaction experiments.

Experiments with complement depleted plasma. Samples of plasma obtained from human blood anticoagulated with 1 U/ml sodium heparin were treated with cobra factor, zymosan, EDTA, or buffer as described previously (28, 30). Protein A-bearing staphylococci were preincubated with plasma samples for 30 min at 37°C and washed three times with Tyrode buffer, pH 7.6. The volume of bacteria coated with plasma proteins was adjusted to the appropriate concentration, and staphylococci were added to platelets separated from plasma proteins.

[1] *Abbreviation used in this paper*: SDS, sodium dodecyl sulfate.

Binding of ^{125}I-protein A to platelets. Purified protein A was labeled with ^{125}I (Amersham Corp.) using the chloramine T method (31). Platelets separated from plasma proteins were incubated at 37°C with ^{125}I-protein A at concentrations from 1 to 10 μg/ml in the absence and presence of IgG and $F(ab)_2$ fragment of IgG, which were used in concentrations of 1 mg/ml and 0.6 mg/ml, respectively. At the end of the 10-min incubation, 40 mM EGTA was added to the incubation mixture, and it was poured into a filtration manifold (Millipore Corp., Bedford, Mass.) using 1-μm nucleopore filters (Arthur H. Thomas Co., Philadelphia, Pa.) which had been presoaked in 0.5% albumin. The samples were filtered under atmospheric pressure and then washed with 20 ml Tyrode buffer, pH 7.4, using a vacuum. The filters were removed and radioactivity associated with platelets was determined in a Searle Gamma Counter (Searle Radiographics, Inc., Des Plaines, Ill.).

Electron microscopy. Electron microscope observations were made on platelets suspended in whole plasma after challenge by staphylococci. After incubation, the samples were mixed thoroughly with an equal volume of 2% glutaraldehyde in phosphate buffer, pH 7.3, and centrifuged at 18,000 *g* for 2 min. The pellets were again incubated in 2% glutaraldehyde for 2–3 h and then centrifuged. After secondary fixation in 1% Millonig's phosphate-buffered OsO_4 (32), the fixed cells were stained en bloc with 0.5% aqueous uranyl acetate. Pellet fragments were then dehydrated and embedded in Araldite (Ciba-Geigy Corp., Ardsley, N. Y.). Thin sections were prepared, stained with uranyl acetate and lead citrate according to Reynolds' formulation (33), and examined in a Philips EM200 electron microscope (Philips Electronic Instruments, Inc., Mahwah, N. J.).

SDS polyacrylamide gel electrophoresis. 7.5% gels were run in 1% SDS according to the procedure of Weber and Osborn (34). Samples with or without 2-mercaptoethanol, used for disulfide reduction, were incubated in buffer containing 1% SDS in a boiling water bath for 5 min. Parallel gels were run with molecular weight markers. Gels were stained with Coomassie Brilliant Blue.

RESULTS

Human platelet aggregation and release of [^{3}H]serotonin induced by protein A-bearing staphylococci in whole plasma. The effect of removal of protein A from staphylococci on their interaction with human platelets. Addition of protein A-bearing staphylococci to platelet-rich plasma induced platelet aggregation and release of [^{3}H]serotonin. As shown in Fig. 1, the effect of staphylococci was concentration dependent. When the ratio of staphylococci:platelets was 1.3, only partial aggregation was recorded (tracing B), and release of [^{3}H]serotonin was 33%. Increasing the ratio of staphylococci:platelets to 2.5 resulted in prompt aggregation (tracing A) and release of [^{3}H]serotonin, which reached a maximum within 5 min. In three independent experiments, the mean±SD release of [^{3}H]serotonin was 94±8%.

When staphylococci were treated with trypsin to remove protein A from their cell wall (see Methods) and substituted into the same system in a ratio of 2.5 staphylococci per platelet, their ability to induce platelet aggregation was abolished (tracing C), and release of serotonin was reduced to 38±12%. The dependence

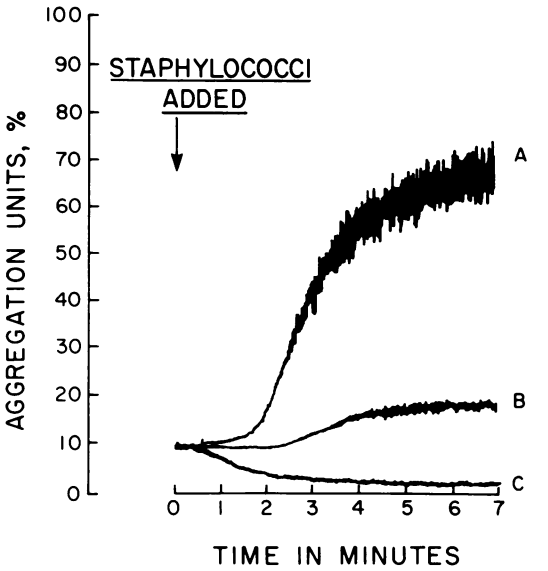

FIGURE 1 Human platelet aggregation induced by two different concentrations of protein A-bearing staphylococci added to platelet-rich plasma before and after treatment with trypsin to remove protein A. Tracing A: 2.5 staphylococci/platelet; Tracing B: 1.3 staphylococci/platelet; Tracing C: 2.5 trypsin-treated staphylococci/platelet.

of human platelet aggregation by staphylococci on protein A was documented further by a blocking experiment in which free, isolated protein A in the concentration of 50 μg/ml completely prevented platelet aggregation by protein A-bearing staphylococci (results not shown).

Electron microscopy of platelets challenged with protein A-bearing staphylococci showed large aggregates of platelets in which staphylococci were embedded. Platelets were almost totally degranulated and showed complex interdigitations with each other and were intimately associated with staphylococci.

Comparison of the effect of protein A-bearing staphylococci and protein A isolated therefrom on aggregation and release reaction of human platelets in whole plasma. The experimental model of staphylococci-induced human platelet injury used in this study enabled us to compare the effect of protein A bound to the staphylococcal cell wall (solid phase) with the effect of protein A isolated in a free, soluble form (fluid phase). Both forms of protein A, i.e., cell-bound and free, were added to platelet-rich plasma; and they demonstrated different patterns of interaction with human platelets. In the solid phase system, protein A induced both aggregation of platelets and release of serotonin. Both changes were dependent on the concentration of staphylococci (Table I). By contrast, in the fluid phase system aggregation of platelets was not detected, and release of [^{3}H]serotonin was somewhat reduced in comparison with protein A-bearing staphy-

TABLE I

Effect of Protein A-bearing Staphylococci and Isolated Protein A on Human Platelets in Whole Plasma

	Concentration of protein A	Number of staphylococci per platelet	Platelet aggregation*	Platelet [^{3}H]serotonin release
	μg			% (±SD)
Solid phase system (staphylococci with bound protein A)				
Platelet-rich plasma (control)		0	0	16±2
Platelet-rich plasma plus staphylococci with cell wall-bound protein A		0.7	0	23±6
		1.3	7	33±7
		2.0	30	69±10
Fluid phase system (isolated protein A)				
Platelet-rich plasma (control)	0 (0)‡		0	20±1
Platelet-rich plasma plus isolated protein A	3 (1)		0	26±5
	10 (3)		0	26±5
	30 (10)		0	41±7
	100 (33)		0	40±3

* Aggregation units expressed as slope value obtained by drawing a tangent line to the steepest rise in the curve and calculating the change per 1 min (29).

‡ Numbers in parentheses represent estimated number of staphylococci per platelet containing equivalent concentrations of isolated protein A.

lococci. As indicated in parentheses accompanying concentrations of protein A in Table I, isolated, soluble protein A was tested in a wide range of concentrations corresponding to the total amount present on staphylococci when used in a ratio of 1 staphylococcus/platelet to 33 staphylococci/platelet.

The role of the Fc fragment of IgG in the interaction between protein A and human platelets. Because staphylococcal protein A represents the bacterial receptor for the Fc fragment of IgG, blocking the Fc receptor should inhibit the interaction between protein A-bearing staphylococci and human platelets. The effect of isolated Fc fragment of IgG on the interaction between protein A-bearing staphylococci and human platelets was examined. Addition of the Fc fragment to a system containing protein A-bearing staphylococci inhibited their interaction with human platelets in whole plasma (Fig. 2). Platelet aggregation was abolished in this system and release of [^{3}H]serotonin was reduced almost twofold with 30 μg/ml of isolated Fc fragment.

Subsequent experiments were performed to establish the role of the Fc fragment in the formation of a complex among protein A, IgG, and human platelets. Isolated protein A labeled with ^{125}I and human platelets separated from plasma proteins were used. ^{125}I-Protein A was not bound to platelets separated from plasma proteins unless IgG was added to the system (Fig. 3). This binding was concentration dependent in regard to protein A and required an intact IgG molecule. Substituting $F(ab)_2$ fragments for IgG did not allow binding of protein A with human platelets, which indicates that the interaction of protein A with human platelets requires the presence of the Fc fragment of the IgG molecule. In parallel experiments, release of [^{3}H]serotonin was measured and, as was expected, addition of iso-

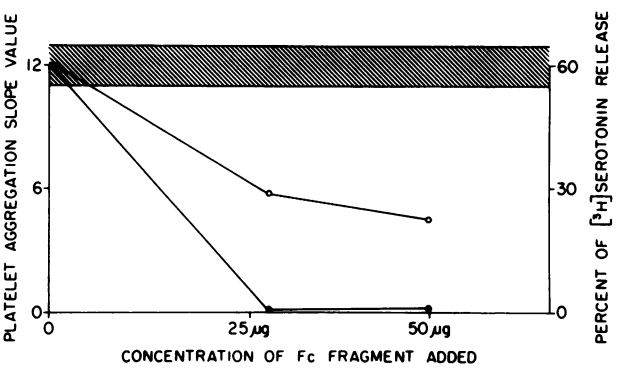

FIGURE 2 Blocking effect of isolated Fc fragment of IgG on the interaction of protein A-bearing staphylococci with human platelets in whole plasma. Aggregation (●) and release of [^{3}H]serotonin (○) were measured. Platelet-rich plasma containing 2 × 10^8/ml platelets was used and 2 staphylococci/platelet were added. Aggregation units are expressed as slope value obtained by drawing a tangent line to the steepest rise in the curve and calculating the change per 1 min. Shaded area indicates control range (±SD) of platelet aggregation and [^{3}H]serotonin release induced by protein A-bearing staphylococci with buffer instead of isolated Fc fragment.

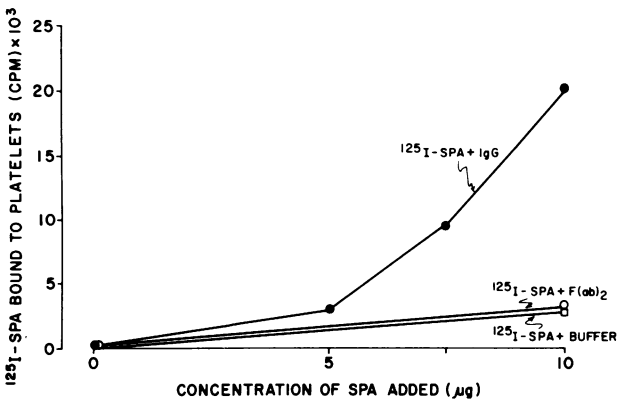

FIGURE 3 Binding of isolated, solubilized ^{125}I-protein A (SPA) to 2 × 10^8/ml platelets separated from plasma proteins. Each point represents the mean binding of ^{125}I-SPA to 2 × 10^8/ml platelets separated from plasma proteins in at least three independent experiments, each performed in triplicate. Concentration of IgG was 1 mg/ml and of F(ab)$_2$ was 0.6 mg/ml.

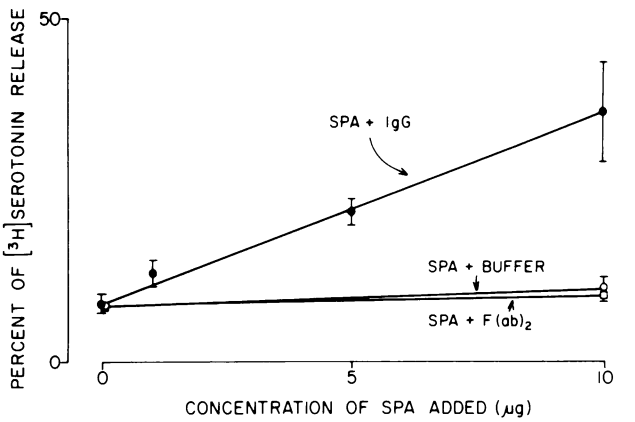

FIGURE 4 Effect of isolated, solubilized protein A (SPA) on human platelets separated from plasma proteins. Each point represents the mean release of [^{3}H]serotonin in at least three independent experiments, each performed in triplicate. Vertical lines represent SD. Concentration of IgG was 1 mg/ml and of F(ab)$_2$ was 0.6 mg/ml.

lated protein A to human platelets separated from plasma proteins did not induce release of [^{3}H]serotonin (Fig. 4). However, in the presence of purified IgG, release of [^{3}H]serotonin was induced and was concentration dependent in regard to protein A. Again, this reaction was dependent on the Fc fragment of IgG, as the F(ab)$_2$ fragment was unable to mediate the interaction of isolated protein A with human platelets.

Addition of isolated Fc fragment of IgG (100 μg/ml) to staphylococci and platelets separated from plasma proteins induced release of 33% [^{3}H]serotonin. A control containing the same ratio of two staphylococci per platelet separated from plasma proteins gave 15±4% release of [^{3}H]serotonin.

The role of the complement system in the interaction between protein A and human platelets. The interaction of protein A with IgG is known to cause fixation of complement (35). Therefore, we examined the possibility that the effect of protein A-bearing staphylococci on human platelets involves the complement system. Staphylococci with cell wall-bound protein A were preincubated in plasma anticoagulated with 1 U/ml heparin to allow binding of plasma proteins. After subsequent washing, opsonized staphylococci were added to human platelets separated from plasma proteins. A ratio of two staphylococci per platelet was used. Staphylococci exposed to intact, heparinized plasma induced normal platelet aggregation; and release of [^{3}H]serotonin (mean±SD) was 52±11%. Release of [^{3}H]serotonin was not accompanied by liberation of the cytoplasmic marker enzyme, lactate dehydrogenase. When staphylococci were preincubated with plasma that had been decomplemented with cobra venom factor, zymosan, or EDTA and then added to human platelets, aggregation remained essentially the same; and release of [^{3}H]-serotonin was 54±7%, 57±10%, and 49±16%, respectively.

DISCUSSION

These studies have demonstrated staphylococci-induced injury to human platelets mediated by two phylogenetically different Fc receptors and their common ligand, IgG, acting through its Fc portion. We have identified the bacterial Fc receptor, protein A, as a structural entity of pathogenic staphylococci responsible for their interaction with human platelets.

Protein A binds IgG through its Fc fragment with an average of 80,000 molecules/staphylococcus (36), thus playing the role of a bacterial Fc receptor. The dependence of the interaction of staphylococci with human platelets on protein A is apparent from the experiments in which Protein A was stripped from the cell wall by trypsin treatment. Because the mucopeptide (murein) of the staphylococcal cell wall and teichoic acids are not substrates for trypsin (37, 38), this experiment provided additional evidence that these basic structural units, which are the main antigenic determinants of the staphylococcal cell wall, are not responsible for aggregation of human platelets in our system.

Several lines of evidence indicate the existence of a human platelet receptor for the Fc fragment of IgG (39–41), and recently an Fc fragment-binding glycoprotein has been isolated from human platelets by affinity chromatography (42). Our data indicate that the function of the Fc fragment receptor on human platelets is influenced by the phase (solid vs. fluid) in which the complex containing the IgG Fc fragment exists. Optimal platelet aggregation and release reaction

mediated by the IgG Fc fragment occurs in whole plasma when the solid phase reactant—namely, cell wall-bound protein A—is employed. This fact is of significance in understanding the role of the Fc fragment of IgG in the mediation of human platelet injury. Previous studies on the role of the Fc fragment in human platelet injury in most instances used soluble antigen-antibody complexes or aggregated IgG and washed platelets (39–41). Neither aggregation nor release of serotonin occurred in whole plasma at physiologic platelet levels, although some binding of aggregated IgG to human platelets was observed (43). Isolated, soluble protein A added to whole plasma in suboptimal, optimal, and supraoptimal concentrations could not induce measurable platelet aggregation, and only release of [^{3}H]serotonin, somewhat reduced, was observed (Table I). Therefore, free protein A in the fluid phase forms a complex with IgG that behaves differently (lack of platelet-aggregating activity) than the complex formed between IgG and cell wall-bound protein A in the solid phase system (full platelet-aggregating activity).

Although the complement system was important for the functional effect of protein A in the rabbit platelet model (28), in the case of the human platelets studied here, complement appears to be nonessential either for binding of ^{125}I-protein A-IgG complexes to human platelets or for their aggregation and release reaction. Fixation of complement by protein A-IgG complexes is reduced when an equimolar ratio of protein A to IgG is attained (44). It is possible that in our solid phase system, fixation of complement could occur; however, it did not depress or promote the interaction of staphylococci with human platelets.

It is apparent from our experiments that IgG binds simultaneously through its Fc fragment to two different cellular Fc receptors. This suggests involvement of at least two constant domains of the Fc fragment. Staphylococci with their Fc receptor, i.e., protein A, emanating from the cell wall interact with the appropriate domain on one heavy chain of the Fc fragment. The other site on a corresponding heavy chain is amplified to react with the platelet receptor for the Fc fragment of IgG. It has been shown that the Fc fragment undergoes significant conformational changes upon binding of protein A (45). Thus, the unique interaction of the IgG molecule with two phylogenetically different Fc fragment receptors, one on the bacterial cell wall and the other on the human platelet membrane, results in the formation of a tertiary complex. This can contribute to thromboembolic complications at the site(s) of intravascular staphylococcal infections.

ACKNOWLEDGMENTS

This work was supported by U. S. Public Health Service grants HL-16646 and HL-08399 and by the Medical Research Service of the Veterans Administration.

REFERENCES

1. Hawiger, J., S. Steckley, D. Hammond, C. Cheng, S. Timmons, and R. M. Des Prez. 1977. The mechanism of thromboembolic events caused by staphylococci: immune injury to human platelets mediated by Protein A and IgG Fc fragment receptor. *Clin. Res.* **25:** 489A. (Abstr.)
2. Rahal, J. J. Jr., H. E. MacMahon, and L. Weinstein. 1968. Thrombocytopenia and symmetrical peripheral gangrene associated with staphylococcal and streptococcal bacteremia. *Ann. Intern. Med.* **69:** 35–43.
3. Hawiger, J., and M. G. Koenig. 1979. Staphylococcal Diseases. *In* Science and Practice of Clinical Medicine. J. P. Stanford, editor. Grune & Stratton, New York. In press.
4. Powell, D. E. B. 1961. Non-suppurative lesions in staphylococcal septicemia. *J. Pathol. Bacteriol.* **82:** 141–149.
5. Johnson, W. E. 1976. The clinical syndrome. *In* Infective Endocarditis. D. Kaye, editor. University Park Press, Baltimore, 87–108.
6. Kalbag, R. M., and A. L. Woolf. 1967. Cerebral Venous Thrombosis. Oxford University Press, London. 287.
7. Lickweg, W. G., S. A. Levinson, and L. Greenfield. 1977. Infections of vascular grafts. Incidence, anatomic locations, etiologic agents, morbidity, and mortality. *In* Infections of Prosthetic Heart Valves and Vascular Grafts. R. J. Duma, editor. University Park Press, Baltimore. 239–251.
8. Hovig, T. 1969. The role of formed elements in thrombosis. *In* Thrombosis. S. Sherry, K. M. Brinkhous, E. Genton, and J. M. Stengle, editors. National Academy of Science, Washington, D. C. 374–391.
9. Clawson, C. C., and J. G. White. 1971. Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. *Am. J. Pathol.* **65:** 367–380.
10. Nagayama, M., M. B. Zucker, and F. K. Beller. 1971. Effects of a variety of endotoxins on human and rabbit platelet function. *Thromb. Diath. Haemorrh.* **26:** 467–473.
11. Elin, R. J., and S. M. Wolff. 1976. Biology of endotoxin. *Ann. Rev. Med.* **27:** 127–141.
12. Yoshida, A., S. Mudd, and N. A. Lenhard. 1963. The common protein agglutinogen of *Staphylococcus aureus*. II. Purification, chemical characterization, and serologic comparison with Jensen's antigen. *J. Immunol.* **91:** 777–782.
13. Sperber, W. K. 1976. The identification of staphylococci in clinical and food microbiology laboratories. *CRC Crit. Rev. Clin. Lab. Sci.* **7:** 121–184.
14. Bjork, I., B. A. Petersson, and J. Sjoquist. 1972. Some physicochemical properties of Protein A from *Staphylococcus aureus*. *Eur. J. Biochem.* **29:** 579–584.
15. Kronvall, G., and R. C. Williams. 1969. Differences in anti-Protein A activity among IgG subgroups. *J. Immunol.* **103:** 828–833.
16. Forsgren, A., and J. Sjoquist. 1969. Protein A from *Staphylococcus aureus*. VII. Physicochemical and immunological characterization. *Acta Pathol. Microbiol. Scand.* **75:** 466–480.
17. James, A. M., and J. E. Brewer. 1968. A protein component of the cell surface of *Staphylococcus aureus*. *Biochem. J.* **108:** 257–262.
18. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. *J. Biol. Chem.* **245:** 3059–3065.
19. Sjoquist, J., B. Meloun, and H. Hjelm. 1972. Protein A isolation from *Staphylococcus aureus* after digestion with lysostaphin. *Eur. J. Biochem.* **29:** 572–578.
20. Hjelm, H., K. Hjelm, and J. Sjoquist. 1972. Protein A from

Staphylococcus aureus, its isolation by affinity chromatography and its uses as an immunoabsorbent for isolation of immunoglobulins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **28:** 73–76.
21. Fahey, J. L., and P. Horbett. 1959. Human gamma globulin fractionation on anion exchange cellulose columns. *J. Biol. Chem.* **234:** 2645–2651.
22. Nisonoff, A. 1962. Conceptual Advances in Immunology and Oncology. Hoeber Medical Division, Harper and Row, New York. 273.
23. Porter, R. R. 1959. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. *Biochem. J.* **73:** 119–126.
24. Franklin, E. C., and F. Prelli. 1960. Structural units of 7S gamma globulin. *J. Clin. Invest.* **39:** 1933–1941.
25. Timmons, S., and J. Hawiger. 1978. Separation of human platelets from plasma proteins including Factor $VIII_{VFW}$ by a combined albumin gradient-gel filtration method using HEPES buffer. *Thromb. Res.* **12:** 297–306.
26. Born, G. V. R. 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (Lond.).* **194:** 927–929.
27. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical quantitation of antigens by a single radial immunodiffusion. *Immunochemistry.* **2:** 235–254.
28. Hawiger, J., S. R. Marney, Jr., D. G. Colley, and R. M. Des Prez. 1972. Complement-dependent platelet injury by staphylococcal Protein A. *J. Exp. Med.* **136:** 68–80.
29. Jenkins, C. S. P., M. Meyers, M. D. Dreyfus, and M. J. Larrieu. 1972. Willebrand factor and ristocetin. I. Mechanism of ristocetin-induced platelet aggregation. *Br. J. Haematol.* **28:** 561–578.
30. Des Prez, R. M., C. S. Bryan, J. Hawiger, and D. G. Colley. 1975. Function of the classical and alternate pathways of human complement in serum treated with ethylene glycol tetraacetic acid and $MgCl_2$-ethylene glycol tetraacetic acid. *Infect. Immun.* **11:** 1235–1243.
31. McConchey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* **29:** 185–189.
32. Millonig, G. 1961. Advantages of a phosphate buffer for OsO_4 solution on fixation. *J. Appl. Physiol.* **32:** 1637. (Abstr.)
33. Reynolds, E. S. 1963. The use of lead citrate at high pH as electron opaque stain in electron microscopy. *J. Cell Biol.* **17:** 208–212.
34. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
35. Sjoquist, J., and G. Stalenheim. 1969. Protein A from *Staphylococcus aureus*: IX. Complement-fixing activity of protein A-IgG complexes. *J. Immunol.* **103:** 467–473.
36. Kronvall, G., P. G. Quie, and R. C. Williams, Jr. 1970. Quantitation of staphylococcal protein A: determination of equilibrium constant and number of protein A residues on bacteria. *J. Immunol.* **104:** 273–278.
37. Salton, M. R. J. 1964. The bacterial cell wall. Elsevier Scientific Publishing Co., Amsterdam. 57.
38. Morse, S. I. 1965. Biological attributes of staphylococcal cell walls. *Ann. N. Y. Acad. Sci.* **128:** 191–213.
39. Israels, E. D., G. Nisli, F. Paraskevas, and L. G. Israels. 1973. Platelet Fc receptor as a mechanism for Ag-Ab complex induced platelet injury. *Thromb. Diath. Haemorrh.* **29:** 434–444.
40. Henson, P., and H. L. Spiegelberg. 1973. Release of serotonin from human platelets induced by aggregated immunoglobulins of different classes and subclasses. *J. Clin. Invest.* **52:** 1282–1288.
41. Pfueller, S. L., and E. F. Luscher. 1972. The effects of aggregated immunoglobulins on human blood platelets in relation to their complement-fixing abilities. I. Studies of immunoglobulins of different types. *J. Immunol.* **109:** 517–525.
42. Cheng, C. M. and J. Hawiger. 1979. Affinity isolation and characterization of IgG Fc fragment-binding glycoprotein from human blood platelets. *J. Biol. Chem.* **254:** 2165–2167.
43. Pfueller, S. L., S. Weber, and E. F. Luscher. 1977. Studies on the mechanism of human platelet release reaction induced by immunologic stimuli. III. Relationship between the binding of soluble IgG aggregates to the Fc receptor and cell response in the presence and absence of plasma. *J. Immunol.* **118:** 514–524.
44. Langone, J. J., P. Boyle, and T. Borsos. 1978. Studies on the interaction between Protein A and immunoglobulin G. I. Effect of Protein A on the functional activity of IgG. *J. Immunol.* **121:** 327–332.
45. Sjoholm, I. 1975. The interaction between Protein A and immunoglobulin G as studied with the Fc fragment of a myeloma protein by circular dichromism. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **52:** 53–56.