

# Complement-Induced Vesiculation and Exposure of Membrane Prothrombinase Sites in Platelets of Paroxysmal Nocturnal Hemoglobinuria

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired stem-cell disorder in which the glycolipid-anchored membrane proteins, including the cell-surface complement inhibitors, CD55 and CD59, are partially or completely deleted from the plasma membranes of mature blood cells. To gain insight into the pathogenesis of thrombosis that is frequently observed in this disorder, the procoagulant responses of PNH platelets exposed to the human terminal complement proteins C5b-9 were investigated. C5b-9 complexes were assembled on gel-filtered platelets by incubation with purified C5b6, C7, C9, and limiting amounts of C8. Platelet microparticle formation and exposure of plasma membrane-binding sites for coagulation factor Va were then analyzed by flow cytometry. PNH platelets exhibiting undetectable levels of surface CD59 antigen showed an approximately 10-fold increase in sensitivity to

C5b-9-stimulated expression of membrane-binding sites for factor Va when compared with platelets from normal controls. Expression of catalytic surface for the prothrombinase complex (VaXa) paralleled the exposure of factor Va-binding sites; the rate of prothrombin conversion by C5b-9-treated PNH platelets exceeded that of C5b-9-treated normal controls by approximately 10-fold at the maximal input of C8 tested (500 ng/mL). These data indicate that PNH platelets deficient in plasma membrane CD59 antigen are exquisitely sensitive to C5b-9-induced expression of prothrombinase activity, and suggest that the tendency toward thrombosis in these patients may be due, at least in part, to the deletion of this complement inhibitor from the platelet plasma membrane.

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**P**AROXYSMAL NOCTURNAL hemoglobinuria (PNH) is a stem-cell disorder characterized clinically by hemolytic anemia and a hypercoagulable state that frequently leads to thrombosis of the veins of the liver, abdominal organs, cerebrum, and skin. The hemolysis is attributed to an unusual sensitivity to the lytic action of complement. The hypercoagulability has been attributed to the hemolysis itself or to defects in the platelets in this disorder. The abnormal blood cells in PNH have been found to be deficient in all proteins tethered to the membrane by the glycosylphosphatidylinositol (GPI) anchor, including those that regulate the activation of complement at the cell surface.<sup>1,2</sup>

The cytolytic activity of human serum resides in a complex of complement proteins C5b, C6, C7, C8, and C9.<sup>3,4</sup> When deposited on cell surfaces, these pore-forming proteins increase ion permeability of the plasma membrane, which can lead to lytic cell death. In addition, the C5b-9 proteins elicit nonlytic stimulatory responses from certain cells, including blood platelets and endothelial cells.<sup>5-7</sup> Human blood and vascular cells are normally protected from autologous activated components of the complement system by cell-surface proteins that inhibit assembly of complement pores. In addition to decay-accelerating factor

(CD55), which accelerates the disassembly of the C3 convertase,<sup>8</sup> the plasma membrane of these cells contains CD59, an inhibitor of the C5b-9 complex.<sup>9,10</sup> This membrane inhibitor, first isolated from erythrocytes, is a glycoprotein with an apparent molecular mass of 18 to 21 Kd, and is tethered to the cell surface by a GPI anchor. CD59 inhibits assembly of the C5b-9 pore through its capacity to restrict C9-binding to C5b-8, and the subsequent polymerization of C9 into a homopolymer.<sup>11,12</sup> A third possible regulatory protein, a 68-Kd protein called homologous restriction factor<sup>13</sup> or C8-binding protein,<sup>14</sup> has been reported to have properties similar to those of CD59, although its identity awaits further molecular characterization.

Assembly of the terminal complement proteins C5b-9 on the surface of blood platelets results in the secretion of the contents of dense and  $\alpha$ -granules and the shedding of vesicles from the platelet surface.<sup>5,6,15</sup> Vesiculation of the platelet plasma membrane has been shown to be an integral part of this cell's procoagulant response, and these vesicles (or microparticles) have been shown to provide principal catalytic surface for assembly of the procoagulant enzyme complexes prothrombinase (VaXa),<sup>15</sup> and tenase (VIIIaIXa).<sup>16</sup>

We have recently shown that blocking the function of CD59 by a specific antibody against CD59 potentiates the activation of normal platelets by the C5b-9 proteins, resulting in increased secretion from the storage granules and the shedding of procoagulant vesicles from the plasma membrane.<sup>17</sup> In this report, we show that PNH platelets with undetectable levels of CD59 antigen show increased sensitivity to activation by the C5b-9 proteins compared with normal controls, resulting in markedly increased production of procoagulant vesicles. This may contribute to the thrombotic tendency observed in patients with this disorder.

## MATERIALS AND METHODS

**Materials.** Bovine serum albumin (BSA; globulin- and fatty acid-free), prostaglandin I<sub>2</sub>, and the chromogenic substrate S2238 (D-Phe-L-pipecolyl-Arg-p-nitroanilide) were from Sigma Chemical (St Louis, MO); fluorescein-5-isothiocyanate (FITC) was from Molecular Probes (Eugene, OR); and phycoerythrin-streptavidin conjugate was from Southern Biotechnology Associates (Birmingham,

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AL). Purified human plasma factor V was prepared and activated with the factor V activator from Russell's viper venom as previously described.<sup>18</sup> Human thrombin, factor Xa, and prothrombin were from Haematologic Technologies (Essex Junction, VT). Murine monoclonal antibody (MoAb) W5, specific for membrane glycoprotein (Gp)Ib, was a gift from Dr Rodger P. McEver (University of Oklahoma Health Sciences Center, Oklahoma City, OK). The MoAb 162 is specific for CD59 (courtesy of Dr Marilyn J. Telen, Duke University, Durham, NC). The light chain of bovine factor Va and MoAb V237, specific for the light chain of factor Va, were gifts from Dr Charles T. Esmon (Oklahoma Medical Research Foundation). All other chemicals were of reagent or analytical grade.

**Solutions.** Buffer 1 consisted of 145 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L MgCl<sub>2</sub>, 0.5 mmol/L sodium phosphate, 0.1% (wt/vol) glucose, 0.1% (wt/vol) BSA, and 5 mmol/L PIPES, pH 6.8. Buffer 2 consisted of 137 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L MgCl<sub>2</sub>, 1 mmol/L CaCl<sub>2</sub>, 0.5 mmol/L sodium phosphate, 0.1% (wt/vol) glucose, 0.1% (wt/vol) BSA, 20 mmol/L HEPES, pH 7.4.

**Platelets.** Venous blood from aspirin-free healthy adult volunteers or from patients with PNH was drawn into 1/7 vol of acid citrate dextrose solution (National Institutes of Health formula A), and prostaglandin I<sub>2</sub> added to a final concentration of 250 ng/mL. Platelet-rich plasma was prepared by centrifugation for 20 minutes at 200g, platelets were sedimented for 20 minutes at 500g, and gel-filtered over Sepharose CL-2B (Pharmacia, Piscataway, NJ) equilibrated with buffer 1.

**Activation of platelets by C5b-9.** Gel-filtered human platelets were suspended in buffer 2 at 3 to 6 × 10<sup>8</sup>/mL. Human complement proteins C5b6,<sup>19</sup> C7,<sup>20</sup> C8,<sup>21</sup> and C9<sup>22</sup> were purified and analyzed for functional activity according to published procedures. To assemble membrane-bound C5b67 complexes, gel-filtered platelets (3 to 6 × 10<sup>8</sup>/mL) were incubated for 3 minutes at 37°C with C5b6 (10 μg/10<sup>8</sup> platelets) and C7 (2.5 μg/10<sup>8</sup> cells). To assemble membrane-bound C5b-9 complexes, C5b67 platelets were suspended to 10<sup>8</sup> platelets/mL in buffer 2. Then, C9 (10 μg/10<sup>8</sup> cells) and C8 at the concentration indicated in the legends to the figures were added, and the cells incubated for 10 minutes at 37°C. In all experiments, comparison was made to identical matched-pair controls (platelets incubated in the absence of the C5b-9 proteins).

**Labeling of MoAbs.** The MoAb V237 was labeled with FITC<sup>15</sup> and MoAb W5 was conjugated with *N*-hydroxysuccinimide biotin ester<sup>23</sup> as previously described.

**Preparation of platelets for flow cytometry.** A quantity of 2 × 10<sup>6</sup> C5b-9-treated or control platelets were incubated in the dark in a total volume of 35 μL for 10 minutes at 23°C in the presence of biotin-W5 (2 μg/mL) and factor Va light chain (2 μg/mL). FITC-V237 (30 μg/mL) and phycoerythrin-streptavidin (5 μL of a 1:10 dilution) were added, and the cells were incubated for an additional 10 minutes. Then, 1-mL aliquots of isotonic saline (Baxter Scientific Products, McGaw Park, IL) were added and the samples analyzed by flow cytometry. For quantitation of CD59 antigen levels, 25 μL of untreated platelet suspension (2.5 × 10<sup>6</sup> platelets) was incubated for 30 minutes with 25 μL of anti-CD59 MoAb 162 (1:1,000 diluted cell supernatant). The cells were washed twice and resuspended to the original volume, and 25 μL of FITC-labeled goat F(ab)<sub>2</sub> antimouse IgG (TAGO, Burlingame, CA) was added. Following incubation for 30 minutes in the dark, samples were analyzed by flow cytometry.

**Flow cytometry.** Samples were analyzed in a Becton Dickinson (Mountain View, CA) FACScan flow cytometer formatted for two-color analysis as described previously.<sup>15,23</sup> In brief, the FL2 (red fluorescence) threshold was first set so as to exclude from analysis background noise and light-scattering particles that were not distinctly positive for platelet GPIb antigen (detected with phycoer-

thrin-labeled MoAb W5; see above). For each sample, 10,000 GPIb-positive events were recorded and analyzed for forward (FSC) and right-angle (SSC) light scatter and for FL1 (green) fluorescence, representing the binding of FITC-labeled antibody to factor Va light chain. For each experiment, two-dimensional analysis gates of forward and right-angle light scatter (FSC × SSC) were set using results for normal platelets (control and complement-activated), so as to maximally discriminate platelet microparticles from platelets,<sup>15</sup> and all samples (normal and PNH) of the same day's experiment analyzed using these gates. The number of microparticles was quantitated as the number of events falling within the microparticle gate, expressed as the percentage of all recorded events falling within either platelet or microparticle gates. Mean FL1 fluorescence per particle was determined for events in each gate (platelet or microparticle), with correction for autofluorescence.

**Prothrombinase activity.** Platelet prothrombinase activity was measured essentially as described.<sup>24</sup> Briefly, C5b-9-activated gel-filtered platelets (normals and patients) were suspended at 2 × 10<sup>7</sup>/mL in 50 mmol/L Tris-Cl, pH 7.9, 175 mmol/L NaCl, 5 mg/mL BSA (TBS-BSA) in the presence of 2.7 μmol/L prothrombin, 2.5 mmol/L CaCl<sub>2</sub>, and 2.0 nmol/L factor Va. The reaction was initiated by the addition of factor Xa (final concentration, 1.0 nmol/L). At times 0, 30, 60, and 120 seconds, 25-μL aliquots of the reaction mixture were removed and diluted into 225 μL of ice-cold TBS-BSA containing 20 mmol/L EDTA. Thrombin generated was assayed using the chromogenic substrate S2238 (final concentration, 0.1 mmol/L) and measuring the rate of change in absorbance at 405 nm using a Vmax microtiter plate reader (Molecular Devices, Menlo Park, CA). Purified human thrombin was used as standard.

## RESULTS

**Generation of microparticles and factor Va-binding sites by activation of complement.** PNH platelets were initially examined for their susceptibility to complement-induced microvesiculation. PNH platelets selected for these studies lacked detectable CD59 antigen. C5b-9 complexes were assembled on gel-filtered platelets by incubation with the purified complement proteins C5b6, C7, C9, and limiting amounts of C8. Platelet activation responses were analyzed by fluorescence-gated flow cytometry, using FITC-labeled MoAb V237 to monitor factor Va-binding sites. Figure 1 shows dot blots of FITC-V237 fluorescence versus forward light scatter (logarithmic scales). As illustrated by these data, platelets from the PNH patients tested were significantly more sensitive to complement C5b-9 when compared with normal controls, as evidenced by a marked increase in (1) the formation of plasma membrane-derived microparticles, and (2) exposure of new binding sites for factor Va on both platelets and microparticles. In these experiments, platelet microparticles were discriminated from platelets by analysis of forward and side-angle light scatter (see Materials and Methods).<sup>15</sup> As is also evident from these data (see untreated samples of Fig 1), PNH platelets generally showed more basal activation in the absence of added complement proteins than normal platelets. This is likely to reflect the effects of prior complement activation at the surface of these cells either in vivo or during processing of platelet-rich plasma, due to deficiencies of the plasma membrane complement regulatory proteins CD55 (decay-accelerating factor) and CD59.

The newly formed population of microparticles was quantitated by setting a forward and side scatter gate so as to

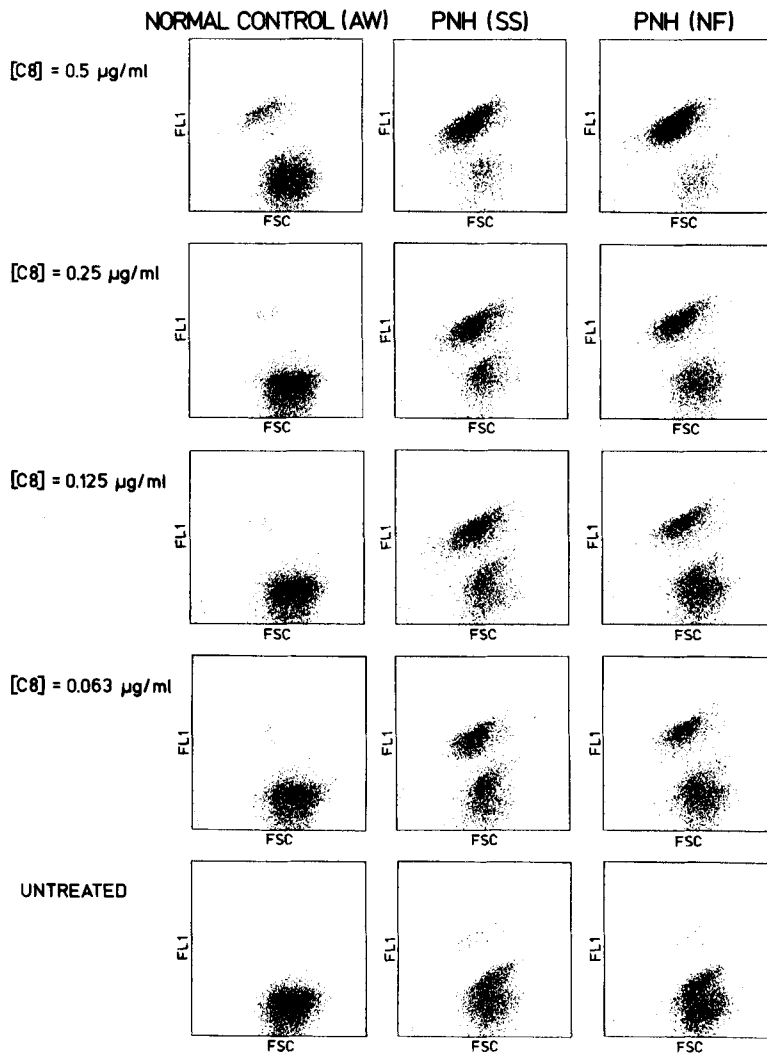


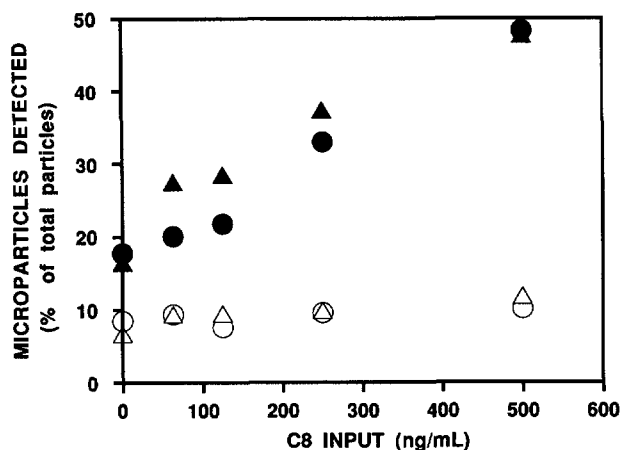
Fig 1. C5b-9–induced platelet microparticle formation. C5b67 complexes were assembled on platelets as described in Materials and Methods. These cells were then incubated for 10 minutes at 37°C in the presence of C9 (10 µg/mL) and C8 at the concentrations indicated, and prepared for flow cytometry (see Materials and Methods). Dot plots of single particle FITC-V237 fluorescence (FL1, ordinate) versus forward scatter (abscissa) for platelets from normal control (AW) and two PNH patients (SS and NF). Data for untreated cells not exposed to the C5b-9 proteins are also shown. Data are from a single experiment, representative of three experiments performed on separate days, using platelets obtained from four individuals with PNH.

exclude the majority of the platelets for analysis, as previously described.<sup>15</sup> As illustrated in Fig 2, a complement dose-dependent increase in the formation of microparticles was observed for platelets from patients with PNH. For comparison, few microparticles were generated in suspensions of normal platelets under these conditions of limited deposition of C5b-9 complexes. It should be noted that, in these experiments, we observed sample-to-sample differences in the extent to which platelets and platelet-derived microparticles were resolved into distinct populations when analyzed by light scatter, and for certain samples (eg, patient PNH SS of Fig 1), the forward scatter of newly formed microparticles overlapped that of small platelets, suggesting heterogeneity in the size of the particles (activated platelets and plasma membrane vesicles) on which the factor Va-binding sites were expressed (see Discussion).

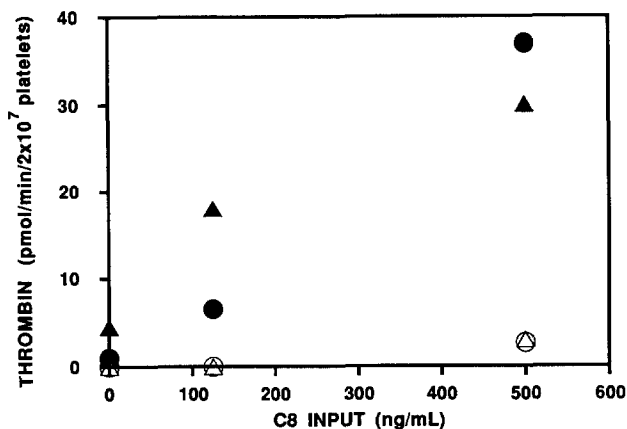
As shown in Fig 3, microparticle formation was accompanied by a complement dose-dependent expression of factor Va-binding sites, monitored by the binding of FITC-labeled V237 in the presence of a saturating amount of exogenously added factor Va light chain. Total binding of FITC-V237 was approximately 10-fold higher for C5b-9–treated PNH

platelets than for normal controls at an input of C8 of 500 ng/mL. Under the conditions of these experiments, increased factor Va-binding sites were observed on both PNH platelets and platelet-derived microparticles.

**Prothrombinase activity.** To test whether these newly formed factor Va-binding sites detected by flow cytometry coincided with increased expression of functional procoagulant surface, the capacity of these C5b-9–treated platelets to assemble the platelet prothrombinase enzyme complex was investigated. As illustrated in Fig 4, the exposure of factor Va-binding sites was paralleled by the expression of catalytic surface for the prothrombinase complex; the rate of prothrombin to thrombin conversion by PNH platelets exposed to the C5b-9 proteins increased in a complement dose-dependent fashion, and again exceeded that of C5b-9–treated normal controls by approximately 10-fold at the maximal input of C8 tested. By contrast to results obtained with C5b-9, we were unable to detect increased sensitivity of the PNH platelets to activation by either collagen (0 to 10 µg/mL) or the calcium ionophore, A23187 (0 to 1 µmol/L; data not shown), suggesting that the increased sensitivity of PNH platelets to activation by C5b-9 is specifically related



**Fig 2.** Quantitation of C5b-9-induced platelet microparticles. Platelets were incubated with the C5b-9 proteins as described for Fig 1. Microparticles were identified by their light-scatter properties and quantitated as described.<sup>15,19</sup> Data are shown for two normal controls (open symbols) and for patients PNH-NY (●) and PNH-NF (▲). Data are from a single experiment, representative of three performed, using platelets obtained from four individuals with PNH.

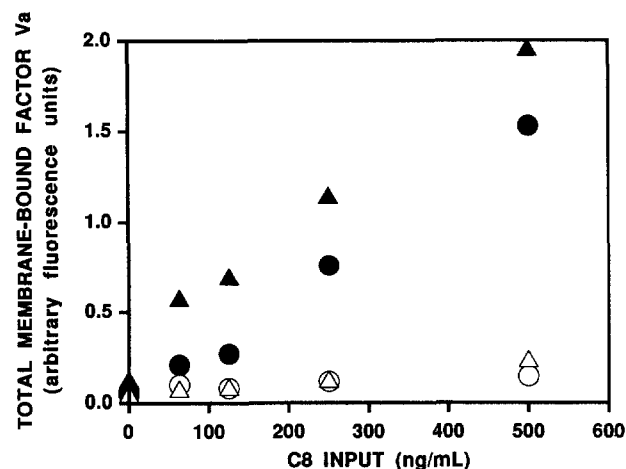


**Fig 4.** C5b-9-induced expression of platelet prothrombinase. Platelets were incubated with the C5b-9 proteins as described for Fig 1, and prothrombinase activity was determined as described in Materials and Methods. The rate of thrombin generation was determined for each C8 concentration using the timed data collections and a standard curve obtained with purified human thrombin. Data are shown for two normal controls (open symbols) and for patients PNH-NY (●) and PNH-NF (▲). Each point represents the average of two separate experiments.

to the loss of the complement-inhibitory function that is normally provided by cell-surface CD59.

#### DISCUSSION

Thrombotic complications are a major cause of morbidity and mortality in patients with PNH. Previous studies have demonstrated that platelets treated with complement



**Fig 3.** C5b-9-induced exposure of membrane-binding sites for factor Va. Platelets were incubated with the C5b-9 proteins and FITC-V237 binding to these cells was measured as described for Fig 1 and in the Materials and Methods. Total FITC-V237 fluorescence was calculated by multiplying the mean single-particle fluorescence by the total number of particles (platelet or microparticle). Data are shown for two normal controls (open symbols) and for patients PNH-NY (●) and PNH-NF (▲). Data are from a single experiment, representative of three performed, using platelets obtained from four individuals with PNH.

proteins C5b-9 release procoagulant microparticles that promote assembly of the prothrombinase enzyme complex.<sup>15</sup> Data of the present study demonstrate that PNH platelets, which lack the CD59 antigen, are significantly more sensitive to activation by complement proteins C5b-9 than platelets from normal controls, as shown by a 10-fold increase in complement-induced exposure of membrane-binding sites for factor Va, which was accompanied by a corresponding increase in membrane-catalyzed prothrombinase activity. The results obtained with PNH platelets are similar to data that we previously obtained using normal platelets in which platelet activation responses were potentiated *in vitro* by a functionally blocking antibody against CD59.<sup>17</sup> Enhanced complement-induced release of serotonin and thromboxane B<sub>2</sub> from PNH platelets has previously been observed by Blaas et al,<sup>25</sup> who attributed this increased sensitivity to activation by the terminal complement proteins to the lack of C8-binding protein in these cells. The relationship between C8-binding protein (or 64-Kd homologous restriction factor) and CD59 and the relative contribution of these factors to homologous restriction of the C5b-9 proteins on blood cells remain unresolved, and await molecular characterization of C8-binding protein.

By contrast to results obtained with normal platelets, the factor Va-binding sites exposed on complement-treated PNH platelets increased prominently on both platelets and platelet-derived microparticles. Furthermore, in certain experiments, the population of microparticles (defined by light scatter) were poorly resolved from remnant PNH platelets (see Fig 1). This apparent difference between normal and PNH platelets is likely to reflect (1) increased fragmentation of PNH platelets, due to loss of complement regulatory function, resulting in membrane fragments that overlap the microparticle gate; or (2) decreased capacity of the remnant PNH platelets to resequester acidic phospholipids

that are exposed upon C9 insertion and induced vesiculation of the plasma membrane,<sup>26</sup> due to inactivation of the aminophospholipid translocase in the remnant cells and membrane fragments. In this context, it is of note that, by contrast to recent results reported for calcium ionophore,<sup>27</sup> we did not observe either impaired vesiculation or diminished expression of prothrombinase activity in PNH platelets exposed to C5b-9 complexes.

This ability of the PNH platelet to shed nascent C5b-9 complexes by vesiculation may account for the normal survival in the circulation of the platelets in this disorder.<sup>28</sup> In the case of erythrocytes, which also lack CD59, C5b-9 complexes accumulate on the membrane and the cells are lysed; this may explain why the survival of PNH erythrocytes is shortened to less than 10% that of normal red blood cells, while platelet survival is unaffected.

The results of the present study indicate that deletion of the regulatory protein, CD59, from platelets is accompanied by a marked increase in the susceptibility of these cells to the effects of the C5b-9 proteins, leading to thrombin generation, which may contribute to the thrombotic risk associated with this disorder.

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#### REFERENCES

- Rosse WF: Phosphatidylinositol-linked proteins and paroxysmal nocturnal hemoglobinuria. *Blood* 75:1595, 1990
- Yamashina M, Ueda E, Kinoshita T, Takami T, Ojima A, Ono H, Tanaka H, Kondo N, Orii T, Okada N, Okada H, Inoue K, Kitani T: Inherited complete deficiency of 20-kilodalton homologous restriction factor (CD59) as a cause of paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 323:1184, 1990
- Müller-Eberhard HJ: Molecular organization and function of the complement system. *Annu Rev Biochem* 57:321, 1988
- Esser AF: The membrane attack pathway of complement. *Year Immunol* 6:229, 1990
- Sims PJ, Wiedmer T: The response of human platelets to activated components of the complement system. *Immunol Today* 12:338, 1991
- Wiedmer T, Ando B, Sims PJ: Complement C5b-9-stimulated platelet secretion is associated with a Ca<sup>2+</sup>-initiated activation of cellular protein kinases. *J Biol Chem* 262:13674, 1987
- Hattori R, Hamilton KK, McEver RP, Sims PJ: Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J Biol Chem* 264:9053, 1989
- Nicholson-Weller A, Burge J, Fearon DT, Weller PT, Austen KF: Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. *J Immunol* 129:184, 1982
- Sugita Y, Nakano Y, Tomita M: Isolation from human erythrocytes of a new membrane protein which inhibits the formation of complement transmembrane channels. *J Biochem (Tokyo)* 104:633, 1988
- Holguin MH, Fredrick LR, Bernshaw NJ, Wilcox LA, Parker CJ: Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest* 84:7, 1989
- Rollins SA, Sims PJ: The complement-inhibitory activity of CD59 resides in its capacity to block incorporation of C9 into membrane C5b-9. *J Immunol* 144:3478, 1990
- Meri S, Morgan BP, Davies A, Daniels RH, Olavesen MG, Waldmann H, Lachmann PJ: Human protectin (CD59), an 18-20 kD complement lysis restricting factor, inhibits C5b-8 catalyzed insertion of C9 into lipid bilayers. *Immunology* 71:1, 1990
- Zalman LS, Wood LW, Müller-Eberhard HJ: Isolation of a human erythrocyte membrane protein capable of inhibiting expression of homologous complement transmembrane channels. *Proc Natl Acad Sci USA* 83:6975, 1986
- Schönermark S, Rauterberg EW, Shin ML, Loke S, Roelcke D, Hänsch GM: Homologous species restriction in lysis of human erythrocytes: A membrane-derived protein with C8-binding capacity functions as an inhibitor. *J Immunol* 136:1772, 1986
- Sims PJ, Faioni EM, Wiedmer T, Shattil SJ: Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J Biol Chem* 263:18205, 1988
- Gilbert GE, Sims PJ, Wiedmer T, Furie B, Furie BC, Shattil SJ: Platelet-derived microparticles express high affinity receptors for factor VIII. *J Biol Chem* 266:17261, 1991
- Sims PJ, Rollins SA, Wiedmer T: Regulatory control of complement on blood platelets: Modulation of platelet procoagulant responses by a membrane inhibitor of the C5b-9 complex. *J Biol Chem* 264:19228, 1989
- Kane WH, Majerus PW: Purification and characterization of human coagulation factor V. *J Biol Chem* 256:1002, 1981
- Podack ER, Müller-Eberhard HJ: Limited proteolysis of C5b-6: Functional stability of the degraded complex. *J Immunol* 124:332, 1980
- Podack ER, Kolb WP, Esser AF, Müller-Eberhard HJ: Structural similarities between C6 and C7 of human complement. *J Immunol* 123:1071, 1979
- Steckel EW, York RG, Monahan JR, Sodetz JM: The eighth component of human complement. Purification and physicochemical characterization of its unusual subunit structure. *J Biol Chem* 255:11997, 1980
- Biesecker G, Müller-Eberhard HJ: The ninth component of human complement: Purification and physicochemical characterization. *J Immunol* 124:1291, 1980
- Shattil SJ, Cunningham M, Hoxie JA: Detection of activated platelets in whole blood using activation-dependent monoclonal antibodies and flow cytometry. *Blood* 70:307, 1987
- Wiedmer T, Esmon CT, Sims PJ: Complement proteins C5b-9 stimulate procoagulant activity through the platelet prothrombinase. *Blood* 68:875, 1986
- Blaas P, Berger B, Weber S, Peter HH, Hänsch GM: Paroxysmal nocturnal hemoglobinuria. Enhanced stimulation of platelets by the terminal complement components is related to the lack of C8bp in the membrane. *J Immunol* 140:3045, 1988
- Chang C-P, Zhao J, Wiedmer T, Sims PJ: Contribution of platelet microparticle formation and granule secretion to the transmembrane migration of phosphatidylserine. *J Biol Chem* 268:7171, 1993
- Whitlow M, Iida K, Marshall P, Silber R, Nussenzweig V: Cells lacking glycan phosphatidylinositol-linked proteins have impaired ability to vesiculate. *Blood* 81:510, 1993
- Devine DV, Siegel RS, Rosse WF: Interactions of platelets in paroxysmal nocturnal hemoglobinuria with complement. Relationship to defects in the regulation of complement and to platelet survival in vivo. *J Clin Invest* 79:131, 1987