Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5873–5877, August 1987
Immunology

Regulation of the activity of platelet-bound C3 convertase of the alternative pathway of complement by platelet factor H

DANA V. DEVINE* AND WENDELL F. ROSSE

Departments of Microbiology and Immunology and Medicine, Duke University Medical Center, Durham, NC 27710

Communicated by D. Bernard Amos, April 27, 1987

ABSTRACT **The alternative pathway of complement is regulated on the surface of homologous blood cells at the C3 amplification step by the membrane protein decay-accelerating factor, as well as by the plasma protein factor H. We have reported elsewhere that platelets from patients with paroxysmal nocturnal hemoglobinuria regulate the activity of the C3 convertase C3bBb, even though they lack decay-accelerating factor. We now report that normal human platelets contain factor H, which was released from the platelet in response to complement deposition or thrombin stimulation. Factor H was localized to the platelet α granules by immunocytochemical techniques. As determined by a solid-phase radioimmunoassay, thrombin-stimulated platelets released ≈54 ng of factor H per 10^8 platelets. The release of factor H in response to complement or thrombin was inhibited by treating the platelets with metabolic inhibitors. Such inhibition resulted in a 3-fold increase in the activity of C3bBb. Platelets that released factor H bound only half as many molecules of radiolabeled factor B to platelet-bound C3b than platelets that could not release factor H. Treatment of platelets with anti-decay-accelerating factor antibody had no effect on the activity of C3bBb unless the release of factor H was blocked. Therefore, so far as we know, human platelets have a unique mechanism for the regulation of the alternative pathway of complement.**

Platelets interact with the complement system in several specific ways. Thrombin-induced platelet aggregation may be enhanced in the presence of purified complement components C3 and C5–9 (1). Complement also enhances the platelet-release reaction induced by anti-platelet immunoglobulin (2). The deposition of complement on the platelet surface in patients with autoimmune disease may contribute to the increased destruction of platelets (3, 4). The platelet, like other blood cells, has evolved mechanisms that protect it from the effects of the activation of homologous complement. When membrane attack complexes, C5b–9, are deposited on the platelet surface, the platelet can recover from complement damage by calcium-dependent exocytosis of C5b–9 (5).

Blood cells, including platelets, can regulate the early stages of complement activation. The C3 convertase complex, C3bBb, which forms the amplification step for C3 deposition on the cell surface, can be regulated by three different proteins: the membrane proteins CR1 (6) and decay-accelerating factor (DAF) (7), and the plasma protein factor H (8). All three proteins act to destabilize the C3bBb complex. CR1 and factor H interact with C3b and act as cofactors for the cleavage of C3b by factor I (9–11). DAF has affinity for the catalytic subunit of the complex, factor Bb (12) and lacks cofactor activity for factor I. Human platelets contain DAF (13, 14) but not CR1. Although platelet DAF is biochemically and immunologically similar to erythrocyte DAF (13, 15), the ability of platelet DAF to regulate the C3 convertases of the alternative and classical pathways on the platelet surface has not been demonstrated. The importance of DAF in the regulation of the classical pathway C3 convertase on platelets can be inferred from studies demonstrating that the platelets from patients with paroxysmal nocturnal hemoglobinuria (PNH) lack DAF and are readily lysed by complement that has been activated by anti-platelet antibodies (16).

Sonicates of platelets have been reported to accelerate the decay of C3bBb from the surface of erythrocytes (17). This activity could be removed from the sonicates with antiserum to factor H (17). Studies from this laboratory on the activity of the alternative pathway C3 convertase on the platelets of patients with PNH demonstrated that not all patients have the increased C3bBb activity that might be expected from platelets deficient in DAF (15). In studies reported here we demonstrate that normal human platelets contain factor H, which can be secreted from platelet α granules and can regulate the activity of platelet-bound C3bBb.

MATERIALS AND METHODS

Materials. Cobra venom factor (18), C3 (19), factor B (20), factor D (21), factor H (21), C3 nephritic factor (22), and DAF (7) were purified using described methods. Purified thrombin was the gift of John W. Fenton II (Albany, NY). Antiserum to erythrocyte DAF was raised in rabbits in our laboratory. This antiserum reacted only with DAF by immunoblot analysis of separated platelet proteins. Polyclonal antisera to factor B, C3, and factor H were purchased from Calbiochem. Polyclonal antiserum to factor I and monoclonal antibodies to factor H and C3d were purchased from Cytotech (San Diego, CA). IgG fractions of polyclonal antisera to C3 and factor H were purchased from Atlantic Antibodies (Westbrook, ME). Monoclonal antibody to C3c was the gift of J. D. Capra (University of Texas at Dallas) and Gordon Ross (University of North Carolina at Chapel Hill). Fluorescein-conjugated $F(ab')_2$ rabbit anti-goat IgG or goat anti-rabbit IgG and peroxidase-conjugated anti-goat or anti-rabbit antisera were purchased from Jackson ImmunoResearch (Avondale, PA). Proteins were labeled with ^{125}I using the Iodogen method (23). All other chemicals were purchased from Sigma.

Platelet Preparation. Platelets were prepared by either of two methods depending on the experiment. For some experiments, blood from healthy volunteers or consenting patients was anticoagulated in EDTA. The platelet-rich plasma was isolated after centrifugation at 110 × *g* and the platelets were washed by centrifugation into the appropriate buffer. For other experiments, blood was anticoagulated in acid citrate/dextrose and the platelets were separated from the plasma proteins by gel filtration on Sepharose CL-2B (24).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DAF, decay-accelerating factor; PNH, paroxysmal nocturnal hemoglobinuria.

*To whom reprint requests should be addressed at: Canadian Red Cross Blood Transfusion Service, Vancouver Centre, 4750 Oak Street, Vancouver, BC V6H 2N9, Canada.

Analysis of Platelet-Bound Complement by Cytofluorometry. Platelets underwent reaction with anti-complement antibodies or nonimmune serum controls for 30 min at room temperature, were washed three times in phosphate-buffered saline (PBS) containing 0.015 M EDTA, and then were incubated in fluorescein-conjugated anti-IgG of the appropriate specificity. Stained platelets were fixed in 1% formalin prior to fluorescence analysis. Cell-associated fluorescence was measured using an Ortho 50H cytofluorograph. Details of the local modifications to this machine can be found in ref. 25. Analysis was restricted to signals from single platelets and amplifiers were set in a linear mode.

Measurement of C3 Convertase Activity. Purified components were used to measure the activity of a C3 nephritic factor stabilized alternative pathway C3 convertase on the platelet surface. Platelets (5×10^8) in Veronal-buffered saline (5.0 mM sodium barbital/0.15 M NaCl/0.1% gelatin, pH 7.5) (GVB) containing 0.015 M EDTA underwent reaction for 30 min at 37°C with 1 mg of C3 and cobra venom factor–factor Bb complexes. These complexes were prepared using purified cobra venom factor, factor B, and factor D as described (15). The amount of platelet-bound C3b at this step was measured by monoclonal anti-C3c antibody binding assay similar to that described (15). These platelets (PC3b) were then washed into GVB containing 2.5 mM $MgCl_2$ and exposed to factor B, factor D, and C3 nephritic factor for 10 min at 37°C to create platelet-bound C3 convertase complexes. After one wash in GVB containing 2.5 mM $MgCl_2$, 1 mg of C3 was added to the platelets and they were incubated for 30 min at 37°C. These platelets (PC3bBbC3NeF) were washed three times in GVB containing 0.015 M EDTA and assayed for platelet-bound C3b. The determination of molecules of antibody bound per platelet was made from the specific activity of the iodinated anti-C3c and the platelet count. Nonspecific antibody binding was defined as the amount of molecules bound per platelet exposed only to C3 (PC3). A conversion ratio was calculated as follows:

$$\frac{\text{molecules bound/PC3bBbC3NeF} - \text{molecules bound/PC3b}}{\text{molecules bound/PC3b} - \text{molecules bound/PC3}}.$$

Metabolic Inhibition of Platelet Release. The release of platelets in response to agonists was blocked by treating platelets with metabolic inhibitors as described by Schmaier *et al.* (26). Platelets (5×10^8) were resuspended in PBS containing 30 mM 2-deoxy-D-glucose, 10 mM D-gluconic acid-δ-lactone and 15 μg of antimycin A per ml and incubated for 30 min at 37°C. For experiments using metabolically inhibited platelets, the inhibitors at the above concentrations were included in the appropriate buffers. These inhibitors had no apparent effect on complement activation.

Solid-Phase RIA for Factor H or C3. Monoclonal antibody to factor H or to C3 was immobilized on polyvinyl chloride 96-well microtiter plates (50 μl of antibody at 8 μg/ml per well) by incubation at room temperature for 90 min. The plate was rinsed with PBS and then incubated in PBS containing 1% bovine serum albumin for 45 min to block other protein-binding sites. Fifty microliters of dilutions of platelet extracts or supernates from stimulated or unstimulated platelets was added to the wells and incubated for 45 min. The unbound protein was rinsed out with PBS containing 1% bovine serum albumin and 50 μl of ^{125}I-labeled polyclonal anti-factor H or anti-C3 was added to each well. After a 30 min incubation, the wells were washed three times and the bound radioactivity was assessed in a scintillation well-type γ counter. A standard curve was constructed using dilutions of purified factor H or C3 from 1 μg/ml to 10 ng/ml. The concentration of factor H or C3 in the platelet samples was determined from the standard curve. Nonspecific binding was assessed by incubating the antigen in wells that were blocked but contained no capture antibody.

Immunoblot Analysis of Complement Proteins Contained in Platelets. Washed platelets were solubilized in 1% Nonidet P-40 and the protein concentration was adjusted to 1 mg/ml as measured by a modified Lowry technique (27). Platelet proteins (100 μg total protein per lane) were separated on $NaDodSO_4$/polyacrylamide gel using a 5–15% acrylamide gradient resolving gel. The proteins were transferred to nitrocellulose according to the method of Towbin *et al.* (28). After blocking unreacted sites with Tris saline containing 3% bovine serum albumin, the nitrocellulose was incubated either with nonimmune serum or with antibody to factor H, factor B, factor I, or C3. The blots were washed in Tris saline and then incubated in peroxidase-conjugated anti-IgG, which reacted with the IgG of the species used to raise the first antibody. Bound peroxidase-conjugated IgG was visualized using the chromogen 4-chloro-1-naphthol. Positive control lanes for each antiserum consisted of purified antigen when available or normal human serum.

Immunocytochemical Localization of Platelet Factor H. Factor H was localized within the platelet by the method of Stenberg *et al.* (29). Briefly, platelet membranes were treated with 1% saponin in PBS to make the membranes permeable to antibodies. After incubation in the IgG fraction of either polyclonal anti-factor H or nonimmune goat IgG diluted in saponin/PBS, the platelets were washed and reacted with peroxidase-conjugated anti-goat IgG. After washing out unbound antibody, the platelets underwent reaction with 3',3'-diaminobenzidine and H_2O_2. The platelets were then fixed in glutaraldehyde, postfixed in osmium, and processed for transmission electron microscopy. The material was examined using a Hitachi 11 electron microscope.

Binding of ^{125}I-Labeled Factor B to Platelet-Bound C3b. Washed platelets (5×10^8) were suspended in GVB containing 0.015 M EDTA and incubated with 1 mg of C3 and cobra venom factor–factor Bb complexes prepared as described above to affix C3b to the platelet surface. They were washed into GVB containing 2.5 mM $MgCl_2$ and a sample was removed to measure platelet-bound C3b. The platelets were then incubated with 0.5 μg of ^{125}I-labeled factor B and factor D for 10 min at 30°C. The unbound ^{125}I-labeled factor B was separated from the bound protein by centrifugation through phthalate esters as described (15). Specific binding was defined as that amount of factor B that was inhibited by the presence of 25 μg (50× molar concentration) of unlabeled factor B. The amount of platelet-bound C3b was determined using the monoclonal antibody binding assay described above.

RESULTS

The factor H identified in immunoblots of whole lysates of washed normal platelets had identical electrophoretic mobility to factor H, which was purified from normal plasma (Fig. 1). Factor H was also detected in immunoblots of the supernatant fluid surrounding platelets stimulated by thrombin (5 units/ml), but not in the supernate from platelets that were incubated in buffer (Fig. 1). Antisera to factor B, factor I, and C3 failed to detect the corresponding antigens in immunoblots of platelet lysates but readily detected their antigens in electrophoresed serum (data not shown). The factor H present in platelets was localized to platelet granules (Fig. 2). The reaction product was seen most intensely in platelets that were disrupted, presumably because the granule membranes were better permeabilized.

Using the solid-phase RIA, the level of platelet factor H released in response to stimulation by thrombin (5 units/ml) was determined to be 54 ± 6 ng of factor H per 10^8 platelets in a total of 27 normal donors (Table 1). The amount of

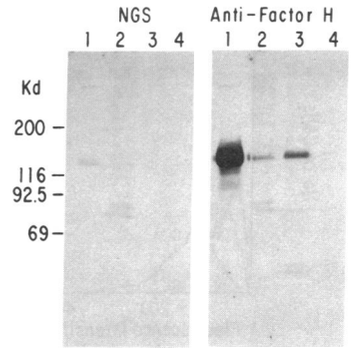

FIG. 1. Immunoblot analysis of platelet factor H. Purified plasma factor H (lanes 1), 100 μg of lysate from washed platelets (lanes 2), or the supernates from 5×10^8 platelets incubated with thrombin (5 units/ml) (lanes 3) or buffer (lanes 4) were electrophoresed in 5–15% acrylamide gradient $NaDodSO_4$/polyacrylamide gels and transferred to nitrocellulose paper. The paper was incubated first with normal goat serum (NGS) or goat anti-factor H antiserum, and then with peroxidase-conjugated anti-goat IgG. Numbers on left are in kDa.

platelet factor H measured in Triton X-100 extracts of 11 donors was 50 ± 9 ng of factor H per 10^8 platelets. Thrombin-stimulated platelets from the same donors released 89% of the factor H measured in detergent extracts. Platelets exposed to C3, cobra venom factor, factor B, and magnesium released 53 ng of factor H per 10^8 platelets, while parallel thrombin-stimulated controls released 69 ng (Table 2). The release of factor H in response to complement deposition was magnesium dependent. The amount of factor H released from platelets reacted with cobra venom factor–factor Bb complexes and C3 in the presence of EDTA was identical to platelets incubated in buffer alone. However, magnesium alone was insufficient to trigger release of factor H. Thrombin-stimulated platelets released 3.9 ng of C3 per 10^8 platelets as measured by the C3 RIA. There were no more than 4 ng of C3 per 10^8 platelets detected in Triton X-100 extracts of platelets.

Experiments to measure the release of factor H from gel-filtered platelets demonstrated that while thrombin readily triggered the release of factor H, 10 μM ADP was unable to cause release from gel-filtered platelets. However, 10 μM ADP was able to induce aggregation in the same preparation of platelets.

Table 1. Quantitation of factor H released from platelets as determined by a solid-phase radioimmunoassay

	ng of factor H released per 10^8 platelets	
	Mean ± 1 SD	Range
Supernate from thrombin-stimulated platelets (n = 27)	53.8 ± 6.1	30–89
Supernate from untreated platelets (n = 27)	3.0 ± 3.1	1–8
Triton X-100-solubilized platelets (n = 11)	49.5 ± 9.2*	30–60

*Thrombin-stimulated factor H release from the same 11 donors was 44.0 ± 12.3.

The thrombin-stimulated release of factor H from platelets could be almost completely blocked by treating the platelets with 2-deoxy-D-glucose, antimycin A and D-gluconic acid-δ-lactone. The amount of factor H released from 10^8 thrombin-stimulated platelets fell from 55 ng to 2 ng after treatment with the metabolic inhibitors. There was also an increase in the activity of the C3 convertase on the surface of metabolically inhibited platelets compared to control platelets that were not treated with inhibitors (Fig. 3). Fluorescence flow cytometry demonstrated that factor H could readily be detected on the surface of platelets bearing C3b. However, when platelets were treated with metabolic inhibitors prior to deposition of C3b, factor H was no longer detected bound to the platelet surface (Fig. 4).

Blocking factor H release by treatment with metabolic inhibitors also affected the binding stoichiometry of C3b and ^{125}I-labeled factor B (Table 3). When the platelets were able to release factor H, the ratio of molecules of factor B bound to molecules of C3b was 0.40. Treatment with metabolic inhibitors resulted in a factor B/C3b ratio of 0.96. Under the conditions of these experiments, there were $2–3 \times 10^{12}$ molecules of platelet-bound C3b, 3×10^{12} molecules of ^{125}I-labeled factor B, and 1.1×10^{12} molecules of factor H.

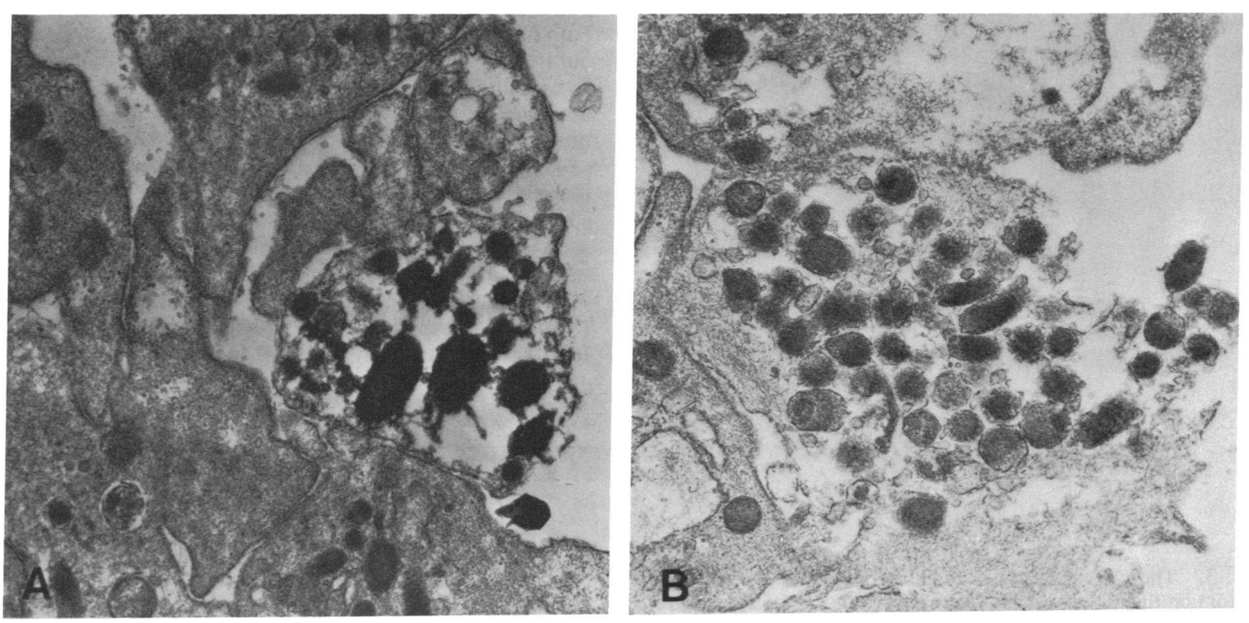

FIG. 2. Immunocytochemical localization of platelet factor H. Washed platelets were treated with saponin and reacted first with the IgG fraction of goat anti-factor H (*A*) or nonimmune goat IgG (*B*) and then with peroxidase-conjugated anti-goat IgG. (×17,000.)

Table 2. Quantitation of factor H released from platelets exposed to complement

Supernate from platelets incubated with	ng of factor H released per 10^8 platelets, mean ± 1 SD (n = 3)
Cobra venom factor–factor Bb complexes,* C3, 2.5 mM $MgCl_2$	53 ± 11.3
Thrombin (5 units/ml)	69 ± 25.7
GVB containing 2.5 mM $MgCl_2$	4 ± 2.0

*Cobra venom factor–factor Bb complexes were formed and chelated prior to the addition of platelets and C3.

This last number was calculated using 53 ng of factor H per 10^8 platelets.

The treatment of normal platelets with anti-DAF failed to alter the activity of the C3 convertase, although this antiserum completely blocked DAF activity on normal erythrocytes (data not shown). In three experiments, the conversion ratio for platelets treated with normal rabbit serum (5.4 ± 2.3) was not significantly different from the conversion ratio of platelets bearing saturating amounts of anti-DAF (4.9 ± 2.6). However, when platelets were treated with metabolic inhibitors to block factor H release and then incubated with anti-DAF, the activity of the C3 convertase increased by 27–61% (n = 3).

DISCUSSION

Previous studies from our laboratory indicated that platelets from patients with PNH, which are deficient in DAF, are still able to regulate the activity of platelet-bound C3 convertase complexes (15). This is unlike the PNH erythrocyte in which the DAF deficiency results in a marked increase in C3bBb activity (20). Since the work of Kenney and Davis (17) suggested that platelets contain factor H, we postulated that platelet factor H might regulate the activity of the alternative pathway C3 convertase. We have confirmed that platelets contain a protein identified by antiserum to factor H (17). In addition, we have shown that this protein has similar electrophoretic mobility to plasma factor H.

Normal donor platelets released an average of 54 ng of factor H per 10^8 platelets in response to thrombin stimulation. This value represents 0.05% of the plasma concentration of 300 μg/ml (30). This fraction is similar to that of other

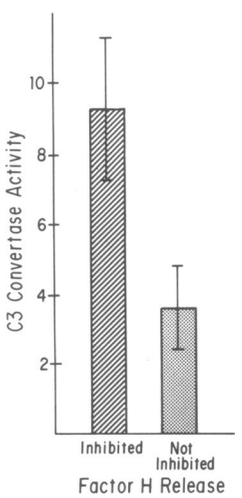

FIG. 3. The effect of the treatment of platelet with metabolic inhibitors on the activity of the alternative pathway C3 convertase. C3 convertase activity was assessed using purified complement proteins and a monoclonal anti-C3c binding assay. Convertase activity is expressed as the conversion ratio defined in the text.

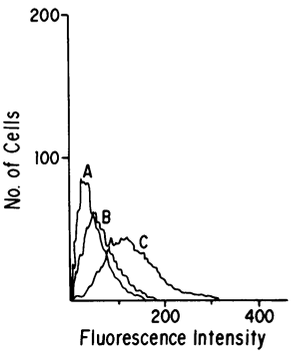

FIG. 4. Cytofluorometric analysis of anti-factor H binding to platelets. Platelets were incubated with metabolic inhibitors (curve B) or buffer (curve C) prior to exposure to cobra venom factor–factor Bb complexes and C3. The platelets were fixed in paraformaldehyde and reacted with either goat anti-factor H or normal goat serum. The binding of anti-factor H to platelets incubated in metabolic inhibitors or buffer but not exposed to complement is shown in curve A. Cytograms of normal goat serum binding to all cell groups were coincident with curve A.

inhibitor proteins that are found both in platelets and in plasma, including C1 inhibitor (26). It is unlikely that the factor H measured by RIA represents protein that is nonspecifically bound to the platelet or trapped in the platelet open canalicular system. The C3 RIA performed on the same platelets never detected >4 ng of C3 per 10^8 platelets even though the plasma concentration of C3 (1.5 mg/ml) is 5 times that of factor H.

The localization of factor H to platelet granules by immunocytochemical staining and electron microscopy further suggests that factor H is a specific platelet protein. Experiments which demonstrate that thrombin, but not ADP, triggered the release of factor H from the granules indicate that these are α granules (31).

The release of factor H from platelets in response to complement deposition is less complete than release in response to thrombin. In addition, the release triggered by complement required the presence of magnesium while thrombin triggered platelet release in the presence of EDTA. This may reflect the potency of thrombin as a platelet agonist.

The release of factor H was blocked by treating the platelets with metabolic inhibitors. This inhibition was seen both in the factor H radioimmunoassay, where thrombin did not trigger release from metabolically inhibited platelets, and in cytofluorometry experiments in which no factor H was detected on the surface of metabolically inhibited platelets bearing C3b. In experiments to assess the functional activity of C3bBb, the inhibition of factor H release resulted in an increase in the activity of the platelet-bound C3 convertase. That platelet factor H was capable of disrupting C3bBb was seen in experiments using radiolabeled factor B. The binding of factor B to platelet-bound C3b was nearly 1:1 when factor H release was blocked, but when the platelet could release factor H in response to complement deposition, there were less than half as many factor B molecules bound to C3b.

Table 3. The effect of metabolic inhibition of platelets on the binding of ^{125}I-labeled factor B to platelet-bound C3b

Platelets incubated with	Molecules of C3b per platelet*	Molecules of ^{125}I-labeled factor B per platelet*	Ratio of factor B/C3b
Metabolic inhibitors	5604 ± 967	5114 ± 628	0.92
Buffer	4118 ± 138	1686 ± 249	0.41

*Values are means ± 1 SD (n = 3).

The inhibition of DAF activity using anti-DAF antibody had no effect on the activity of the platelet-bound alternative pathway C3 convertase. However, if the platelets were prevented from releasing factor H, then the effect of inhibiting DAF with anti-DAF was reflected in an increase in the C3 convertase activity.

These studies indicate that platelets have a mechanism for the regulation of the alternative pathway of complement not found in erythrocytes. In settings where the platelet either lacks DAF—i.e., PNH—or DAF is unable to effectively regulate the activity of C3bBb, the release of factor H can regulate platelet-bound C3bBb. Although the plasma concentration of factor H is 300 μg/ml, the release of factor H from the platelet α granules may produce a local increase in factor H concentration either at the platelet surface or within a thrombus. High local concentration is suggested by the ability of platelet factor H to disrupt a C3 nephritic factor-stabilized C3 convertase. Such convertases are highly resistant to plasma factor H unless the factor H concentration is quite high (8). It is also possible that platelet factor H is more active than plasma factor H. Increase in activity has been reported for plasma factor H treated with proteases (32), which are also present in platelets (33).

Triggering the platelet release reaction *in vivo* may severely shorten the lifespan of the platelet as well as cause platelet aggregation and the release of platelet products that participate in coagulation reactions. The release of factor H may function to regulate more than C3bBb on the releasing platelet. Platelet α granules also contain factor D, the serine protease that cleaves factor B to its active form. Platelet factor D apparently can inhibit the binding of thrombin to the platelet surface (34). The simultaneous release of factor H may function to inhibit the localized activation of complement by the alternative pathway caused by both the generation of C3b by thrombin (1) as well as high local concentrations of factor D.

We thank Frank Cornew for performing the cytofluorimetric analysis. This work was supported by National Institutes of Health Grant 1 R01 AM31379 awarded to W.F.R.

1. Polley, M. J. & Nachman, R. L. (1978) *J. Exp. Med.* **147,** 1713–1726.
2. Cines, D. B. & Schreiber, A. D. (1979) *Blood* **53,** 567–577.
3. Kurata, Y., Curd, J. G., Tamerius, J. D. & McMillan, R. (1985) *Br. J. Haematol.* **60,** 723–733.
4. Tsubakio, T., Tani, P., Curd, J. G. & McMillan, R. (1986) *Br. J. Haematol.* **63,** 293–300.
5. Sims, P. J. & Wiedmer, T. (1986) *Blood* **68,** 556–561.
6. Iida, K. & Nussenzweig, V. (1981) *J. Exp. Med.* **153,** 1138–1150.
7. Nicholson-Weller, A., Burge, J., Fearon, D. T., Weller, P. F. & Austen, K. F. (1982) *J. Immunol.* **129,** 184–189.
8. Weiler, J. M., Daha, M. R., Austen, K. F. & Fearon, D. T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3268–3272.
9. Medof, M. E., Iida, K., Mold, C. & Nussenzweig, V. (1982) *J. Exp. Med.* **156,** 1739–1754.
10. Ross, G. D., Lambris, J. D., Cain, J. A. & Newman, S. L. (1982) *J. Immunol.* **129,** 2051–2060.
11. Pangburn, M. K., Schreiber, R. D. & Muller-Eberhard, H. J. (1977) *J. Exp. Med.* **146,** 257–270.
12. Pangburn, M. K. (1986) *J. Immunol.* **136,** 2216–2221.
13. Nicholson-Weller, A., March, J. P., Rosen, C. E., Spicer, D. B. & Austen, K. F. (1985) *Blood* **65,** 1237–1244.
14. Medof, M. E., Kinoshita, T., Silber, R. & Nussenzweig, V. (1985) *J. Exp. Med.* **162,** 75–92.
15. Devine, D. V., Siegel, R. S. & Rosse, W. F. (1987) *J. Clin. Invest.* **79,** 131–137.
16. Aster, R. H. & Enright, S. E. (1969) *J. Clin. Invest.* **48,** 1199–1210.
17. Kenney, D. M. & Davis, A. E., III (1981) *Clin. Immunol. Immunopathol.* **21,** 351–363.
18. Ballow, M. & Cochrane, C. G. (1969) *J. Immunol.* **103,** 944–952.
19. Tack, B. F. & Prahl, J. W. (1976) *Biochemistry* **15,** 4513–4521.
20. Parker, C. J., Baker, P. J. & Rosse, W. F. (1982) *J. Clin. Invest.* **69,** 337–346.
21. Lambris, J. D., Dobson, N. J. & Ross, G. D. (1980) *J. Exp. Med.* **152,** 1625–1644.
22. Schreiber, R. D., Gotze, O. & Muller-Eberhard, H. J. (1976) *Scand. J. Immunol.* **5,** 703–713.
23. Fraker, P. J. & Speck, J. C. (1978) *Biochem. Biophys. Res. Commun.* **80,** 849–857.
24. Tangen, O., Berman, H. J. & Marfey, P. (1971) *Thromb. Diath. Haemorrh.* **25,** 269–276.
25. Devine, D. V., Falk, R. J. & Balber, A. E. (1986) *Infect. Immun.* **52,** 223–229.
26. Schmaier, A. H., Smith, P. M. & Colman, R. W. (1985) *J. Clin. Invest.* **75,** 242–250.
27. Markwell, M. A., Haas, S. M., Bieber, L. L. & Tolberi, N. E. (1978) *Anal. Biochem.* **87,** 206–210.
28. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
29. Stenberg, P. E., Shuman, M. A., Levine, S. P. & Bainton, D. F. (1984) *Histochem. J.* **16,** 983–1001.
30. Whaley, K. (1985) *Methods in Complement for Clinical Immunologists* (Churchill Livingstone, New York), pp. 1–20.
31. Holmsen, H. (1982) in *Hemostasis and Thrombosis*, eds. Colman, R. W., Hirsh, J., Marder, V. J. & Salzman, E. W. (Lippincott, Philadelphia), pp. 390–403.
32. Hong, K., Kinoshita, T., Dohi, Y. & Inoue, K. (1982) *J. Immunol.* **129,** 647–652.
33. Legrand, Y., Pignaud, G. & Caen, J. P. (1977) *FEBS Lett.* **76,** 294–298.
34. Davis, A. E., III, & Kenney, D. M. (1979) *J. Clin. Invest.* **64,** 721–728.