Platelet Activation by C1q Results in the Induction of α_{IIb}/β_3 Integrins (GPIIb-IIIa) and the Expression of P-Selectin and Procoagulant Activity

By Ellinor I. B. Peerschke,* Kenneth B. M. Reid,§ and Berhane Ghebrehiwet*‡

*From the Departments of *Pathology and ‡Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794; and §Medical Research Council Immunochemistry Unit, Department of Biochemistry, Oxford University, Oxford OX1 3QU, UK*

Summary

C1q receptors (C1qR) have been identified on a variety of somatic and cultured cells including peripheral blood platelets. Since platelets are likely to encounter both circulating C1q multimers and C1q associated with the extracellular matrix after complement activation by the classical pathway, the present study was designed to assess the effect of fluid phase and immobilized C1q on platelet function. Platelet adhesion to C1q-coated surfaces was accompanied by the induction of fibrinogen receptors. Scatchard analysis of fibrinogen binding to adherent platelets revealed the binding of ~10,000 molecules of fibrinogen per platelet with a K_d of 0.1 ± 0.03 μM (mean ± SD, n = 4). Furthermore, fluid phase C1q multimers were noted to aggregate platelets at doses > 5 μg/ml. This aggregation was preceded by a rise in inositol-1,4,5-trisphosphate (IP_3) (6.9 ± 2.4 pmoles/10^9 platelets at 15 s, n = 4), and activation of GPIIb–IIIa complexes supporting fibrinogen binding. Platelet aggregation in response to C1q multimers was accompanied by the aspirin-inhibitable release of granule contents and P-selectin (CD62) expression. Platelet aggregation was inhibited by the collagenous domain of C1q (c-Clq) and a monoclonal antibody directed against C1q receptors, suggesting the direct involvement of the 67-kD platelet C1qR. Antibodies against the very late antigen 2 or CD36 collagen receptors were without effect. Platelet exposure to C1q multimers was also accompanied by the expression of procoagulant activity, as demonstrated by the dose-dependent shortening of the kaolin recalcification time of normal plasma from 108 ± 12 s in the presence of unstimulated platelets to 62 ± 14 s in the presence of platelets that had been preincubated (5 min, 37°C) with 100 μg/ml multimeric C1q (n = 3). These data suggest that platelet interactions with C1q multimers or immobilized C1q, resulting in the activation of GPIIb–IIIa fibrinogen binding sites and the expression of P-selectin as well as platelet procoagulant activity, are likely to contribute to thrombotic events associated with complement activation and inflammation.

C1q is a 460,000-mol wt glycoprotein present in plasma at ~75 μg/ml (1). C1q circulates as a calcium-dependent complex with $C1r_2$ and Cls_2, forming the first component of complement, C1 (2). Upon activation of C1 by circulating immune complexes or certain bacteria and viruses, $C1r_2$ and Cls_2 are disassembled by C1-inactivator (3), leaving complex-bound C1q with its collagen-like tail exposed and potentially available for binding to C1q receptors (C1qR) (4). Whereas the extravascular localization of free C1q is limited under normal conditions, selective accumulation of this protein has been observed in inflamed and injured tissues, particularly after vascular injury or enhanced vascular permeability (5, 6). Moreover, the secretion of C1q by lymphokine-activated monocytes or macrophages has been documented (7, 8).

The interaction of C1q with its receptors on somatic and cultured cells has been reported to elicit a variety of biological responses (4). In platelets, the interaction with monomeric C1q was first reported to inhibit collagen-induced platelet aggregation (9, 10). The structural similarities between the NH_2 terminus of C1q and collagen (11) suggested that C1q and collagen binding sites were identical. Recent studies (12), however, have demonstrated the existence of distinct platelet membrane receptors for C1q and collagen. Under certain conditions, however, these sites demonstrate cross-reactivity with regard to ligand binding (12).

Since virtually all biologically important C1 activating substances, including immune complexes, RNA tumor viruses, and lipid A–rich LPS bind multiple C1q molecules, C1q is likely to circulate in multimeric or aggregated form after ac-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/08/0579/09 $2.00
Volume 178 August 1993 579–587

tivation of the classical complement pathway (13, 14). Unlike C1q monomers, C1q multimers have been suggested to support platelet aggregation (9). Because C1q has also been reported as a transient component of the extracellular matrix (15), the present study was designed to evaluate the response of platelets both to C1q multimers in the fluid phase, and to immobilized C1q on plastic surfaces.

Materials and Methods

Platelet Preparation

Blood was collected from human volunteers after obtaining informed consent. The blood was anticoagulated with 0.1 vol 3.2% sodium citrate in the presence or absence of 1 mM aspirin. Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 280 *g* (15 min, 22°C). Washed platelets (WP)[1] were obtained after acidifying the PRP to pH 6.5 with 1 M citric acid, centrifuging the sample (1,000 *g*, 20 min, 22°C), and resuspending the resulting platelet pellet in 0.01 M Hepes-buffered modified Tyrode's solution containing no added calcium, 2 mM $MgCl_2$, and 2 mg/ml BSA (HBMT) (Sigma Chemical Co., St. Louis, MO) (16).

Purification of Protein Ligands

C1q was isolated from human serum as described by Reid (17). Digestion of C1q with pepsin to obtain the collagen-like domain (c-C1q) was performed as previously described (18). Purified C1q (1 mg/ml) in 0.23 M sodium acetate buffer, pH 5.2, was dialyzed against 0.01 M PBS, pH 7.2, for 48 h at 4°C. The resulting material appeared cloudy and was designated multimeric/aggregated C1q (agg-C1q). To rule out effects of bacterial contamination of agg-C1q preparations during dialysis, similar studies were performed using microfiltered (0.2 μm) C1q, autoclaved dialysis buffers, and glassware. Sterile agg-C1q supported similar dose-dependent platelet activation as nonsterile preparations (data not shown). Aggregated IgG (agg-IgG) was prepared by heat treatment (60°C, 20 min) of protein A-purified, human IgG. Agg-IgG was tested for its ability to activate complement by hemolytic assay (19). Fibrinogen was purified from fresh frozen plasma (20) and iodinated with ^{125}I and an immobilized oxidant (*N*-chlorobenzenesulfonamide : iodobeads; Pierce Chemical Co., Rockford, IL) according to the instructions provided by the manufacturer.

Antibodies to the 67-kD C1qR designated II1/D1 were purified from ascites (21). mAbs recognizing the very late antigen (VLA) 2 collagen receptor (6F1) (22) were obtained as a gift from Dr. B. Coller, State University of New York (SUNY) at Stony Brook. mAbs (IOP36) against CD36, platelet glycoprotein IV (GPIV), were purchased from AMAC, Inc. (Westbrook, ME).

Preparation of Protein-coated Surfaces

Microtiter wells were exposed to purified C1q (80 μg/ml) or BSA (80 μg/ml) for 16 h at 4°C. Unreactive sites were blocked for 60 min at 37°C with 0.1% BSA. Wells were rinsed with HBMT before exposure to platelets.

Platelet Function Studies

Platelet Aggregation. Platelet aggregation was monitored at 37°C in a dual channel aggregometer (Chronolog Corp., Havertown, PA) after addition of 5–100 μg/ml agg-C1q. In some experiments, platelets were preincubated (5 min, 22°C) with c-C1q (100 μg/ml) or mAbs, II1/D1, 6F1, IOP36 (20 μg/ml) directed against the C1qR, the VLA-2 collagen receptor, or the CD36/GPIV collagen receptor, respectively. In other experiments, platelets were preincubated with mAbs (50 μg/ml), recognizing the platelet FcγRII receptor (AMAC, Inc.), before exposure to agg-C1q.

Fibrinogen Binding to WP Suspensions. Fibrinogen binding to WP was evaluated in an unstirred system (20) after platelet stimulation with agg-C1q in the presence of increasing amounts of ^{125}I-fibrinogen. Nonspecific binding was assessed in the presence of 10 mM EDTA or excess unlabeled fibrinogen (20).

Fibrinogen Binding to Adherent Platelets. WP (1.25×10^7) were added to C1q-coated microtiter wells. After 30-min adhesion (22°C), nonadherent platelets were removed, the wells rinsed three times with PBS, and platelet adhesion quantified in duplicate wells using the bicinchoninic acid protein assay (23). Additional wells were exposed to increasing concentrations of ^{125}I-fibrinogen. After 60 min at 22°C, wells were rinsed three times, and residual radioactivity quantified using a gamma counter. Nonspecific fibrinogen binding was evaluated in the presence of excess unlabeled fibrinogen. The specificity of ^{125}I-fibrinogen binding was further evaluated in the presence of 100 μM arginine, glycine, aspartic acid (RGDS; Peninsula Labs., Inc., Belmont, CA), or after platelet exposure to a mAb (20 μg/ml) recognizing the α_{IIb}/β_3 integrin, GPIIb–IIIa (10E5, a gift from Dr. B. Coller) (24).

Serotonin Release. Platelets were labeled with ^{14}C-serotonin, 5-hydroxy(side chain-2-^{14}C)tryptamine creatinine sulphate (Amersham Corp., Arlington Heights, IL), in whole blood (25). ^{14}C-serotonin release was measured at maximum platelet aggregation in the presence of 5 μM imipramine to prevent reuptake of released serotonin. Samples were fixed with an equal volume of 2% formalin, placed on ice, centrifuged for 3 min at 12,000 *g*, and the supernatant radioactivity evaluated in a beta counter.

P-Selectin Expression. The secretion of platelet α granules with concomitant expression of the platelet α granule membrane protein designated P-selectin (CD62) was assessed after stimulating platelets with saturating concentrations of agg-C1q (100 μg/ml) (26) at 37°C for 10 min. Platelet suspensions were subsequently fixed with paraformaldehyde (27), washed, and exposed to a PE-labeled monoclonal antiCD62 antibody (Becton Dickinson Immunocytometry Systems, San Jose, CA) (30 min, 22°C). Platelets were washed three times in PBS and evaluated qualitatively for fluorescence at the Center for Imaging and Analysis (SUNY at Stony Brook) using a microscope/camera (Axiophot; Carl Zeiss, Inc., Thornwood, NY). Resting PGE_1 (10 μM)-treated platelets, or platelets preincubated with 100 μg/ml monomeric C1q, served as negative controls. Platelets stimulated with 50 mU/ml thrombin (a gift from Dr. John Fenton II, New York State Department of Health, Albany, NY) served as positive controls. Thrombin was neutralized with excess hirudin before platelet fixation.

Inositol-1,4,5-Triphosphate (IP_3) Production. WP (2×10^9/ml) were incubated at 37°C in the presence of 0.1 mg/ml apyrase and stimulated with 100 μg/ml agg-C1q. The reaction was terminated by adding 0.2 ml 1 M TCA per 1 ml of platelet suspension. After a 15-min incubation on ice, samples were centrifuged (1 min, 12,000 *g*, 4°C). The supernatant (1 ml) was removed, and IP_3 quantified using an IP_3 ^3H-radioreceptor assay kit (New England Nuclear Research Products, Boston, MA) as described by the manufacturer.

Platelet Procoagulant Activity. Platelet factor 3 (PF3) availability was measured by incubating 0.1 ml pooled normal plasma (George King Biomedical Co., Overland Park, KS) with 0.1 ml kaolin (20

[1] *Abbreviations used in this paper:* agg, aggregated; GPIV, glycoprotein IV; IP_3, inositol-1,4,5-triphosphate; PF3, platelet factor 3; VLA, very late antigen; WP, washed platelet.

Table 1. *Inhibition of Agg-C1q Induced Fibrinogen Binding to Platelets in Suspension and Fibrinogen Binding to Platelets Adhering to C1q-Coated Surfaces*

Inhibitor	Inhibition of fibrinogen binding induced by:	
	Immobilized C1q	Agg-C1q
	%	
10E5	95 ± 7	ND
RGDS	92 ± 18	ND
c-C1q	ND	85 ± 12
II1/D1	ND	87 ± 14
6F1	ND	5 ± 11
Anti-Fcγ RII	ND	7 ± 9
Anti-CD36/GPIV	ND	0 ± 8

Platelets were preincubated (5 min, 22°C) with inhibitors: 100 μg/ml c-C1q, 20 μg/ml 10E5, II1/D1, 6F1, or Anti-CD36, 50 μg/ml anti-Fcγ RII, or 100 μM RGDS. Platelets were subsequently stimulated with 25 μg/ml agg-C1q in the presence of ^{125}I-fibrinogen. Values represent mean ± SD, n = 3.

mg/ml) (Sigma Chemical Co.). After 5 min at 37°C, 0.2 ml of platelets was added, followed by $CaCl_2$ (0.1 ml of a 0.035 M stock solution). Clot formation was monitored using a semi-automated impedance device (fibrometer; BBL, Division of Becton Dickinson & Co., Cockeysville, MD).

Results

Platelet adhesion to C1q-coated surfaces (59,000–68,000 platelets/mm^2) resulted in specific and saturable fibrinogen binding. Maximal binding was achieved after 60 min at 22°C. Half-maximal binding was noted within 10 min. Binding

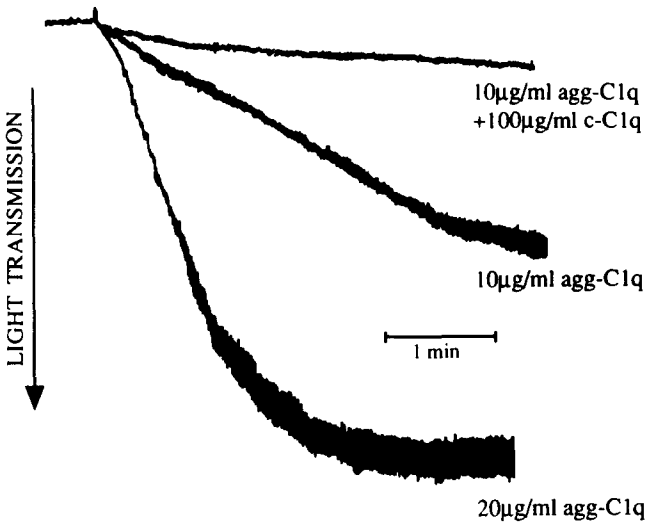


Figure 1. Response of WP to aggregated C1q (*agg-C1q*) in the presence of 0.1 mg/ml fibrinogen. Aggregometer tracings depict the platelet response to 10 and 20 μg/ml agg-Clq in the presence or absence of 100 μg/ml c-C1q.

was inhibited in the presence of 100 μM RGDS and preincubation of platelets with a mAb (10E5) recognizing the platelet membrane GPIIb–IIIa complex (Table 1). Assuming a 1:1 relationship between bound fibrinogen and GPIIb–IIIa, Scatchard analysis (28) of fibrinogen binding isotherms revealed the exposure of 10,000 ± 3,000 platelet membrane receptors, binding fibrinogen with a K_d of 0.1 ± 0.03 μM (n = 3), consistent with GPIIb-IIIa–fibrinogen interactions previously characterized in the fluid phase (20).

In the fluid phase, platelet exposure to C1q multimers resulted in dose-dependent platelet aggregation (Fig. 1). Platelet aggregation in response to agg-C1q occurred without delay, and was accompanied by ^{125}I-fibrinogen binding and ^{14}C-serotonin release (Table 2). Maximal fibrinogen binding was

Table 2. *Washed Platelet Responses to Agg-C1q: Effect of Aspirin*

Response	Platelets			
	Stimulated with agg-C1q		Unstimulated	
	ASA	Non-ASA	ASA	Non-ASA
Aggregation (%)	64 ± 24	>90	0	0
^{14}C-serotonin release (%)	32 ± 12	84 ± 18	0.4 ± 0.2	0.1 ± 0.3
Fibrinogen binding (molecules/platelet)	27,270 ± 3690	41,240 ± 1648	243 ± 124	285 ± 58

Platelet aggregation and serotonin release in response to 20 μg/ml agg-C1q were measured 5 min after stirring samples in an aggregometer at 37°C, in the presence of 100 μg/ml fibrinogen and 5 μM imipramine. Values are expressed as percent relative to maximal aggregation or total platelet ^{14}C-serotonin, respectively. Fibrinogen binding was measured 60 min after platelet stimulation at 37°C, in the presence of 250 μg/ml ^{125}I-fibrinogen without stirring. Unstimulated platelets served as controls. The responses of platelets pretreated with 1 mM aspirin (ASA) were compared to untreated platelets (Non-ASA). Data represent mean ± SD, n = 5.

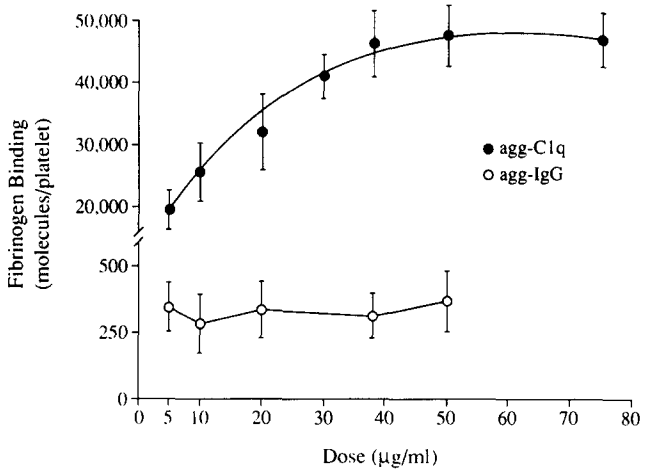


Figure 2. ^{125}I-fibrinogen binding to washed platelets stimulated with increasing concentrations of agg-C1q or aggregated IgG (*agg-IgG*). WP were incubated with agg-C1q or agg-IgG for 60 min at 22°C in the presence of 250 μg/ml ^{125}I-fibrinogen. Fibrinogen binding was quantified as described in Materials and Methods.

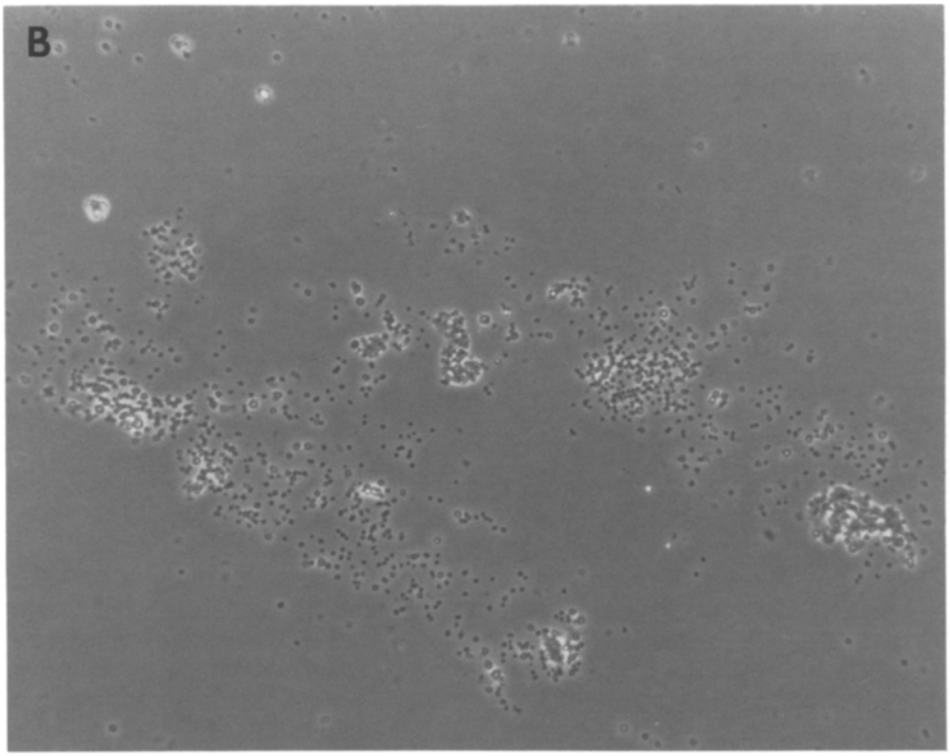
obtained in the presence of 30–75 μg/ml agg-C1q (Fig. 2). Fibrinogen binding to platelets in suspension was always higher than fibrinogen binding to platelets adhering to C1q-coated surfaces. This may reflect the extent of platelet stimulation and/or the potentially decreased accessibility of fibrinogen receptors on adherent platelets which bind fibrinogen only to their nonadherent surface. Platelet aggregation, fibrinogen binding, and serotinin release were all inhibited but not abrogated after pretreatment of platelets with 1 mM aspirin, confirming the involvement of cyclo-oxygenase–dependent synthesis of thromboxane A_2 (29) and release of platelet granule contents. The release of α granules was inferred from qualitative analysis of cell surface P-selectin expression by direct immunofluorescence (Fig. 3). Interestingly, the extent of P-selectin expression by 100 μg/ml agg-C1q (Fig. 3 *E*) appeared similar to that noted after platelet stimulation with a standard dose of thrombin (Fig. 3 *F*).

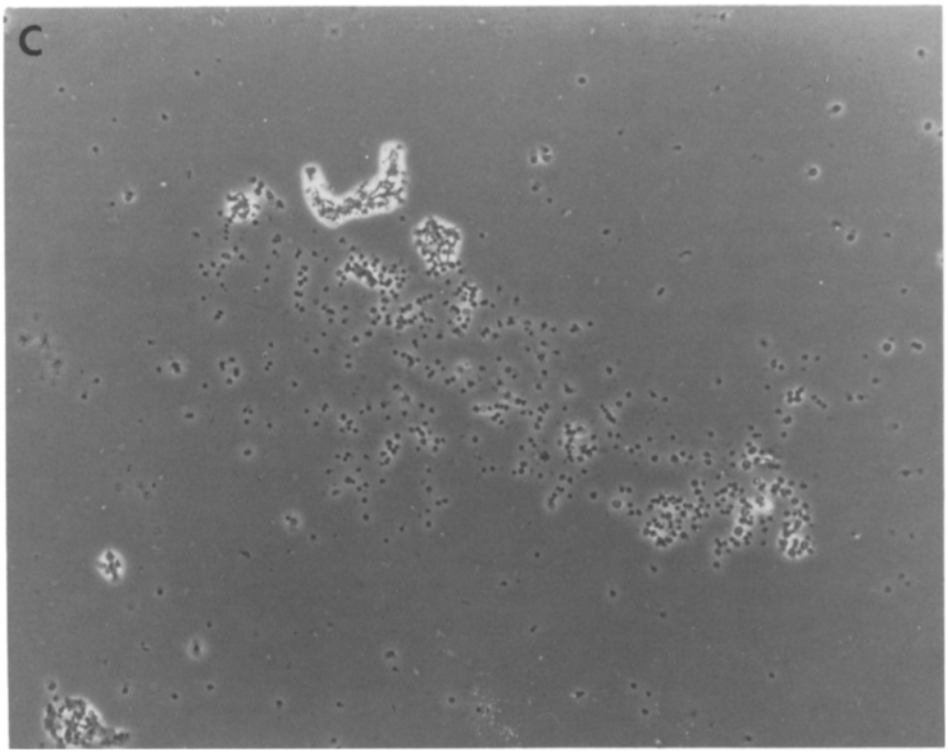
To rule out platelet activation via occupancy of FcγRII receptors (30) by potential trace agg-IgG contamination, fibrinogen binding was compared in response to agg-C1q or similar concentrations of agg-IgG. As summarized in Fig. 2, agg-IgG was without effect at 22°C in unstirred systems. Moreover, agg-C1q–induced fibrinogen binding was inhibited in the presence of the collagen-like NH_2-terminal domain of C1q (c-C1q) or mAbs (II1/D1) directed against the 67-kD C1q receptor (Table 1). mAbs directed against the FcγRII receptor, the VLA-2 collagen receptor, or CD36/GPIV, also reported to function in platelet–collagen interactions (31), were without effect (Table 1).

Platelet stimulation with agg-C1q was associated with rapid IP_3 production (Fig. 4). To optimize IP_3 generation, platelets were stimulated with 100 μg/ml agg-C1q. The reaction was terminated at 2, 15, and 30 s. Maximal IP_3 production was observed within 15 s of platelet stimulation (Fig. 4). By 30 s, IP_3 levels decreased almost to baseline.

Platelet stimulation with agg-C1q was also accompanied by the expression of surface membrane procoagulant activity. As summarized in Table 3, shortening of the kaolin recalcification time of pooled normal plasma was observed in the presence of platelets activated with increasing concentrations of agg-C1q. Clotting times in the presence of un-

Figure 3. Phase contrast views of unstimulated (*A*) platelets and platelets stimulated with 100 μg/ml agg-C1q (*B*), or 50 mU/ml human thrombin (*C*). Immunofluorescence views demonstrating P-selectin expression on unstimulated platelets (*D*), platelets stimulated with 100 μg/ml agg-Clq (*E*), or 50 mU/ml thrombin (*F*) using a PE-conjugated anti CD-62 mAb. Same views as 3 *A–C*. ×200.

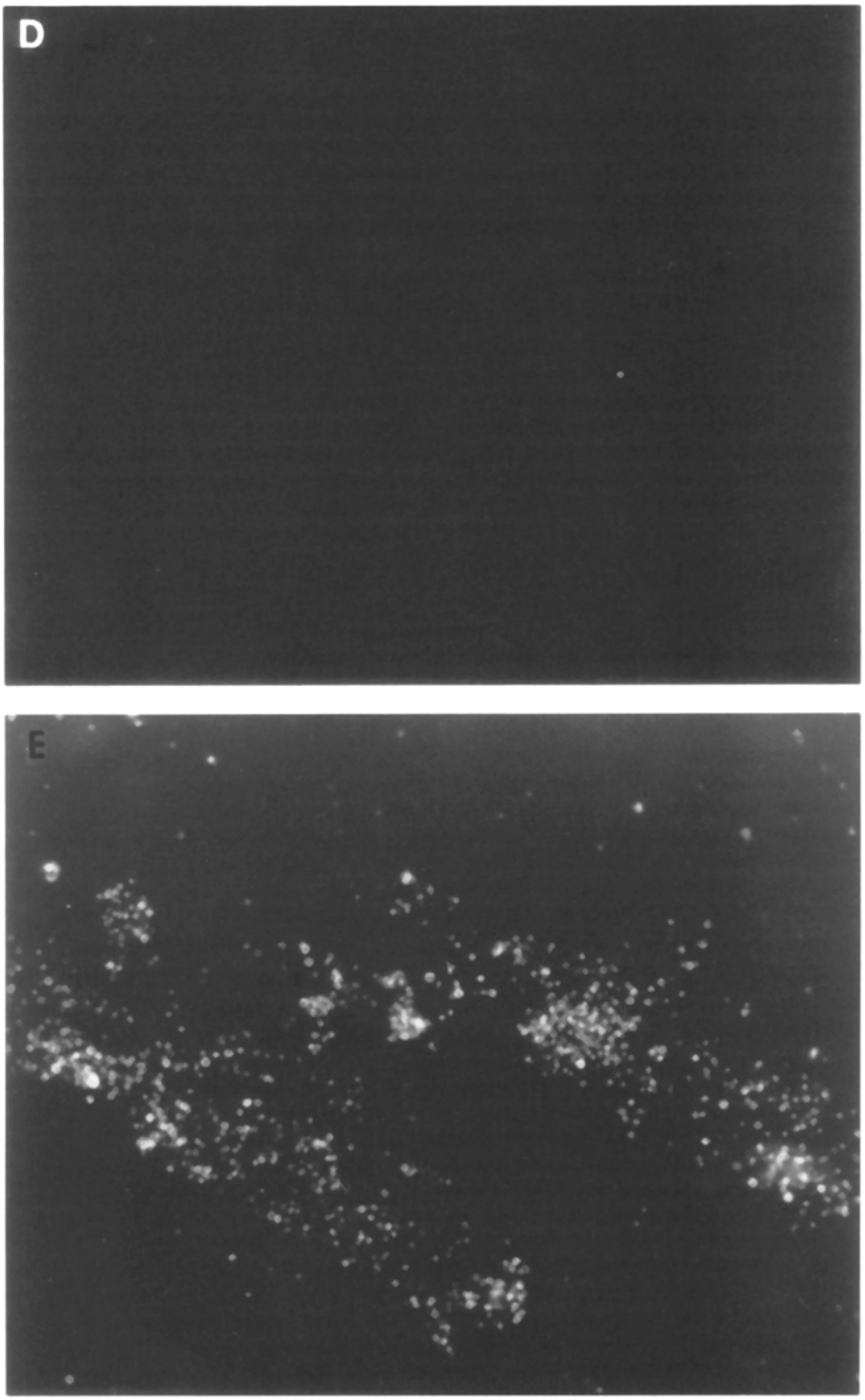




stimulated platelets or thrombin-stimulated platelets served as negative and positive controls, respectively.

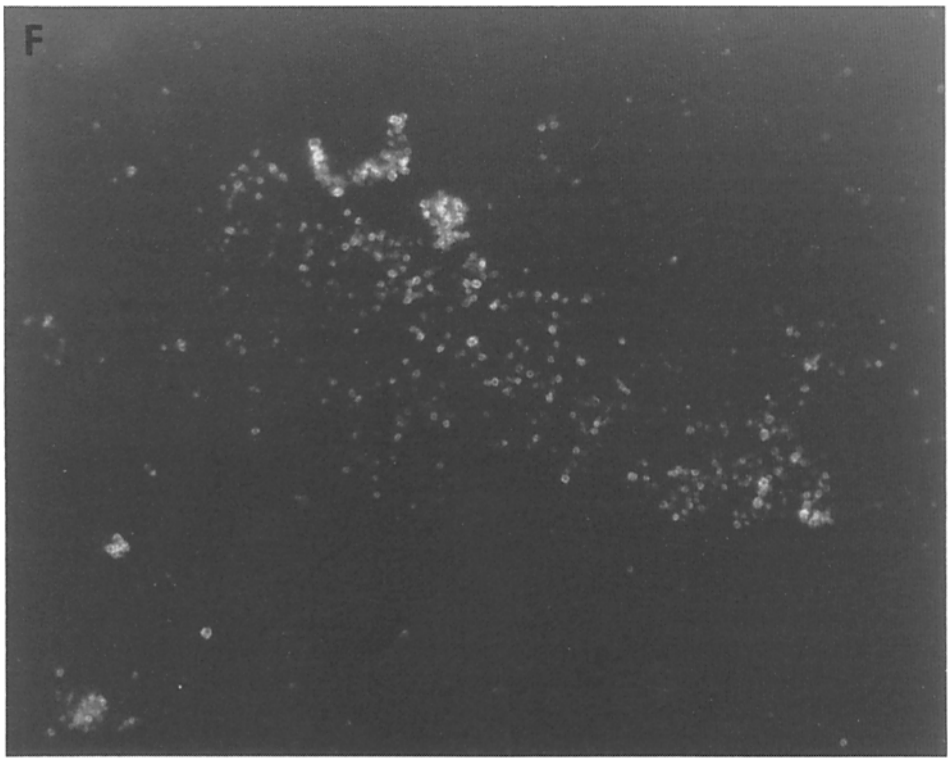
Discussion

C1q receptors are present on numerous somatic and cultured cells where they mediate a variety of cell-specific reactions (4). Platelets possess ~4,000 C1q binding sites per cell and bind C1q with a K_d of 3.5×10^{-7} M in a divalent cation independent manner (26). The present study characterizes platelet responses to agg-C1q in solution and to immobilized C1q on plastic surfaces. The data demonstrate the direct involvement of the 67-kD platelet C1qR in C1q-induced



platelet stimulation. This stimulation involves several surface membrane–associated events including IP_3 production, activation of GPIIb–IIIa fibrinogen receptors, cyclo-oxygenase–mediated thromboxane A_2 formation supporting the release of platelet granule contents, and the expression of P-selectin and procoagulant activity.

Previous studies (9, 10) have described the interaction of soluble C1q monomers with human platelets. The structural



similarity between C1q and collagen suggested a potential role for platelet C1q receptors in modulating collagen-induced platelet functions. Interestingly, studies with highly purified C1q monomers and monoclonal as well as polyclonal anti-C1qR antibodies demonstrated that whereas C1q and anti-C1qR antibodies inhibited collagen-induced platelet aggregation and secretion at low concentrations of collagen, they did not affect the adhesion of platelets to collagen fibrils or collagen-coated surfaces (32). Moreover, the effect of anti C1qR Abs and an Ab directed against the platelet VLA-2 receptor clearly indicated that platelet adhesion to Type I collagen and C1q was mediated by distinct receptors (12). This concept is further supported by observations made in the present study demonstrating specific inhibition of agg-C1q–induced platelet stim-

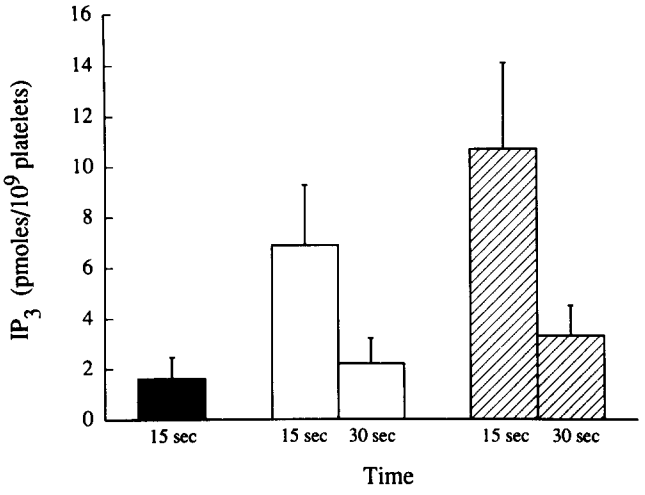


Figure 4. IP_3 production 15 and 30 s after platelet stimulation (37°C) with 100 μg/ml agg-C1q (*open bars*) or 0.5 mU/ml human thrombin (*hatched bars*). Baseline IP_3 levels were established using unstimulated platelets (*filled bar*), incubated with 10 μM PGE_1. Error bars depict one SD.

Table 3. *Effect of Platelet Stimulation with Agg-C1q on the Kaolin Recalcification Time of Normal Pooled Plasma*

Addition	Clotting time
	s
Buffer	114 ± 13
Resting platelets	108 ± 9
Platelets + Thrombin	56 ± 15
Platelets + Agg-C1q	
20 μg/ml	92 ± 12
30 μg/ml	78 ± 11
100 μg/ml	62 ± 14

Washed platelets were stimulated with 0.10 U/ml human thrombin or agg-IgG (20–100 μg/ml). Thrombin was neutralized with excess hirudin before adding 0.2 ml of the platelet suspension to 0.2 ml normal plasma preincubated 5 min (37°C) with 10 mg/ml kaolin. Clotting was initiated by adding 0.1 ml $CaCl_2$ (0.035 M). Values represent mean ± SD, $n = 3$.

ulation by anti C1qR antibodies, whereas anti-VLA-2 Abs and Abs against the CD36 collagen receptor were without effect.

Since circulating platelets are more likely to encounter C1q multimers (14) or C1q immobilized to extracellular matrix components (15) than C1q monomers, the ability of agg-Clq and C1q coated surfaces to activate platelets provides strong support for the hypothesis that occupancy of the C1qR may contribute to thrombotic events associated with complement activation and inflammation. For example, platelet aggregation is the sine qua non for hemostatic or thrombotic platelet plug formation. Platelet aggregation requires occupancy of surface membrane agonist receptors capable of signal transduction. The present study suggests that the platelet C1qR may be such a receptor, as C1qR occupancy was associated with rapid IP_3 formation. IP_3 is an intracellular messenger that serves to raise cytosolic calcium (33). Increases in intraplatelet calcium levels are associated with changes in platelet membrane GPIIb–IIIa expression from an inactive to an active conformation. Indeed, platelet activation with agg-C1q or immobilized C1q in the present study was accompanied by specific and saturable fibrinogen binding. This bound fibrinogen is thought to be primarily responsible for platelet cohesion.

Platelet stimulation by agg-C1q was also accompanied by the release of platelet-dense granule serotonin. α granule secretion was inferred from cell surface P-selectin expression. Dense bodies contain not only serotinin, but also ADP which is an important agonist and may function to attract additional platelets to the site of a growing thrombus by expression of active GPIIb–IIIa complexes (34). In addition, dense bodies contain biogenic amines that may participate in inflammatory reactions. In contrast, the release of α granule contents includes a number of adhesive proteins such as fibrinogen, fibronectin, and thrombospondin, that may serve to reinforce platelet–platelet interactions (35). Secretion of α granule contents is also accompanied by the surface expression of an α granule membrane protein, P-selectin, previously referred to as GMP 140 or PADGEM (36, 37). P-selectin has been shown to mediate the adhesion of neutrophils and monocytes to activated platelets in a calcium-dependent manner (37).

Platelet α granule secretions also contain the active form of coagulation factor V (Va) (38). This, in combination with surface membrane changes leading to the exposure of negatively charged phospholipids (phosphatidylserine, phosphatidylinositol), encompasses PF3 procoagulant activity (39). PF3 activity is essential for efficient thrombin generation leading to fibrin clot formation. Indeed, exposure of platelets to agg-C1q in the present study lead to dose-dependent PF3 generation, as measured by the kaolin recalcification time, a standard assay for the evaluation of platelet procoagulant activity (40).

Since platelet C1q receptors were previously found to participate in immune complex localization via immune complex association with the globular head region of C1q (41), the question arises as to whether C1qR occupancy by the collagen-like NH_2-terminal region of C1q is physiologically beneficial or detrimental. Based on available data, low concentrations of C1q multimers (<5–10 μg/ml), formed after binding immune complexes, certain bacteria or viruses, may participate in clearance mechanisms, and/or antigen presentation to lymphoid cells of the reticuloendothelial system. In contrast, high concentrations of agg-C1q appear likely to contribute to thrombotic and inflammatory reactions.

The authors are grateful to Joan Hennessey and Jean A. Wainer for expert technical assistance.

This work was supported in part by grants 900994 (E.I.B. Peerschke and B. Ghebrehiwet) sponsored by the American Heart Association-Wyeth Ayerst Laboratories, and 900696 (B. Ghebrehiwet) from the American Heart Association (Dallas, TX).

Address correspondence to Dr. Ellinor I. Peerschke, University Hospital, L-3, State University of New York at Stony Brook, Stony Brook, NY 11794–7300.

Received for publication 16 February 1993 and in revised form 23 April 1993.

References

1. Lepow, I.H., G.B. Naff, E.W. Todd, J. Pensky, and C.F. Hinz. 1963. Chromatographic resolution of the first component of human complement with three activities. *J. Exp. Med.* 117:983.
2. Ziccardi, R.J., and N. Cooper. 1977. The subunit composition and composition properties of human C1. *J. Immunol.* 118:2047.
3. Sim, R.B., G.J. Arlaud, and M.G. Colomb. 1979. C1 inhibitor dependent dissociation of human complement component C1 bound to immune complexes. *Biochem. J.* 179:449.
4. Ghebrehiwet, B. 1987. C1q receptor. *In* Methods in Enzymology. G. DiSabato, editor. Vol. 150, Academic Press, Inc., San Diego. 558–568.
5. Lindgren, S., A.B. Laurell, and S. Eriksson. 1984. Complement components and activation in primary biliary cirrhosis. *Hematology.* 4:9.
6. Rossen, R.D., J.L. Swain, L.H. Michael, S. Weakly, E. Giannini, and M.L. Entman. 1985. Selective accumulation of the first component of complement and leukocytes in ischemic canine heart muscle: a possible initiator of an extramyocardial mechanism of ischemic injury. *Circ. Res.* 57:119.

7. Bensa, J.C., A. Revoul, and M.G. Colomb. 1983. Biosynthesis in vitro of complement subcomponent C1q, C1s and C1 inhibitor by resting and stimulated human monocytes. *Biochem. J.* 216:385.
8. Tennner, A.J., and D.B. Volkin. 1986. Complement subcomponent C1q secreted by cultured monocytes has subunit structure identical with that of serum C1q. *Biochem. J.* 233:451.
9. Cazenave, J.P., S.N. Assimeh, R.H. Painter, M.A. Packham, and J.F. Mustard. 1976. C1q inhibition of the interaction of collagen with human platelets. *J. Immunol.* 116:162.
10. Csako, G., and E.A. Suba. 1987. Free platelet count and size distribution during C1q inhibition of collagen-induced platelet aggregation. *Thromb. Haemostasis.* 58:682.
11. Reid, K.B.M. 1989. C1q: genes, biosynthesis and biology. *Behring Inst. Mitt.* 84:8.
12. Peerschke, E.I.B., and B. Ghebrehiwet. 1990. Platelet C1q receptor interactions with collagen and C1q coated surfaces. *J. Immunol.* 145:2984.
13. Hughes-Jones, N.C., and B. Gardner. 1978. The reaction between the complement subcomponent C1q, IgG complexes, and polyionic molecules. *Immunology.* 34:459.
14. Cooper, N.R., and D.C. Morrison. 1978. Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. *J. Immunol.* 120:1862.
15. Bordin, S., B. Ghebrehiwet, and R.C. Page. 1990. Participation of C1q and its receptor in adherence of human diploid fibroblasts. *J. Immunol.* 145:2520.
16. Tangen, O., M.L. Andrae, and B.E. Nilssen. 1973. Nucleotide leakage from platelets in artificial media: prevention by albumin and other macromolecules and relation to ADP-induced platelet aggregation. *Scand. J. Haematol.* 11:241.
17. Reid, K.B.M. 1981. Preparation of human C1q, a subcomponent of the first component of the classical pathway of complement. *Methods Enzymol.* 80:16.
18. Reid, K.B.M. 1976. Isolation, by partial pepsin digestion, of the three collagen-like regions present in subcomponent C1q of the first component of human complement. *Biochem. J.* 155:5.
19. Ghebrehiwet, B., B.P. Randazzo, J.T. Dunn, M.S. Silverberg, and A.P. Kaplan. 1983. Mechanisms of activation of the classical pathway of complement by Hageman factor fragment. *J. Clin. Invest.* 71:1450.
20. Peerschke, E.I.B., M.B. Zucker, R.A. Grant, J.J. Egan, and M.M. Johnson. 1980. Correlation between fibrinogen binding to human platelets and platelet aggregability. *Blood.* 55:841.
21. Ghebrehiwet, B. 1986. Production and characterization of a murine monoclonal IgM antibody to human C1q receptors (C1qR). *J. Immunol.* 137:618.
22. Coller, B.S., J.H. Beer, L.E. Scudder, and M.H. Steinberg. 1989. Collagen-platelet interactions: evidence for a direct interaction of collagen with platelet GPIa/IIa and an indirect interaction with platelet GPIIb/IIIa mediated by adhesive proteins. *Blood.* 74:182.
23. Tuszynski, G.P., and A. Murphy. 1990. Spectrophotometric quantitation of anchorage dependent cell numbers using bicinchoninic acid protein assay reagent. *Anal. Biochem.* 184:189.
24. Coller, B.S., E.I. Peerschke, L.E. Scudder, and C.A. Sullivan. 1983. A murine monoclonal antibody that completely blocks the binding of fibrinogen to platelets produces a thrombasthenic-like state in normal platelets and binds GPIIb and/or GPIIIa. *J. Clin. Invest.* 72:325.
25. Peerschke, E.I. 1982. Induction of human platelet fibrinogen receptors by epinephrine in the absence of released ADP. *Blood.* 60:71.
26. Peerschke, E.I.B., and B. Ghebrehiwet. 1987. Human blood platelets possess specific binding sites for C1q. *J. Immunol.* 138:1537.
27. Du, X., E. Plow, A. Frelinger, T. O'Toole, J. Loftus, and M. Ginsberg. 1991. Ligands "activate" integrin $alpha_{IIb}/beta_3$ (platelet GPIIb-IIIa). *Cell.* 65:409.
28. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. NY. Acad. Sci.* 51:660.
29. Quick, A.J. 1966. Salicylates and bleeding: the aspirin tolerance test. *Am. J. Med. Sci.* 252:265.
30. Kelton, J.G., J.W. Smith, A.V. Santos, W.G. Murphy, and P. Horsewood. 1987. Platelet IgG Fc receptor. *Am. J. Hematol.* 25:299.
31. Tandon, N.N., U. Kralisz, and G.A. Jamieson. 1989. Identification of glycoprotein IV (CD36) as a primary receptor for platelet collagen adhesion. *J. Biol. Chem.* 264:7576.
32. Peerschke, E.I.B., and B. Ghebrehiwet. 1990. Modulation of platelet responses to collagen by C1q receptors. *J. Immunol.* 144:221.
33. Brass, L.F., D.R. Manning, and S.J. Shattil. 1991. GTP-binding proteins and platelet activation. *In* Progress in Hemostasis and Thrombosis, Vol. 10. B.S. Coller, editor. W.B. Saunders Co., Philadelphia. 127–174.
34. Peerschke, E.I.B. 1985. The platelet fibrinogen receptor. *Sem. Hematol.* 22:241.
35. Mosher, D.F., D.M. Pesciotta, J.C. Loftus, and R.W. Albrecht. 1985. Secreted alpha granule proteins: the race for receptors. *In* Platelet Membrane Glycoproteins. J.N. George, A.T. Nurden, and D.R. Phillips, editors. Plenum Press, New York. 171–191.
36. Johnston, G.I., G.A. Bliss, P.J. Newman, and R.P. McEver. 1990. Structure of the human gene encoding granule membrane protein GMP 140, a member of the selectin family of adhesion receptors for leukocytes. *J. Biol. Chem.* 265:21381.
37. Larsen, E., A. Celi, G.E. Geibert, B.C. Furie, J.K. Erban, R. Bonfanti, D. Wagner, and B. Furie. 1989. PADGEM protein: a receptor that mediates the interactions of activated platelets with neutrophils and monocytes. *Cell.* 59:305.
38. Holmsen, H. 1982. Platelet secretion. *In* Hemostasis and Thrombosis. Basic Principles and Clinical Practice. R.W. Colman, J. Hirsch, V.J. Marder, and E.W. Salzman, editors. J.B. Lippincott Co., Philadelphia. 390–403.
39. Zwaal, R.F.A. 1978. Membrane and lipid involvement in blood coagulation. *Biochim. Biophys. Acta.* 515:163.
40. Hardisty, R.M., and R.A. Hutton. 1965. The kaolin clotting time of platelet-rich plasma: a test of platelet factor-3 availability. *Br. J. Haematol.* 11:258.
41. Peerschke, E.I.B., and B. Ghebrehiwet. 1992. Platelet interactions with C1q in whole blood and in the presence of immune complexes or aggregated IgG. *Clin. Immunol. Immunopathol.* 63:45.