JCI The Journal of Clinical Investigation

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R B Stricker, … , L Corash, M A Shuman

J Clin Invest. 1987;79(6):1589-1594. https://doi.org/10.1172/JCI112993.

Research Article

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Evidence That a 210,000-Molecular-Weight Glycoprotein (GP 210) Serves as a Platelet Fc Receptor

Raphael B. Stricker, Patrick T. Reyes, Laurence Corash, and Marc A. Shuman
Department of Laboratory Medicine, Cancer Research Institute, University of California, San Francisco, California 94143

Abstract

We previously identified a 210,000-mol-wt platelet glycoprotein (GP 210) that is missing from Bernard-Soulier platelets, and found that an antibody against GP 210 inhibits ristocetin-induced platelet agglutination. We now show by immunoblotting that GP 210 binds heat-aggregated rabbit and human IgG, as well as keyhole limpet hemocyanin (KLH)–anti-KLH and ovalbumin (OA)–anti-OA immune complexes. Immune complex binding to GP 210 was preserved on chymotrypsin-treated platelets that lacked glycoprotein Ib (GP Ib). In contrast, ristocetin-induced platelet agglutination resulted in disappearance of immunologically detectable GP 210 and loss of immune complex binding, even though GP Ib remained intact. Purified Fc fragments inhibited binding of anti–GP 210 antibody to intact platelets and to GP 210 on immunoblots. The Fc fragments also blocked immune complex binding to GP 210. Conversely, anti–GP 210 antiserum and $F(ab)_2$ fragments inhibited binding of fluorescein-labeled Fc fragments to intact platelets. We conclude that GP 210 functions as a platelet Fc receptor.

Introduction

Previous studies have suggested that platelet glycoprotein Ib (GP Ib)[1] functions as an Fc receptor (1, 2). GP Ib is a 170,000-mol-wt glycoprotein that binds von Willebrand factor (F VIII:vWF) when platelets are exposed to ristocetin (1). In the Bernard-Soulier syndrome, platelets are deficient in GP Ib and lack the ability to respond to ristocetin. Moore et al. (1) showed that binding of aggregated IgG to platelets inhibits ristocetin-induced platelet agglutination, and suggested that the IgG complex interferes with ristocetin-induced binding of F VIII:vWF to GP Ib. Also, binding of keyhole limpet hemocyanin (KLH)–anti-KLH immune complex to platelets is blocked by prior platelet exposure to ristocetin and F VIII:vWF (2). This observation further implicates GP Ib in immune complex binding. Recently, however, Pfueller et al. (3) showed that when platelets are treated with a concentration of chymotrypsin that hydrolyzes GP Ib, they retain their ability to bind immune complexes. This finding suggests that the Fc receptor may be distinct from GP Ib. Moreover, Cheng and Hawiger (4) isolated a 255,000-mol-wt platelet membrane glycoprotein that binds to Fc fragments immobilized on a Sepharose column.

We recently described a patient with an autoantibody directed against a 210,000-mol-wt platelet membrane glycoprotein (GP 210) (5). The $F(ab)_2$ portion of the antibody bound to GP 210 and inhibited ristocetin-induced platelet agglutination. Also, we noted that, because heat-aggregated human IgG bound to this platelet membrane protein, the protein may have Fc receptor properties. In the present report, we have determined that GP 210 functions as an Fc receptor on platelets.

Methods

Antibody, immune complex, and Fc fragment preparations. Rabbit IgG and human IgG were prepared from serum by using Protein A–Sepharose CL6B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) (6). 2 mg/ml IgG was aggregated by heating at 63°C for 10 min. KLH and ovalbumin (OA) were obtained from Sigma Chemical Co., St. Louis, MO. Rabbit anti-KLH was purchased from Cappel Laboratories, Malvern, PA, whereas rabbit anti-ovalbumin was obtained from Miles Scientific Div., Miles Laboratories Inc., Naperville, IL. KLH–anti-KLH and OA–anti-OA immune complexes were prepared as previously described (2) by using 2 mg/ml specific antibody for the formation of immune complex. Human Fc fragments were obtained from Dako Corp., Santa Barbara, CA, and rabbit Fc fragments were obtained from Immuno-Search, Toms River, NJ. Human Fc fragments labeled with fluorescein isothiocyanate (FITC) were obtained from Rockland, Inc., Gilbertsville, PA. The Fc fragments were shown to be free of residual IgG by polyacrylamide gel electrophoresis (PAGE).

Serum antibody against GP 210 was obtained as previously described (5). Rabbit anti–GP Ib antibody (7) was a generous gift of Dr. Joan Fox, Gladstone Foundation Laboratories, San Francisco, CA. This antibody was raised against the glycocalicin moiety of GP Ib as previously described in detail (7). The antibody does not react with GP Ibβ, GP V, or GP IX on immunoblots (7). 32 mg/ml ammonium sulfate–purified antibody was diluted 1:500 in Tris-buffered saline (TBS) that contained 1% gelatin (J. T. Baker Chemical Co., Phillipsburg, NJ) for use in immunoblotting. $F(ab)_2$ from the rabbit anti–GP Ib antibody was prepared as previously described (5).

Platelet preparation. Washed O-positive platelets were prepared as previously described (5). Chymotrypsin-treated platelets were prepared according to the method of Greenberg et al. (8) by incubating 1×10^9 platelets with 10 U/ml α-chymotrypsin (Sigma Chemical Co.) for 30 min at 37°C. At this concentration GP Ib was selectively removed from the platelet surface (8). By immunoblotting, these platelets were shown to retain GP IIb/IIIa and GP V. Platelets from two patients with the Bernard-Soulier syndrome (9) were the generous gift of Dr. Margaret Johnson of the Medical Center of Delaware, Newark, DE. The control and chymotrypsin-treated platelets were solubilized in 2% sodium dodecyl sulfate (SDS) sample buffer at a concentration of 1×10^9/ml (1.2 mg/ml). The Bernard-Soulier platelets were used at a concentration of 8×10^8/ml (3.6 mg/ml). Freshly isolated control platelets were also solubilized in Nonidet P-40 (NP-40) as follows: after isolation and washing in Tyrode's buffer (5), 2×10^9 platelets were dissolved in 1 ml phosphate buffer that contained 1% NP-40 for 30 min at 4°C. The lysate was centrifuged at 50,000 *g* for 30 min at 4°C, and the supernatant that contained

Address correspondence to Dr. R. B. Stricker, M-1282, Cancer Research Institute, University of California, San Francisco, CA 94143.

Received for publication 24 December 1985 and in revised form 22 January 1987.

1. Abbreviations used in this paper: FITC, fluorescein isothiocyanate; F VIII:vWF, von Willebrand factor; GP, glycoprotein; KLH, keyhole limpet hemocyanin; NP-40, Nonidet P-40; OA, ovalbumin; TBS, Tris-buffered saline.

J. Clin. Invest.
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0021-9738/87/06/1589/06 $1.00
Volume 79, June 1987, 1589–1594

solubilized platelet membranes was mixed with an equal volume of 4% SDS sample buffer in the presence or absence of 200 mM dithiothreitol. The samples were stored at −70°C until use.

Ristocetin-agglutinated platelets were prepared as follows: control platelets were separated by differential centrifugation and resuspended at 500,000/μl in autologous platelet-poor plasma. Either 1.5 mg/ml ristocetin (Pacific Hemostasis, Bakersfield, CA) or saline buffer was added, and platelet agglutination proceeded at 37°C for 10 min. Agglutination was determined by platelet aggregometry and was seen only in the ristocetin-treated sample and not in the saline control. The platelets were then washed and solubilized in the same manner as fresh platelets.

Immunoblotting. Immunoblotting studies were performed as previously described (5). 50 μl solubilized platelet proteins were electrophoresed in unreduced SDS–5% or 12% polyacrylamide gels and transferred to nitrocellulose (5). For reduced gels, platelets were treated with 200 mM dithiothreitol before electrophoresis. Blocking was performed by using 5% gelatin in TBS for 1 h at 37°C, after which the nitrocellulose was incubated with test serum, IgG, $F(ab)_2$, or immune complexes (aggregated IgG, KLH–anti-KLH, OA–anti-OA) diluted 1:100 in TBS–1% gelatin for 2 h at room temperature. After washing in TBS, the samples were incubated with a 1:500 dilution of biotin-conjugated goat $F(ab)_2$ anti–human or anti–rabbit immunoglobulin (Tago Inc., Burlingame, CA) for 1 h. After further washing in TBS, the nitrocellulose was incubated with avidin-conjugated peroxidase (Cappel Laboratories) diluted 1:500 in TBS–1% gelatin for 1 h. Color development was carried out by using 4-chloronaphthol as substrate (5); the nitrocellulose was then dried and photographed. High molecular weight standards (Bio-Rad Laboratories, Richmond, CA and Pharmacia Fine Chemicals) were simultaneously electrophoresed and transferred with the samples. These were stained with 0.05% amido black and served as molecular weight markers for the blots.

Blocking experiments were carried out as follows: the nitrocellulose membranes were incubated with 2 mg/ml human or rabbit Fc fragments diluted 1:100 in TBS–1% gelatin for 1 h at room temperature. The membranes were then washed and incubated with anti–GP 210 antibody or immune complex for 2 h as described above. Rabbit Fc was used to block human antibody, whereas human Fc was used to block the rabbit immune complexes. However, neither of the Fc preparations could be visualized directly on the nitrocellulose by using the goat anti–immunoglobulin reagents, since these reagents bound primarily to intact IgG and not to Fc fragments.

Flow cytometry. Competitive binding of immune complexes and Fc fragments to intact platelets was studied by using a flow cytometric technique, as previously described (10). In the first set of experiments, 2×10^8/ml washed control platelets were fixed with 1% paraformaldehyde and incubated with control buffer, 2 mg/ml monomeric rabbit IgG, 2 mg/ml heat-aggregated rabbit IgG, or 2 mg/ml rabbit Fc fragments for 30 min at room temperature. The platelets were then washed and incubated with 2 mg/ml human anti–GP 210 antibody for 30 min at room temperature. Binding of the human antibody, which does not crossreact with rabbit IgG, was detected by using biotinylated goat $F(ab)_2$ directed against human IgG (Tago Inc.). The platelets were then incubated with avidin linked to FITC (Tago Inc.) and analyzed in a flow cytometer. Binding of anti–GP 210 antibody was expressed as mean platelet fluorescence on a logarithmic scale. Inhibition of binding was expressed as the percent decrease in mean fluorescence that was induced by preincubation with immune complex or Fc fragments as compared with baseline binding of anti-GP 210 to the fixed platelets.

In the second set of experiments, paraformaldehyde-fixed platelets were incubated with serum, 2 mg/ml $F(ab)_2$ fragments, or 2 mg/ml unlabeled human Fc fragments for 30 min at 37°C. The platelets were then washed and incubated with 2 mg/ml FITC-labeled human Fc fragments for 30 min at 37°C. Fc binding to platelets was expressed as mean platelet fluorescence, as measured by flow cytometry. Inhibition of Fc binding was expressed as the percent decrease in mean fluorescence induced by serum, $F(ab)_2$ fragments, or unlabeled Fc fragments compared to controls. Experiments were performed in duplicate on separate occasions using two different platelet donors.

Results

Figs. 1 and 2 demonstrate the effects of chymotrypsin and ristocetin on platelet GP Ib and GP 210 by using antibodies against these antigens. Fig. 1 shows that rabbit antiserum against GP Ib, bound to several membrane components in control platelets (Fig. 1, lane *A*): a major 170,000-mol-wt band corresponding to GP Ib, and a 210,000–260,000-mol-wt broad nonhomogeneous complex. In Bernard-Soulier platelets, which are known to be deficient in GP Ib, binding to the 170,000-mol-wt protein was not seen (Fig. 1, lane *B*). Also, the antibody failed to bind to the lower portion of the 210,000–260,000-mol-wt complex. Chymotrypsin-treated platelets in which GP Ib was cleaved retained the entire 210,000–260,000-mol-wt complex (Fig. 1, lane *C*). Platelets exposed to ristocetin reacted with anti–GP Ib (Fig. 1, lane *D*), but immunoreactivity of the lower portion of the 210,000–260,000-mol-wt complex was diminished. Platelets treated only with aggregation buffer (Fig. 1, lane *E*) appeared no different than control platelets (Fig. 1, lane *A*).

Binding of anti–GP 210 antibody to the same platelet preparations is shown in Fig. 2. Antibody to GP 210 bound to control platelets (lanes *A* and *E*) and chymotrypsin-treated platelets (lane C), but not to Bernard-Soulier platelets (lane *B*) or ristocetin-treated platelets (lane *D*). Thus, GP 210 was deficient in Bernard-Soulier platelets that also lacked GP Ib (Fig. 1, lane *B*), and was no longer immunologically detectable after exposure of platelets to ristocetin, although GP Ib remained intact (Fig. 1, lane *D*). This finding suggests that GP 210 was altered after exposure of platelets to ristocetin; however, we could not detect GP 210 by immunoblotting in the plasma supernatant after ristocetin-induced platelet agglutination (data not shown).

Fig. 3 shows binding of immune complexes to control platelet proteins that were transferred to nitrocellulose. 2 mg/ml normal rabbit IgG did not bind to GP 210 (lane *A*). In contrast, heat-aggregated rabbit and human IgG (lanes *B* and *C*, respectively),

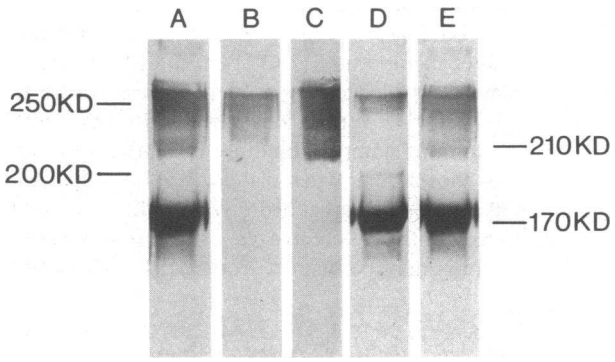

Figure 1. Effects of chymotrypsin and ristocetin on platelet GP Ib. Solubilized platelets were subjected to SDS-PAGE, transferred to nitrocellulose, and incubated in stepwise fashion with rabbit anti–GP Ib antibody, biotin-conjugated goat $F(ab)_2$ anti–rabbit IgG, and avidin peroxidase as described in Methods. (lane *A*) Control platelets; (lane *B*) Bernard-Soulier platelets; (lane *C*) chymotrypsin-treated platelets; (lane *D*) platelets exposed to ristocetin; and (lane *E*) platelets treated with aggregation buffer alone. Note major 170,000-mol-wt band and broad 210,000–260,000-mol-wt complex in control platelets (lanes *A* and *E*). Bernard-Soulier platelets (lane *B*) and chymotrypsin-treated platelets (lane *C*) are missing the 170,000-mol-wt band, whereas ristocetin-treated platelets retain this band (lane *D*).

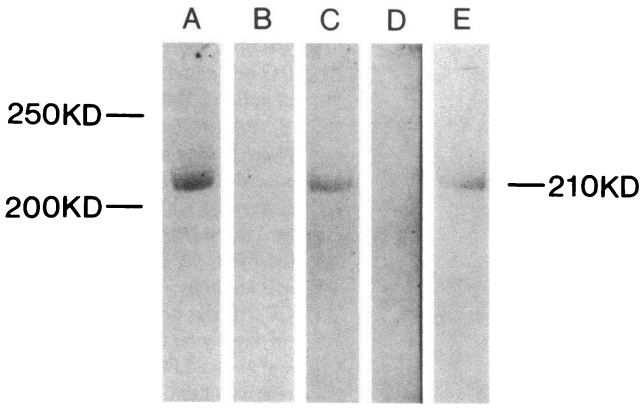

Figure 2. Effects of chymotrypsin and ristocetin on platelet GP 210. Solubilized platelets were subjected to SDS-PAGE, transferred to nitrocellulose, and incubated with anti-GP 210 antiserum, biotin-conjugated goat $F(ab)_2$ anti-human IgG, and avidin peroxidase as described in Methods. (lane *A*) Control platelets; (lane *B*) Bernard-Soulier platelets; (lane *C*) chymotrypsin-treated platelets; (lane *D*) platelets exposed to ristocetin; and (lane *E*) platelets treated with aggregation buffer alone. Note binding to GP 210 in control platelets (lanes *A* and *E*) and chymotrypsin-treated platelets (lane *C*), but not in Bernard-Soulier platelets (lane *B*) or ristocetin-treated platelets (lane *D*).

KLH–anti-KLH (lane *D*), and OA–anti-OA (lane *E*) all bound to GP 210. In addition, the OA complex bound to a second 250,000-mol-wt protein (lane *E*). Binding of heat-aggregated human IgG to GP 210 was blocked by preincubation with rabbit anti–GP Ib antibody (lane *F*). Preincubation with control rabbit IgG did not affect immune complex binding (data not shown). $F(ab)_2$ prepared from the anti–GP Ib antibody (lane *G*) also bound to GP 210, and blocked binding of both heat-aggregated human IgG and anti–GP 210 antibody to GP 210 on immunoblots (data not shown). Uncomplexed anti–KLH and uncomplexed anti–OA failed to bind to GP 210 (data not shown).

Also, when nitrocellulose-fixed platelet proteins were sequentially incubated with KLH or OA followed by antibody against the respective antigen, antibody binding to GP 210 was not seen. Thus, GP 210 bound aggregated IgG, antigen–antibody complexes, and antibody raised against GP Ib; it did not bind extraneous uncomplexed antibody or antigen alone in the absence of antibody.

Fig. 4 demonstrates binding of a specific immune complex (KLH–anti-KLH) to various platelet preparations. The immune complex bound to GP 210 in normal platelets (lane *A*) but not in Bernard-Soulier platelets that were deficient in both GP Ib and GP 210 (lane *B*). Heat-aggregated rabbit and human IgG as well as OA–anti-OA immune complexes also failed to bind to the Bernard-Soulier platelets (data not shown). Immune complex binding to GP 210 occurred in chymotrypsin-treated platelets (lane *C*) that were deficient in GP Ib. In contrast, binding did not occur in normal platelets after exposure to ristocetin (lane *D*). These platelets retained GP Ib (as shown in Fig. 1), but GP 210 appeared to be altered or lost, since immune complex no longer bound to it. Platelets treated only with aggregation buffer (lane *E*) bound KLH–anti-KLH in the same manner as control platelets (lane *A*).

Fig. 5 shows the results of binding studies that used reduced and unreduced control platelets solubilized in either SDS or NP-40. Fig. 5 *A* shows that anti–GP 210 antibody bound to GP 210 in both unreduced (lane *A*) and reduced (lane *B*) SDS–solubilized-platelet proteins separated by SDS-PAGE in 5% gels. However, in the reduced sample, GP 210 appeared as a doublet instead of a single band. In contrast, we did not detect antibody binding to GP 210 in NP-40–solubilized platelets under nonreducing (lane *C*) or reducing (lane *D*) conditions. Fig. 5 *B* shows anti–GP 210 antibody binding to platelet proteins separated by SDS-PAGE in 12% gels. Anti-GP 210 failed to bind to lower molecular weight SDS–solubilized platelet proteins under nonreducing (lane *A*) or reducing (lane *B*) conditions. However, the antibody bound to a 40,000-mol-wt protein in NP-40–solubilized unreduced (lane *C*) or reduced (lane *D*) platelet samples. Thus,

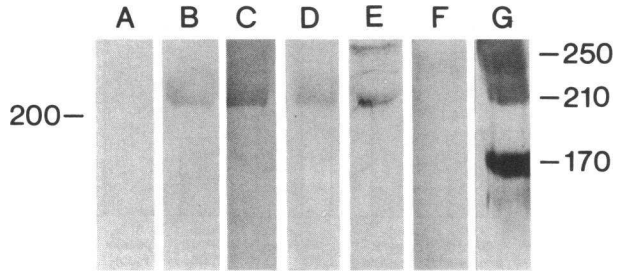

Figure 3. Binding of immune complexes to platelet proteins that were transferred to nitrocellulose. Control platelets were electrophoresed and transferred to nitrocellulose as described in Methods. The nitrocellulose was then incubated with the following: (lane *A*) 2 mg/ml normal rabbit IgG; (lane *B*) 2 mg/ml heat-aggregated rabbit IgG; (lane *C*) 2 mg/ml heat-aggregated human IgG; (lane *D*) KLH–anti-KLH; (lane *E*) OA–anti-OA; (lane *F*) rabbit anti-GP Ib followed by heat-aggregated human IgG; and (lane *G*) the $F(ab)_2$ portion of rabbit anti–GP Ib antibody. Note binding of immune complexes to GP 210 (lanes *B–E*). The OA immune complex also bound to a second 250,000-mol-wt protein (lane *E*). Binding of aggregated IgG was blocked by preincubation with anti–GP Ib antibody (lane *F*). $F(ab)_2$ made from GP Ib antibody also bound to GP 210 (lane *G*).

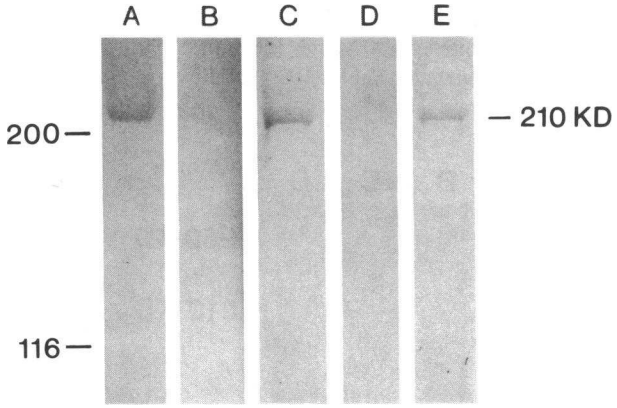

Figure 4. Binding of KLH–anti-KLH immune complex to various platelet preparations. Immunoblotting was performed as described in Fig. 1. KLH–anti-KLH was incubated with the following: (lane *A*) control platelets; (lane *B*) Bernard-Soulier platelets; (lane *C*) chymotrypsin-treated platelets; (lane *D*) platelets exposed to ristocetin; and (lane *E*) platelets exposed to aggregation buffer only. The immune complex bound to GP 210 in control platelets (lanes *A* and *E*) and chymotrypsin-treated platelets (lane *C*), but not in Bernard-Soulier platelets (lane *B*) or ristocetin-agglutinated platelets (lane *D*).

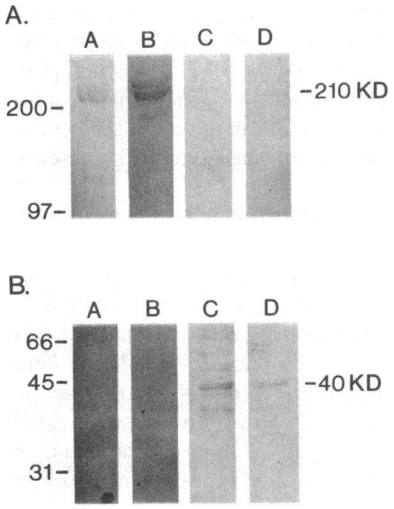

Figure 5. Effects of detergent solubilization and disulfide bond reduction on binding of anti-GP 210 to platelet proteins. Solubilized platelet proteins were subjected to SDS-PAGE in either 5% gels (*A*) or 12% gels (*B*) before transfer to nitrocellulose. In *A* and *B*, anti-GP 210 was incubated with unreduced SDS-solubilized platelets (lane *A*), reduced SDS-solubilized platelets (lane *B*), unreduced NP-40-solubilized platelets (lane *C*), and reduced NP-40-solubilized platelets (lane *D*). Note binding to GP 210 alone in SDS-solubilized platelets and binding to 40,000-mol-wt protein in NP-40-solubilized platelets.

GP 210 was retained in reduced SDS-solubilized platelet preparations, whereas NP-40 solubilization of platelets abolished antibody binding to GP 210 and resulted in antibody binding to a 40,000-mol-wt protein.

Fig. 6 shows the results of blocking studies that used human Fc fragments and rabbit immune complexes on immunoblots. Binding of KLH–anti-KLH to GP 210 is shown in lane *A*. This binding was blocked by preincubation with Fc fragments (lane *B*). Similarly, binding of OA–anti-OA to GP 210 and to the 250,000-mol-wt protein is shown in lane *C*. Binding to both of these sites was blocked by preincubation with human Fc (lane *D*). Thus, Fc fragments blocked binding of the immune complexes. Binding of human anti-GP 210 was also blocked by preincubation with either rabbit Fc fragments or KLH–anti-KLH immune complex (data not shown).

Figs. 7 and 8 show the results of blocking experiments that used intact platelets. In Fig. 7, binding of anti-GP 210 antibody (bar A) was inhibited by 90% after incubation with equal concentrations of aggregated rabbit IgG (bar B) or rabbit Fc fragments (bar C). 2 mg/ml monomeric rabbit IgG failed to block binding of anti-GP 210 to the intact platelets (data not shown). In Fig. 8, binding of FITC-labeled Fc fragments was measured after preincubation of platelets with serum (Fig. 8 *A*) or $F(ab)_2$ and unlabeled Fc fragments (Fig. 8 *B*). Anti-GP 210 antiserum or $F(ab)_2$ fragments inhibited Fc binding to platelets by 70–75%. Preincubation of platelets with control serum, control $F(ab)_2$ fragments, and serum or $F(ab)_2$ fragments from another patient with anti-platelet antibody failed to inhibit Fc binding. Preincubation of platelets with unlabeled Fc fragments inhibited binding of the FITC-labeled Fc fragments by > 80%. According to the results of these blocking experiments, immune complexes and Fc fragments bound to GP 210 on intact platelets as well as on immunoblots and inhibited anti-GP 210 antibody binding

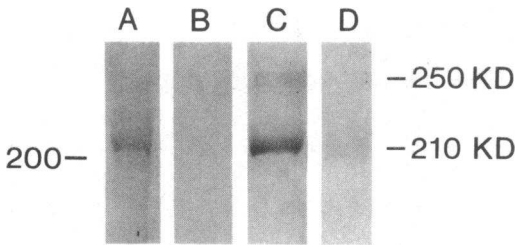

Figure 6. Effect of human Fc fragments on binding of KLH and OA immune complexes to GP 210. Unreduced platelet proteins were subjected to SDS-PAGE, transferred to nitrocellulose, and then incubated with KLH–anti-KLH (lane *A*), human Fc fragments followed by KLH–anti-KLH (lane *B*), OA–anti-OA (lane *C*), and human Fc fragments followed by OA–anti-OA (lane *D*). Note that Fc fragments blocked binding of KLH complex to GP 210 as well as binding of OA complex to GP 210 and 250,000-mol-wt protein.

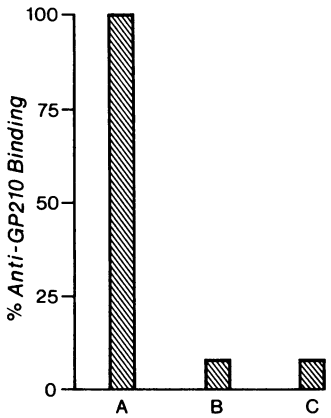

Figure 7. Flow cytometric analysis of the effects of aggregated IgG or Fc fragments on binding of anti-GP 210 to platelets. 2×10^8/ml paraformaldehyde-fixed platelets were incubated with control buffer (bar A), 2 mg/ml aggregated rabbit IgG (bar B), or 2 mg/ml rabbit Fc (bar C) followed by 2 mg/ml anti-GP 210 antibody. Binding of anti-GP 210 was detected with biotinylated goat anti-human IgG and avidin-FITC. Note that binding of anti-GP 210 to platelets was blocked by preincubation with aggregated rabbit IgG or Fc fragments.

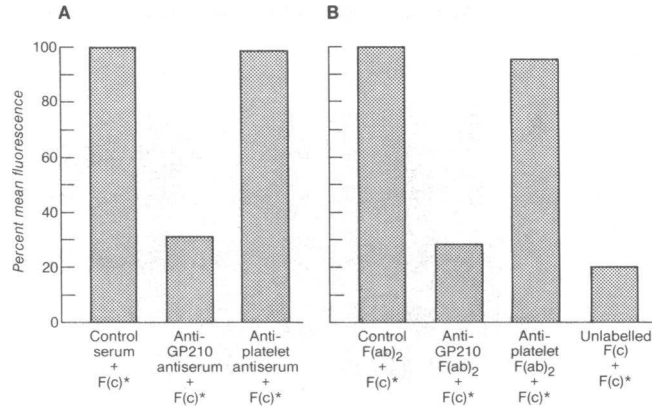

Figure 8. Inhibition of Fc binding to intact platelets by anti-GP 210 antibody. 2×10^8/ml paraformaldehyde-fixed platelets were incubated with serum, 2 mg/ml $F(ab)_2$ fragments, or 2 mg/ml unlabeled human Fc fragments followed by 2 mg/ml FITC-labeled human Fc fragments. Fc binding was determined by mean platelet fluorescence, as detected by flow cytometry. Note that anti-GP 210 antiserum or $F(ab)_2$ fragments blocked binding of labeled Fc fragments to platelets, whereas control serum, control $F(ab)_2$ fragments, and serum or $F(ab)_2$ fragments from another patient with anti-platelet antibody failed to block Fc binding. Preincubation of platelets with unlabeled Fc fragments also blocked labeled Fc binding. F(c)*, FITC-labeled Fc fragments.

to platelets. Conversely, anti-GP 210 inhibited binding of Fc fragments to intact platelets. Since binding of the Fc fragments was not completely inhibited by the anti-GP 210 antibody, it is possible that platelets have additional Fc receptors.

Discussion

Previously we showed that platelet GP 210 binds heat-aggregated human IgG (5). In the present study, we have demonstrated that GP 210 also binds KLH–anti-KLH and OA–anti-OA immune complexes. Immune complex binding to GP 210 was blocked by anti-GP 210 antibody and human Fc fragments. We previously demonstrated that GP 210 is missing from Bernard-Soulier platelets that also lack GP Ib, and have now shown that these platelets did not bind the immune complexes that were tested. In contrast, immune complexes bound to GP 210 in chymotrypsin-treated platelets that lacked GP Ib, further implicating GP 210 rather than GP Ib as an Fc receptor on platelets. Moreover, platelets exposed to ristocetin retained GP Ib, but did not bind immune complexes. We have demonstrated for the first time that GP 210 is altered when platelets are exposed to ristocetin.

Although it has been known for some time that platelets have Fc receptors that bind immune complexes (11), uncertainty persists over the nature of the Fc receptor. Karas et al. (12) previously measured binding of pure polymers of IgG to platelets by using an elegant stoichiometric assay. Binding of antigen–antibody complexes was not examined, however, and the nature of the binding site was not investigated. In a more recent study, Beardsley et al. (13) noted that aggregated IgG binds to a 200,000-mol-wt platelet protein on unreduced gels. Antigen–antibody and Fc fragment binding to this protein was not investigated, however. By using affinity chromatography with immobilized Fc fragments, Cheng and Hawiger (4) isolated a 255,000-mol-wt platelet membrane protein under nonreducing conditions in SDS-PAGE. The isolated protein was shown to bind aggregated IgG in a continuous sucrose density gradient. Although this 255,000-mol-wt protein appears to differ from GP 210, the protein that was demonstrated by Coomassie dye showed a broad staining region, and its molecular weight did not appear to be precise. Also, isolated Fc fragments possess binding properties distinct from those of aggregated IgG, since the entire Fc domain may be available for binding in these fragments (13). Thus, the Fc fragments may have bound to a large protein complex that contained more than one Fc binding site.

Our findings suggest that platelets have more than one type of Fc receptor. This hypothesis has been proposed to explain variations in Fc binding among lymphocyte subpopulations (14) and other leukocytes (15, 16). Moore and Nachman (2) showed that, although binding of KLH–anti-KLH complexes to platelets is blocked by F VIII:vWF and ristocetin, binding of OA–anti-OA is not blocked by these agents; consequently, some immune complexes may bind to more than one Fc receptor on the platelet membrane. Our results show binding of both types of complexes to GP 210, although the OA–anti-OA complex also appeared to bind to a second 250,000-mol-wt protein. The Fc binding site identified by Cheng and Hawiger (4) may have contained both of these proteins. A possible explanation for the difference in immune complex binding may be related to antigenic size: aggregated IgG ($1–2 \times 10^6$ mol wt) (17) or the large KLH antigen ($3.0–7.5 \times 10^6$ mol wt) could have sterically hindered Fc binding to the 250,000-mol-wt protein, whereas the smaller OA molecule (43,000 mol wt) may not have inhibited this interaction. The isolated Fc fragments that were used by Cheng and Hawiger (4) would not be affected by the presence of a large aggregate or antigen and might have a higher affinity for the 250,000-mol-wt binding site.

In contrast to our findings in normal platelets, we have been unable to identify an Fc receptor on Bernard-Soulier platelets. Pfueller et al. (3) showed that latex particles coated with IgG and fibrinogen bind to these platelets, which implies immune complex binding. No other complexes were tested, however, and the latex particles may have bound via immobilized fibrinogen to the platelet surface, since control fibrinogen–latex particles also bound to the Bernard-Soulier platelets. Identification of immune complex binding sites on these platelets requires further investigation.

Our results also demonstrate the importance of the solubilizing detergent in determining the molecular weight of membrane proteins that are separated by SDS-PAGE, as suggested by Metzger and his colleagues (18, 19). In a recent study, Rosenfeld et al. (20) incubated a mouse monoclonal anti-Fc receptor antibody with radiolabeled NP-40–solubilized platelets. This antibody immunoprecipitated a 40,000-mol-wt platelet protein in the presence of NP-40. We have now shown that human anti-GP 210 antibody binds to a similar molecular weight protein in NP-40–solubilized platelets that were subjected to immunoblotting (Fig. 5). Thus, GP 210 may represent a complex between a large membrane protein and a lower molecular weight Fc receptor. An analogous membrane complex that is composed of three proteins has been identified as the IgE receptor on human mast cells (19).

Our current findings suggest a structural and/or functional relationship between GP 210 and GP Ib. Previously we showed that an autoantibody against GP 210 blocks ristocetin-induced platelet agglutination by F VIII:vWF (5). Kunicki et al. (21) and Nachman et al. (22) have shown that a 210,000-mol-wt platelet membrane glycoprotein copurifies with GP Ib on wheat germ affinity chromatography; thus, GP 210 may be associated with GP Ib in the platelet membrane. Binding of immune complexes to GP 210 may therefore sterically hinder interaction of GP Ib with ristocetin or F VIII:vWF and block aggregation. This mechanism would explain the findings of Moore et al. (1). Although Nachman et al. (22) suggested a structural similarity between GP 210 and GP Ib based on tryptic peptide mapping, the exact nature of GP 210 and its relationship to GP Ib remains unknown. Selective chymotrypsin-induced cleavage of GP Ib and not GP 210 in intact platelets suggests that GP 210 is anatomically distinct from GP Ib in the platelet membrane. However, the possibility that GP 210 represents a complex between 170,000-mol-wt GP Ib and the 40,000-mol-wt Fc receptor described by Rosenfeld et al. (20) has not been excluded.

In summary, we have shown that GP 210 serves as an Fc receptor on platelets. GP 210 is functionally distinct from GP Ib, which explains why selective loss of GP Ib does not abolish immune complex binding to platelets. In contrast, absence of GP 210 in Bernard-Soulier platelets and its alteration after exposure of normal platelets to ristocetin may explain the failure of immune complexes to bind to these platelets. The composition of GP 210 and its relationship to GP Ib in the platelet membrane remain to be determined.

Acknowledgments

The authors thank Dr. Joan Fox of the Gladstone Foundation Laboratories, San Francisco, CA for providing the anti-GP Ib antibody. We also thank Dr. Margaret Johnson of the Medical Center of Delaware, Newark, DE for providing Bernard-Soulier platelets, and Margaret Rheinschmidt, Faye Weimer, Patricia Meers, and Debra Singer for technical assistance.

This work was supported by grants 1F32 HL-06871-01 CLN and HL-21403 of the National Heart, Lung and Blood Institute. Dr. Stricker is the recipient of Research Fellowship 86-N37A from the American Heart Association, California Affiliate. Dr. Shuman was the recipient of a Research Career Development Award, 1 K04 HL-00802, from the National Institutes of Health while this research was conducted.

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