Platelet Aggregation by Group B Streptococci

By YUKIO USUI,* YUKIO OHSHIMA AND KOSAKU YOSHIDA

Department of Microbiology, St Marianna University School of Medicine, 2-16-1, Sugao, Miyamaye-ku, Kawasaki 213, Japan

(Received 29 October 1986; revised 20 January 1987)

Forty-six strains of group B streptococci (GBS), including various serotypes and non-serotypable strains, were tested for their ability to induce platelet aggregation in human platelet-rich plasma; four strains, all belonging to type III, showed a positive reaction. The characteristics of the reaction were investigated in these four positive strains. Aggregation was dependent on the ratio of bacteria to platelets, being maximal at a ratio of 4.3. Platelet aggregation was inhibited by EDTA (100% inhibition at 3.1 mM), indomethacin (100% inhibition at 10 mM), acetylsalicylic acid (93–100% inhibition at 5.0 mM) and quinacrine (100% inhibition at 0.25 mM). Thus the reaction was cation-dependent and required cyclooxygenase activity. Assays for cytosolic lactate dehydrogenase did not indicate platelet lysis. GBS induced the release of [³H]serotonin, which was maximal (68–78%) at 10 min after the reaction was started. Experiments with gel-filtered platelets suggested that GBS-induced platelet aggregation required both fibrinogen and heat-resistant (56 °C, 30 min) serum factors. Type-specific antisera prevented the platelet aggregation activity of heat-killed bacteria, but not of live bacteria. Trypsin digestion of the bacterial cells caused an almost complete loss of the platelet aggregation activity.

INTRODUCTION

Platelets are known to respond to various aggregation agents such as ADP, arachidonate, collagen and thrombin (Holmsen *et al.*, 1969). Also, platelet aggregation is induced by viruses (Jerushalmy *et al.*, 1961), antigen–antibody complexes (Henson, 1970), lipopolysaccharides (Morrison *et al.*, 1978) and bacteria (Clawson & White, 1971). Bacteria causing platelet aggregation include staphylococci (Hawiger *et al.*, 1972, 1979), streptococci (Kurpiewski *et al.*, 1983) and listeria (Czuprynski & Balish, 1981), as well as several species of Gram-negative bacteria. The relation between aggregation and infection has been discussed (Czuprynski & Balish, 1981; Hawiger *et al.*, 1979; Kurpiewski *et al.*, 1983).

We recently showed that a type III strain of group B streptococci (GBS) exhibiting positive clumping factor (CF) reaction induced intravascular coagulation in mice (Usui *et al.*, 1986), suggesting the possible induction of disseminated intravascular coagulation (DIC). Since a decrease of platelet count is generally recognized as one of the diagnostic criteria of DIC, it is of interest to examine the ability of the strain to cause platelet aggregation *in vitro*. However, little is known about GBS–platelet interaction except for the recent data reported by Wood & Gray (1986).

In this paper, we report some properties of platelet aggregation induced by strains of GBS.

METHODS

Bacterial strains. GBS were isolated from clinical specimens at the Clinical Laboratory, St Marianna University School of Medicine Hospital. The organisms were bacitracin-resistant and were identified as GBS by both the

Abbreviations: ASA, acetylsalicylic acid; CF, clumping factor; DPO, 2,5-diphenyloxazole; GAS, group A streptococci; GBS, group B streptococci; GFP, gel-filtered platelets; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

microprecipitin test (Swift *et al.*, 1943) and serological grouping by the Phadebact-Streptococcus test (Pharmacia), which is based on the method of Christensen *et al.*, (1973). A strain of group A streptococci (GAS) obtained from the clinical specimens was used as a control.

Culture. The bacteria were grown for approximately 20 h in Todd-Hewitt broth (Difco). They were harvested by centrifugation (7000 *g*, 4 °C, 30 min), washed three times with 0.01 M-phosphate buffered saline (PBS), pH 7.4, and resuspended in the same buffer. Bacterial numbers were estimated by nephelometry and reference to a standard curve of light scattering versus viable count. Bacteria were killed by heating at 60 °C for 10 min before use unless otherwise stated.

Chemicals. 5-Hydroxy[G-³H]tryptamine creatinine sulphate (19.6 Ci mmol⁻¹; 725.2 GBq mmol⁻¹) was from Amersham, human fibrinogen (grade L) from AB KABI, Na₂EDTA, indomethacin, acetylsalicylic acid (ASA), trypsin and 2,5-diphenyloxazole (DPO) from Wako Pure Chemical Industries, quinacrine dihydrochloride and NADH (disodium salt) from Sigma, Triton X-100 from Nakarai Chemicals, and Sepharose-2B from Pharmacia. Other chemicals were all reagent grade.

Preparation of human platelets. Human blood was collected into 0.1 vol. 3.8% (w/v) sodium citrate from drug-free donors and was used as a source of platelets. Only plastic tubes and pipettes were used. Citrated blood was gently mixed, then centrifuged at 150 *g* for 10 min at room temperature. Platelet-rich plasma (PRP) was removed, and the platelet concentration was adjusted to 300 000 µl⁻¹ by addition of platelet-poor plasma (PPP) obtained by centrifuging the PRP at 1640 *g* for 10 min. Platelet numbers were counted as described by Brecher & Cronkite (1950). PRP was stored in capped plastic tubes at room temperature until used, and experiments were completed within 4 h. Gel-filtered platelets (GFP) were prepared by passing 2.0 ml portions of the PRP preparation through a Sepharose-2B column according to the method of Timmons & Hawiger (1978). In the case of GFP, numbers of platelets were adjusted to 300 000 µl⁻¹ by the addition of HEPES buffer, pH 7.35. For the preparation of serum, blood drawn from the volunteers was kept for 1 h at 37 °C before transfer to 4 °C to facilitate clot contraction. Blood cells were then removed by centrifugation at 1500 *g* for 20 min, and serum was obtained by means of a sterile pipette.

Platelet aggregation. A tetra-channel aggregometer (NKK Hema Tracer 1 model Pat-4A PAT-4M) was used to measure platelet aggregation. In each experiment, PPP was used to adjust the instrument to 100% transmission to represent 100% aggregation. A mixture of 200 µl of either PRP or GFP, and 22 µl 0.01 M-PBS, pH 7.4, was used to represent 0% transmission (zero aggregation). In the actual experiments, 200 µl PRP or GFP was added to a cuvette, which was placed in the aggregometer with stirring at 1000 r.p.m., and allowed to warm to 37 °C. Then 22 µl of the bacterial suspension was added, and the density of the bacterial suspension was readjusted to zero. Platelet aggregation was observed for at least 10 min. To test whether the platelet preparation was normal and adequate for the reaction to proceed, ADP was added to a final concentration of 1.5 µM.

In the inhibition experiments, indomethacin, ASA, quinacrine or EDTA was preincubated with the PRP for 10 min at room temperature before transfer to the 37 °C environment in the aggregometer. To observe the effects of plasma components, the GFP (0.2 ml) were kept for 10 min at room temperature together with one of several plasma components (40 µl). When necessary, serum was heated at 56 °C for 30 min and the precipitates formed were discarded after centrifugation.

Release of serotonin and lactate dehydrogenase. Serotonin within platelets was labelled by incubation of PRP with 1 µCi (37 kBq) [³H]serotonin for 30 min at room temperature. After the addition of bacterial suspension, aggregation was recorded as usual. From a separate tube containing a larger volume of the same mixture in a 37 °C water bath, 300 µl portions were removed at intervals of approximately 1 min and added to 50 µl 933 mM-formaldehyde containing 51 mM-EDTA in 1.5 ml centrifuge tubes held in ice. The tube was centrifuged at 12 000 *g* for 2 min. Duplicate 50 µl samples were removed and added to 5 ml scintillation fluid (1 vol. Triton X-100 and 2 vols toluene containing 0.4%, w/v, DPO), and radioactivity was measured by an Aloka Liquid Scintillation System LSL-753. Total radioactivity in the samples was determined by using uncentrifuged controls. Release of radiolabelled serotonin was calculated as described by Holmsen & Dangelmaier (1977).

Lactate dehydrogenase released from platelets during these experiments was determined by the method reported by Bergmeyer *et al.* (1965). A solution of Triton X-100 was used to lyse the platelets to determine the total amount of intracellular lactate dehydrogenase.

Assay of CF activity. This was done by the method reported previously (Usui *et al.*, 1982). Briefly, a bacterial suspension containing about 8.5×10^9 c.f.u. ml⁻¹ and a fibrinogen solution (3 mg ml⁻¹) were prepared immediately before the assay. Then the bacterial suspension was mixed on glass slides with equal volumes of serial twofold dilutions of fibrinogen solution. Within 2 min of the reaction, the reciprocal of the maximum dilution causing clumping of the bacteria was recorded.

Trypsin digestion of bacteria. Heat-killed (60 °C for 10 min) bacteria were adjusted to 1.0×10^9 c.f.u. ml⁻¹ in 0.01 M-PBS, pH 7.4 and incubated with trypsin at a concentration of 0.01 mg ml⁻¹ at 37 °C for the required time. After incubation, bacteria were washed three times with 0.01 M-PBS, pH 7.4, before resuspension to 9.0×10^9 c.f.u. ml⁻¹.

Preparation of rabbit antisera. GBS strains were cultured in Todd-Hewitt broth at 37 °C for 24 h, then the

organisms were washed once by centrifugation and resuspended in 0.01 M-PBS, pH 7.4. The organisms were treated with formalin and then the turbidity was adjusted by nephelometry to $OD_{430} = 0.5$, which corresponded to $2.1-2.4 \times 10^8$ c.f.u. ml⁻¹. Quantities of 0.2 ml were injected intravenously into rabbits on three successive days during the first week. In the second week, the vaccine was increased to 0.5 ml; thereafter, 1.0 ml was injected on three successive days in each week for one month. Two weeks after the final immunization the rabbits were exsanguinated, and the sera were separated. The sera obtained were pre-absorbed with type Ia, Ib, Ic, and II organisms to eliminate the reaction with group antigens on the cells. The pooled pre-absorbed serum had an agglutination titre against the homologous strain of 1280.

Inhibition of the platelet aggregation activity with type-specific antisera. Equal volumes of the bacterial suspension and type III pre-absorbed sera were mixed, incubated at 37 °C for the required time, and then subjected to the platelet aggregation assay.

RESULTS

Platelet aggregation activity in GBS. When 46 strains (7 type Ia, 1 type Ib, 3 type Ic, 10 type II, 22 type III, and 3 non-typable strains) were tested for reactivity with platelets, four strains, all belonging to type III and positive for the CF reaction (Usui *et al.*, 1982), were also positive for platelet aggregation within 10 min. The values of platelet aggregation induced by the four strains, SMU-11, 50, 53 and 56, were 80, 86, 82 and 90%, respectively. The strains that were negative for platelet aggregation were all CF-negative.

Effects of the ratio between the counts of bacteria and platelets on platelet aggregation. When the bacterial concentration exceeded 8.1×10^7 c.f.u. ml⁻¹, a significant increase in the aggregative response developed, with a maximum at about 1.3×10^9 c.f.u. ml⁻¹ (final concentration). This optimal cell number corresponded to a bacterial c.f.u./platelets ratio of 4.3 and was similar with all the strains used in these experiments (Fig. 1).

Serotonin release. The release of serotonin from platelets was detected after 2 min incubation with GBS. With each of the GBS strains, after 10 min incubation (when aggregation was mostly complete) 68–78% of the intracellular serotonin was released from the platelets (Table 1, Fig. 2). Similarly, 70.6% of the serotonin was released from platelets after 10 min incubation with a GAS strain (Table 1).

Lactate dehydrogenase release. To determine whether platelets were lysed by the action of GBS, the activity of lactate dehydrogenase was measured 10 min after commencement of the aggregation experiment. The platelets contained the expected amount of the enzyme (1002 U), whereas the supernatant fluids from the PBS control and the four GBS bacteria/platelet mixtures had similar levels, 113 and 181–256 U, respectively (Table 1). In the case of the GAS strain, 249 U of enzyme was detected (Table 1).

Effects of EDTA, ASA, indomethacin and quinacrine on the platelet aggregation induced by GBS. Platelet aggregation by the four strains of GBS was totally inhibited by 3.1 mM-EDTA but not by 0.67 mM-EDTA (Table 2). Also two cyclooxygenase inhibitors and one phospholipase A₂ inhibitor (Roth & Majerus, 1975; Vargaftig, 1977) were examined for their ability to inhibit platelet aggregation by GBS. ASA (0.1 mM) inhibited aggregation by 78–89% whereas quinacrine (0.025 mM) gave 74–86% inhibition, depending on the strain used. Higher doses of the reagents, 10 mM-ASA or 0.25 mM-quinacrine, completely inhibited the aggregation induced by the four strains of bacteria tested, as did 10 mM-indomethacin (Table 2).

Requirement for plasma components. To test the effects of plasma components on GBS-induced platelet aggregation, separate lots of GFP were supplemented with one of the following components: human fibrinogen, heated serum (56 °C, 30 min) or PPP. In the experiments with GBS strains, GFP alone or GFP supplemented with fibrinogen were not sufficient for complete platelet aggregation. However, in the case of the GAS strain, supplementation of GFP with fibrinogen was sufficient for aggregation to occur (Table 3). Supplementation with heated serum was also ineffective for platelet aggregation by GBS. Only supplementation with PPP or a mixture of fibrinogen and heated serum achieved complete platelet aggregation in the case of GBS (Table 3).

Fig. 1.

Fig. 1. Percentage aggregation of platelets in PRP upon exposure to different concentrations of four strains of GBS during a 10 min incubation. ■, SMU-11; ●, SMU-50; ○, SMU-53; ▲, SMU-56.

Fig. 2.

Fig. 2. Release of serotonin (○—○; bars indicate range) and percentage aggregation of human platelets exposed to GBS strain SMU-56 (spiked line) over a 10 min incubation. Bacterial numbers (final concentration) were 1.3×10^9 c.f.u. ml⁻¹.

Table 1. Release of serotonin and lactate dehydrogenase (LDH) during platelet aggregation by four strains of GBS and one strain of GAS

The results are means $\pm$ range of two determinations. Bacterial numbers (final concentration) were $1.2\text{--}1.4 \times 10^9$ c.f.u. ml⁻¹ for the GBS strains and 9.5×10^8 c.f.u. ml⁻¹ for the GAS strain.

Strain	Serotonin release (%)	LDH (U ml ⁻¹)*	Platelet aggregation (%)
GBS			
SMU-11	75.6 $\pm$ 2.3	224.4 $\pm$ 82.7	80 $\pm$ 2
SMU-50	78.3 $\pm$ 1.6	181.9 $\pm$ 30.7	86 $\pm$ 3
SMU-53	68.3 $\pm$ 2.5	215.0 $\pm$ 30.7	82 $\pm$ 3
SMU-56	76.6 $\pm$ 1.7	256.4 $\pm$ 8.8	90 $\pm$ 3
GAS			
SMU-054	70.6 $\pm$ 1.9	248.9 $\pm$ 19.1	75 $\pm$ 3

* LDH was assayed after 10 min of the commencement of the reaction. Control values: saline (negative control), 113.4 $\pm$ 9.7; Triton X-100 (positive control), 1001.7 $\pm$ 19.6.

Effects of type-specific antisera on the aggregation ability of GBS. When undiluted type-specific antisera were preincubated for 10 min with heat-killed bacteria of strain SMU-50 or SMU-56 before contact with PRP, platelet aggregation was mostly prevented. After 30 min incubation, the aggregation induced by these strains was completely inhibited (Table 4). Platelet aggregation induced by strain SMU-50 was inhibited to 0, 1, 2.5, 8 and 54% of the control aggregation (after 5 min of the reaction) by undiluted, 10-fold, 20-fold, 40-fold and 80-fold diluted antisera, respectively (Fig. 3). However, when living bacteria were used in place of heat-killed bacteria, type-specific antisera were not inhibitory even after prolonged incubation (65 min) (Table 4).

Effects of trypsin digestion of GBS on their platelet aggregation ability and CF activity and the agglutination titre of type-specific antisera. Bacteria of the four GBS strains positive for platelet aggregation were incubated with trypsin solution for several minutes and tested for their

Fig. 3. Effects of different doses of type-specific antisera on the platelet-aggregating activity of GBS strain SMU-56. The numbers next to the traces indicate the dilution (fold) of the antiserum. Undil., undiluted antiserum; PBS, 0.01 M-PBS (pH 7.4) (control). Bacterial numbers (final concentration) were 1.3×10^9 c.f.u. ml⁻¹.

Table 2. *Effects of EDTA, ASA, quinacrine and indomethacin on the platelet aggregation induced by GBS strain SMU-50*

The results are means $\pm$ range of two determinations. Bacterial numbers (final concentration) were 1.1×10^9 c.f.u. ml⁻¹. The following concentrations of reagents were also tested and completely inhibited platelet aggregation: EDTA, 3.1 and 10 mM; ASA, 5 and 10 mM; quinacrine, 0.25 and 1 mM. Similar results were obtained with the other three GBS strains tested, except that 5 mM-ASA inhibited aggregation by about 95% in the case of strains SMU-53 and 56.

Reagent	Concn (mM)	Platelet aggregation (%)	Inhibition (%)
EDTA	0.67	50 $\pm$ 2	41
ASA	0.1	9 $\pm$ 1	89
Quinacrine	0.025	22 $\pm$ 2	74
Indomethacin	10	0	100
PBS (control)		85 $\pm$ 3	0

Table 3. *Effects of supplementation with plasma components on aggregation of GFP by four strains of GBS and one strain of GAS*

The results are means $\pm$ range of two determinations. Bacterial numbers (final concentration) were $1.0\text{--}1.4 \times 10^9$ c.f.u. ml⁻¹ for the GBS strains and 9.5×10^8 c.f.u. ml⁻¹ for the GAS strain.

Strain	Platelet aggregation (%)			
	GFP supplemented with			GFP alone
	Fib.*	Fib.* + htd serum†	PPP	
GBS				
SMU-11	7 $\pm$ 1	70 $\pm$ 2	73 $\pm$ 2	0
SMU-50	9 $\pm$ 1	65 $\pm$ 2	74 $\pm$ 2	0
SMU-53	7 $\pm$ 1	70 $\pm$ 2	77 $\pm$ 3	0
SMU-56	8 $\pm$ 1	68 $\pm$ 2	80 $\pm$ 3	0
GAS				
SMU-054	55 $\pm$ 2	70 $\pm$ 2	75 $\pm$ 2	3 $\pm$ 1

* Fibrinogen (final concentration 3 mg ml⁻¹).

† Heated serum (56 °C, 30 min).

Table 4. *Effects of type-specific antisera on the platelet-aggregating ability of two strains of GBS*

The results are means $\pm$ range of two determinations. Bacterial numbers (final concentration) were 1.2×10^9 c.f.u. ml⁻¹ for SMU-50 and 0.8×10^9 c.f.u. ml⁻¹ for SMU-56.

Strain	Addition of antiserum*	Incubation time (min)	Aggregation (%)	Inhibition (%)
SMU-50				
heat-killed	+	10	6 $\pm$ 1	92.9
heat-killed	+	30	0	100
heat-killed	-	10	85 $\pm$ 2	0
living	+	10	70 $\pm$ 3	12.5
living	+	65	70 $\pm$ 3	12.5
living	-	10	80 $\pm$ 3	0
SMU-56				
heat-killed	+	10	15 $\pm$ 1	81.9
heat-killed	+	30	0	100
heat-killed	-	10	62 $\pm$ 2	0
living	+	10	80 $\pm$ 2	0
living	+	65	79 $\pm$ 2	1.3
living	-	10	80 $\pm$ 2	0

* +, Anti-type III serum (undiluted) used; -, 0.01 M-PBS (pH 7.4) used.

aggregation abilities. In the case of strain SMU-50 (9.0×10^8 c.f.u. ml⁻¹, final concentration), the platelet aggregation activity decreased from 85% to 5% after 5 min trypsin digestion. The CF assay revealed that 5 min trypsin digestion was enough to remove the CF activity on the cell surface. In contrast, the type antigen, measured by agglutination titre using anti-type III serum, was not lost by the trypsin treatment, although the titre decreased to one-fourth (titre 320) of the control value (titre 1280). A similar trend was observed with the three other strains.

DISCUSSION

GBS-induced platelet aggregation was reported by Wood & Gray (1986) to be dependent on the GBS/platelet ratio, with an optimal ratio of 1.5. In the present experiments, maximum aggregation was observed at a ratio of about 4.3 for all the strains tested, although the same procedure as reported by Wood & Gray (1986) was used. This difference may be due to variation in platelets from different individuals.

The presence of EDTA, ASA or quinacrine has been reported to inhibit GAS-induced platelet aggregation (Kurpiewski *et al.*, 1983). Also, bacterium-induced platelet aggregation has been reported to require plasma components such as fibrinogen (Des Prez *et al.*, 1980; Kurpiewski *et al.*, 1983), immunoglobulin G (Des Prez *et al.*, 1980; Hawiger *et al.*, 1979), or complement system components (Hawiger *et al.*, 1972; Sheth *et al.*, 1980; Skerl *et al.*, 1981). The results described above indicate that GBS-induced platelet aggregation requires both cations and an intact arachidonate pathway, as well as the presence of fibrinogen and heat-resistant (56 °C, 30 min) serum components in the reaction mixture. These properties are similar to those of GAS-induced platelet aggregation (Kurpiewski *et al.*, 1983), except the requirement for heat-resistant serum components in GBS. In addition, participation of a bacteriolytic substance such as haemolysin seemed unlikely in the GBS-platelet reaction.

Fibrinogen receptor is present on some species of bacteria (Kronvall *et al.*, 1979; Schönbeck *et al.*, 1981; Yoshida *et al.*, 1979). In staphylococci, fibrinogen receptor has long been called CF because of its effects when bacteria are mixed with fibrinogen or plasma (Much, 1908). We have found a similar material in GBS and tentatively called it CF (Usui *et al.*, 1982). The rate of distribution of CF-positive strains among GBS was relatively low (4 out of 76; unpublished data). Interestingly, the strains positive for platelet aggregation were all positive for CF activity, and aggregation-negative strains were all negative for CF activity. Thus, the CF of GBS could participate in the reaction since fibrinogen is required for platelet aggregation to proceed. However, it is not known how these plasma factors participate in the platelet aggregation by GBS.

As inhibition of GBS-induced platelet aggregation by type-specific rabbit antisera was reported by Wood & Gray (1986), we did similar experiments. Although results which supported their data were obtained when heat-killed bacteria were used, little or no inhibition was observed when live bacteria were used. The fact that type-specific antisera inhibit the reaction retrospectively supports the idea that the type III antigen itself is responsible for the aggregation phenomenon. However, only four out of 22 strains of type III GBS showed a positive reaction and a crude type III antigen extract failed to induce platelet aggregation (unpublished data). Furthermore, although the platelet-aggregating activity was easily destroyed by a brief treatment with trypsin, the net amount of the type III antigen on the cell surface (measured by agglutination titre) was not significantly affected by the treatment, unlike the case of CF activity. These data suggest that another factor (or factors) different from type III antigen is (are) associated with the phenomenon of platelet aggregation by GBS, although the basis of the different effects of the antisera on heat-killed and live bacteria needs to be elucidated.

The extraction and purification of the substance inducing this phenomenon are in progress in our laboratory.

We thank Dr M. Yamamoto, Department of Laboratory Medicine, St Marianna University School of Medicine, for use of the aggregometer.

REFERENCES

- BERGMEYER, H. U., BERNT, E. & HESS, B. (1965). Lactic dehydrogenase. In *Methods of Enzymatic Analysis*, pp. 736–741. Edited by H. U. Bergmeyer. New York: Academic Press.
- BRECHER, G. & CRONKITE, E. P. (1950). Morphology and enumeration of human blood platelets. *Journal of Applied Physiology* **3**, 365–377.
- CHRISTENSEN, P., KAHLMEYER, G., JONSSON, S. & KRONVALL, G. (1973). New method for the serological grouping of streptococci with specific antibodies absorbed to protein-A-containing staphylococci. *Infection and Immunity* **7**, 881–885.
- CLAWSON, C. C. & WHITE, J. G. (1971). Platelet interaction with bacteria. I. Reaction phases and effects of inhibitors. *American Journal of Pathology* **65**, 367–380.
- CZUPRYNSKI, C. J. & BALISH, E. (1981). Interaction of rat platelets with *Listeria monocytogenes*. *Infection and Immunity* **33**, 103–108.
- DES PREZ, R. M., STECKLEY, S., STROUD, R. M. & HAWIGER, J. (1980). Interaction of *Histoplasma capsulatum* with human platelets. *Journal of Infectious Diseases* **142**, 32–39.
- HAWIGER, J., MARNEY, S. R., COLLEY, D. G. & DES PREZ, R. M. (1972). Complement-dependent platelet injury by staphylococcal protein A. *Journal of Experimental Medicine* **136**, 68–80.
- HAWIGER, J., STECKLEY, S., HAMMOND, D., CHENG, C., TIMMONS, S., GLICK, A. D. & DES PREZ, R. M. (1979). Staphylococci-induced human platelet injury mediated by protein A and immunoglobulin G Fc fragment receptor. *Journal of Clinical Investigation* **64**, 931–937.
- HENSON, P. M. (1970). Mechanisms of release of constituents from rabbit platelets by antigen-antibody complexes and complement. *Journal of Immunology* **105**, 476–489.
- HOLMSEN, H. & DANGELMAIER, C. A. S. (1977). Adenine nucleotide metabolism of blood platelets. X. Formaldehyde stops centrifugation-induced secretion after A23187-stimulation and causes breakdown of metabolic ATP. *Biochimica et biophysica acta* **497**, 46–61.
- HOLMSEN, H., DAY, H. J. & STORMORKEN, H. (1969). The blood platelet release reaction. *Scandinavian Journal of Haematology* (Supplement) **8**, 3–26.
- JERUSHALMY, Z., KOHN, A. & DE VRIES, A. (1961). Interaction of myxoviruses with human blood platelets *in vitro*. *Proceedings of the Society for Experimental Biology and Medicine* **106**, 462–464.
- KRONVALL, G., SCHÖNBECK, C. & MYHRE, E. (1979). Fibrinogen binding structure in β -hemolytic streptococci group A, C, and G. *Acta pathologica et microbiologica scandinavica* **87**, 303–310.
- KURPIEWSKI, G. E., FORRESTER, L. J., CAMPBELL, B. J. & BARRETT, J. T. (1983). Platelet aggregation by *Streptococcus pyogenes*. *Infection and Immunity* **39**, 704–708.
- MORRISON, D. C., KLINE, L. F., OADES, Z. G. & HENSON, P. M. (1978). Mechanisms of lipopolysaccharide-initiated rabbit platelet responses: alternative complement pathway dependence of the lytic response. *Infection and Immunity* **20**, 744–751.
- MUCH, H. (1908). Über eine Vorstufe des Fibrinfermentes in Kulturen von *Stafylokokkus Aureus*. *Biochemische Zeitschrift* **14**, 143–155.
- ROTH, G. J. & MAJERUS, P. W. (1975). The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. *Journal of Clinical Investigation* **56**, 624–632.
- SCHÖNBECK, C., BJORCK, L. & KRONVALL, G. (1981). Receptors for fibrinogen and aggregated β_2 -microglobulin detected in strains of group B streptococci. *Infection and Immunity* **31**, 856–861.
- SHETH, N. K., KURUP, V. P. & BARRON, B. A. (1980). The role of *Aspergillus fumigatus* antigens in blood coagulation and platelet function. *Microbios* **28**, 91–96.
- SKERL, K. G., CALDERONE, R. A. & SREEVALSAN, T. (1981). Platelet interactions with *Candida albicans*. *Infection and Immunity* **34**, 938–943.
- SWIFT, H. F., WILSON, A. T. & LANCEFIELD, R. C.

- (1943). Typing group A hemolytic streptococci by M-precipitin reactions in capillary pipettes. *Journal of Experimental Medicine* **78**, 127-133.
- TIMMONS, S. & HAWIGER, J. (1978). Separation of human platelets from plasma proteins including factor VIII by a combined albumin gradient-gel filtration method using HEPES buffer. *Thrombosis Research* **12**, 297-306.
- USUI, Y., YOSHIDA, K. & SAN CLEMENTE, C. L. (1982). Characterization of partially purified group B streptococcal clumping factor. *Zentralblatt für Bakteriologie, Microbiologie und Hygiene (I Abt. Orig. A)* **252**, 299-309.
- USUI, Y., ICHIMAN, Y., YOSHIDA, K. & OIKAWA, K. (1986). Possible induction of disseminated intravascular coagulation in the mouse by group B streptococcal clumping factor. *British Journal of Experimental Pathology* **67**, 629-635.
- VARGAFTIG, B. B. (1977). Carragenan and thrombin trigger prostaglandin synthetase-independent aggregation of rabbit platelets: inhibition by phospholipase A₂ inhibitors. *Journal of Pharmacy and Pharmacology* **29**, 222-228.
- WOOD, E. G. & GRAY, B. M. (1986). Type-specific antibody prevents platelet aggregation induced by group B streptococci type III. *Journal of Laboratory and Clinical Medicine* **107**, 322-326.
- YOSHIDA, K., TAKAHASHI, M., OHTOMO, T., USUI, Y. & NARIKAWA, S. (1979). Factors causing the clumping reaction of streptococcal strains with human plasma. *Journal of Infectious Diseases* **139**, 242-245.