*Biochimica et Biophysica Acta* 818 (1985) 285–290
Elsevier



BBA 72724

# Identification of $Pl^{A1}$ alloantigen domain on a 66 kDa protein derived from glycoprotein IIIa of human platelets

Elizabeth Kornecki [b,*], Si-Yin Chung [a,**], John C. Holt [a], Czeslaw S. Cierniewski [a,***], George P. Tuszynski [a,+] and Stefan Niewiarowski [a]

[a] *Thrombosis Research Center, Temple University Health Sciences Center, Philadelphia, PA 19140 and* [b] *Department of Psychiatry, Neurosciences Research Unit, University of Vermont, Burlington, VT 05405 (U.S.A.)*

(Received February 26th, 1985)

Key words: Alloantigen domain; Glycoprotein IIIa; Proteinase treatment; (Human platelet)

**Incubation of platelets with chymotryptin leads to the exposure of fibrinogen receptors and to the appearance of a 66 kDa membrane component on the surface of platelets. Both glycoprotein IIIa (GP IIIa) and a 66 kDa component were precipitated from detergent extracts of solubilized, surface radiolabeled chymotrypsin-treated platelets by human anti-$Pl^{A1}$ antisera. Moreover, the presence of the $Pl^{A1}$ antigen was identified on GP IIIa (but not on GP IIb) and on a 66 kDa protein by means of immunoblot procedures using platelet Triton X-114 extracts and these purified proteins. Anti-$Pl^{A1}$ antiserum did not recognize GP IIIa on the surface of intact (untreated) platelets nor the 66 kDa protein on the surface of chymotrypsin-treated platelets of $Pl^{A1}$-negative individuals. The present data demonstrate directly that the 66 kDa protein is derived from GP IIIa and contains the $Pl^{A1}$ alloantigen.**

## Introduction

The $Pl^{A1}$ antigen, which is detected on the platelet surface in 98% of the population [1], is the most critical platelet alloantigen in the development of post transfusion purpura and in neonatal alloimmune thrombocytopenic purpura. The majority of patients with these syndromes have been shown to be $Pl^{A1}$ negative and to have anti-$Pl^{A1}$ antibodies in their plasma [2–4].

The $Pl^{A1}$ antigen has been shown to be expressed on glycoprotein IIIa [5–8]. Kunicki and Aster [9] and Kunicki et al. [10] reported that genes controlling expression of GP IIb/GP IIIa and $Pl^{A1}$ antigen activity are most likely not identical and that thrombasthenic platelets are phenotypically $Pl^{A1}$ null because they lack the glycoprotein carrier (GP IIIa) upon which these alloantigenic determinants are normally expressed. Similar observations were reported by Van Leeuwen et al. [11].

Our previous studies have demonstrated that treatment of platelets with chymotrypsin or pronase leads to the exposure of fibrinogen receptors [12] and to the appearance of a 66 kDa platelet membrane component on the surface of platelets [13]. We observed a direct correlation between the exposure of a 66 kDa protein and enhanced fibrinogen-induced platelet aggregation and fibrinogen binding to proteolytically-treated platelets [13].

* To whom correspondence should be addressed.
** Present address: Biomedical Product Dept., DuPont NEN, 331, Trebel Cove Rd., North Billerica, MA, U.S.A.
*** Present address: Department of Biophysics, Medical School of Lodz, Lodz, Poland.
+ Present address: Lankenau Research Center, Lankenau Hospital, Philadelphia, PA, U.S.A.

In order to further investigate the origin of the 66 kDa protein, we studied the interaction of human anti-$Pl^{A1}$ antisera with membrane components of proteolytically-treated platelets and with isolated components of platelet membranes. Our study indicates that the $Pl^{A1}$ antigen domain resides on a 66 kDa protein which is derived from GP IIIa by limited proteolysis.

## Materials and Methods

*1. Collection of blood.* Blood was taken from healthy individuals who signed informed consent forms approved by Institutional Human Experimentation Committee. $Pl^{A1}$-negative donor was kindly provided by Dr. M. Dahlke, American Red Cross, Philadelphia, PA.

*2. Isolation of platelet suspensions.* Platelets, from blood freshly collected in the anticoagulant acid-citrate dextrose, were washed by the method of Mustard et al. [14] and suspended in Tyrode albumin (0.35%) solution (pH 7.35). Chymotrypsin-treated platelets were prepared as previously described [12,13]. In brief, suspensions of washed platelets ($10^9$ platelets/ml) were incubated with 500 $\mu$g chymotrypsin per ml (Grade IS, Sigma Chemical Company, St. Louis), for 45 min at 37°C in the presence of prostaglandin $E_1$ ($10^{-6}$ M). The chymotrypsin was inhibited with PMSF (25 $\mu$mol/$\mu$mol enzyme) and eliminated by extensive washing of the platelets in Tyrode solution.

*3. Antisera.* Anti-human platelet membrane antisera were raised in rabbits by injecting platelet membranes isolated from intact platelets or from platelets treated with chymotrypsin or pronase [13]. These antisera blocked fibrinogen-induced platelet aggregation and $^{125}$I-fibrinogen binding to platelets.

Anti-human $Pl^{A1}$ antisera were kindly supplied by Dr. R. Aster, Blood Center of Southeastern Wisconsin, Milwaukee, WI (serum from patient W.), and by Dr. M. Dahlke, American Red Cross, Philadelphia, PA (serum from patient J.). The contamination with antibodies reacting with HLA antigens was insignificant. The IgG fractions of the antisera were prepared by Bio-Rad DEAE-Affi-Gel-Blue column chromatography or ammonium sulfate precipitation. Fab fragments were prepared by papain digestion as described previously [13]. Anti-human GP IIb antiserum raised in rabbits was kindly supplied by Dr. J.J. Sixma (University Hospital, Utrecht, The Netherlands). Antisera to GP IIIa were raised in our laboratory. The rabbits were injected intradermally with 50 $\mu$g antigen emulsified in complete Freund's adjuvant. The same doses of antigen, suspended in complete Freund's adjuvant, were given during booster injections made every second week.

*4. Iodogen-catalyzed $^{125}$I-iodination* of intact and chymotrypsin-treated platelets was performed according to Tuszynski et al. [15].

*5. SDS-polyacrylamide gel electrophoresis* was performed according to the method of Laemmli [16] in 7.5% polyacrylamide slab gels. The gels were stained for protein with Coomassie brilliant blue, destained in 10% acetic acid, alcohol, dried in vacuum and exposed to Kodak X-Omat R film with DuPont Cronex Lighting Plus intensifying screens for approx. 24 h and developed using a Kodak X-Omat developer.

*6. Immunoblotting.* Completed gels were transferred electrophoretically (6 V/cm for 15 h at 4°C) onto nitrocellulose paper for staining with specific antibody. The transfer buffer contained 25 mM Tris, 192 mM glycine, pH 8.2, 20% v/v methanol [17]. For Amido Black staining, completed transfers were incubated in 0.1% Amido Black in 45% methanol, 10% acetic acid and destained in water. For antibody staining, they were incubated at 20°C on a rocker as follows: 5 min in 0.15 M NaCl, 0.01 M Tris-glycine (pH 8.3), which was the buffer used throughout; 5 min in 0.15% gelatin (Fisons, Loughborough, U.K.) in buffer; 1–2 h in gelatin containing first antibody (IgG or serum); 20 min in several changes of buffer to remove excess first antibody; 1 h in 30 $\mu$l peroxidase-conjugated goat anti-rabbit IgG (Cappel Laboratories, Chester, PA); 20 min in several changes of buffer to remove second antibody; 5 min in substrate solution (10 mg 4-chloro-1-naphthol dissolved in 3 ml methanol and added to 47 ml buffer containing 30 vol. $H_2O_2$). All volumes were 5–10 ml, the minimum to ensure uniform wetting of the paper. Sensitivity was of the order of 25 ng.

*7. Immunoprecipitation of $^{125}$I-labeled platelet proteins* by anti-platelet membrane antibodies was performed according to the method of Kessler [18]

with previously described modifications [13,19].

*8. Purification of glycoprotein IIb and glycoprotein IIIa.* Platelet suspensions were prepared from 48 units of outdated platelets. Subsequently, these platelets were lysed with glycerol in the presence of proteolytic enzyme inhibitors, according to Barber and Jamieson [20]; the membrane fractions were isolated by means of sucrose gradient centrifugation. An aliquot (2 ml) of platelet membrane-suspension (3 mg protein/ml) was extracted with Triton X-114 at a final concentration of 1% [21]. GP IIb and GP IIIa were separated in the Triton X-114 phase. Final separation of GP IIb and GP IIIa was achieved by means of preparative SDS-polyacrylamide gel electrophoresis. GP IIb and GP IIIa bands were cut from the gel and eluted electrophoretically into SDS electrode buffer (pH 8.3), containing 0.1% SDS [22]. The yield of GP IIb and GP IIIa from 1 unit of blood was approx. 100 and 200 $\mu$g per $10^{11}$ platelets, respectively.

*9. Purification of a 66 kDa platelet membrane component.* Platelet suspensions prepared from 12 units of fresh blood were treated with chymotrypsin as described above. The remaining GP IIb and GP IIIa and the newly formed 66 kDa component were extracted with Triton X-114 at a final concentration of 1%. Final separation of a 66 kDa component was achieved by means of preparative SDS-polyacrylamide gel electrophoresis [13]. The recovery of 66 kDa component was approx. 50 $\mu$g per $10^{11}$ platelets from 1 unit of blood.

## Results

Fig. 1 shows the autoradiogram of SDS-polyacrylamide gels of immunoprecipitates of surface $^{125}$I-radiolabeled intact and chymotrypsin-treated platelets with the use of anti-human platelet membrane antisera and anti-PI$^{A1}$ antisera obtained from two patients with post-transfusion purpura. It can be seen that anti-human platelet membrane antisera precipitated GP IIb and GP IIIa from intact platelets. On the other hand, the immunoprecipitates obtained from chymotrypsin-treated platelets contained GP IIb, GP IIIa and a 60 kDa component. Human anti-PI$^{A1}$ antisera immunoprecipitated GP IIIa from intact platelets, whereas both GP IIIa and a kDa protein were immunoprecipitated from chymotrypsin-treated platelets. Both

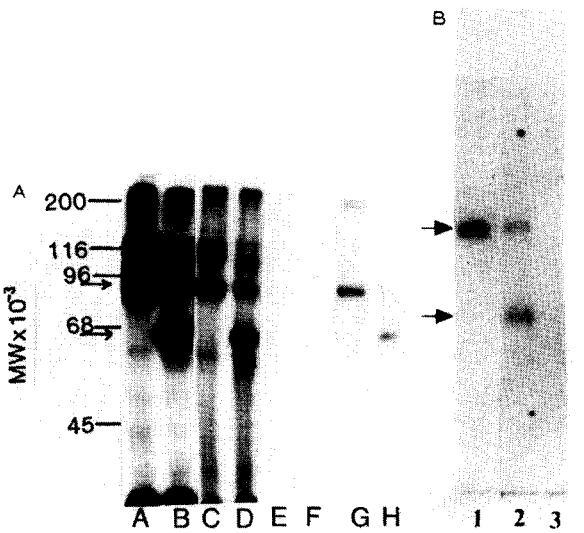


Fig. 1. Autoradiograms showing immunoprecipitation of GP IIIa and a 66000 $M_r$ protein from platelets using human anti-PI$^{A1}$ and anti-human platelet membrane antibodies. (A) Aliquots (10 $\mu$l) of detergent solubilized, surface radiolabeled intact and chymotrypsin-treated platelets were incubated with 10 $\mu$l of antibody. The immune complexes were precipitated with 100 $\mu$l of *Staphylococcus aureus*, solubilized in 1% Triton X-100 and 0.2% SDS and applied without reduction on 7.5% gels for SDS-polyacrylamide gel electrophoresis. Molecular weight markers are shown on the left. Each sample was analysed in duplicate. A, Total surface protein pattern of intact platelets; B, total surface protein pattern of chymotrypsin-treated platelets; C, immunoprecipitate of intact platelet extracts using rabbit anti-platelet membrane antiserum; D, immunoprecipitate of chymotrypsin-treated platelet extracts using anti-platelet membrane antiserum; E and F, control immunoprecipitates using nonimmune rabbit serum and human IgG, respectively; G, immunoprecipitate of intact platelet extracts using anti-PI$^{A1}$ IgG obtained from the serum of patient W.; H, immunoprecipitate of chymotrypsin-treated platelet extracts using anti-PI$^{A1}$ IgG from patient W. Top arrow indicates position of GP IIIa and bottom arrow indicates position of 66000 $M_r$ protein. (B) The same procedure for radiolabeling and immunoprecipitation were followed as described in (A) above. 1, Immunoprecipitate of radiolabeled intact platelet extracts using anti-PI$^{A1}$ serum from patient J. 2, Immunoprecipitate of radiolabeled chymotrypsin-treated platelet extracts using anti-PI$^{A1}$ serum from patient J. 3, Control immunoprecipitate of radiolabeled chymotrypsin-treated platelet extracts using non-immune-human serum. Top arrow indicates the position of GP IIIa and the bottom arrow indicates the position of the 66 kDa protein.

autoradiograms (Figs. 1A and 1B) show the pattern of migration of membrane proteins in the non-reduced system. In the reduced system, the mobilities of GP IIIa and the 60 kDa component

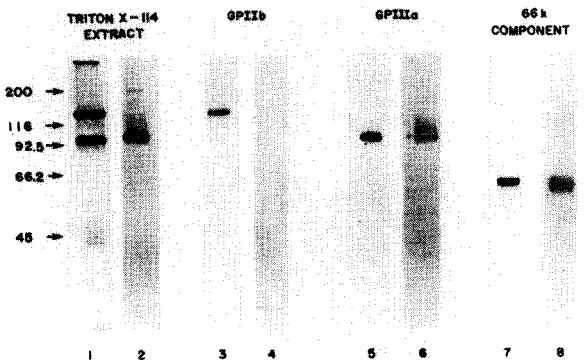


Fig. 2. Identification of $Pl^{A1}$ antigen on GP IIIa and 66 kDa components by means of purified protein. Aliquots of 20 $\mu$l of Triton X-114 extracts of human platelet membranes, purified GP IIb, purified GP IIIa and purified 66 kDa component were subject to SDS-polyacrylamide gel electrophoresis according to Laemmli [16] in a nonreduced system. The concentration of protein in each sample amounted to 80–100 $\mu$g/ml. Each sample was run in duplicate. After electrophoresis, the gels were either stained with Coomassie blue or the bands were transferred to nitrocellulose paper and stained with antibody as described in Materials and Methods. Lanes 1, 3, 5, 7 show the Coomassie blue protein staining pattern of the following: Triton X-114 extract of platelet membranes, purified GP IIb, purified GP IIIa, and 66000 $M_r$ component, respectively. Numbers 2, 4, 6, 8 indicate immunoblots of the same material stained with anti-$Pl^{A1}$ IgG obtained from patient J. The molecular weight markers: myosin (200 kDa), $\beta$-galactosidase (116 kDa), phosphorylase (92.5 kDa), bovine serum albumin (66.2 kDa) and ovalbumin (45 kDa) are indicated by the arrows.

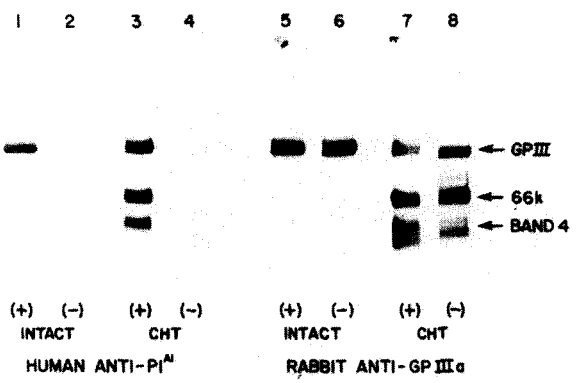


Fig. 3. Identification of $Pl^{A1}$ antigen in Triton X-114 extracts of intact or of chymotrypsin-treated platelets of $Pl^{A1}$ positive and $Pl^{A1}$ negative individuals. Whole platelet lysates (samples 3 and 7) or Triton X-114 extracts (all other samples) were fractionated by SDS-polyacrylamide gel electrophoresis and resolved proteins were transferred to nitrocellulose paper: lanes 1, 3, 5, 7 represent material obtained from $Pl^{A1}$-positive platelets and lanes 2, 4, 6, 8, material from $Pl^{A1}$-negative platelets. Extracts of intact platelets were shown in lanes 1, 2, 5, 6, and those of chymotrypsin-treated platelets in lanes 3, 4, 7, 8. Lanes 1–4 were stained with human anti-$Pl^{A1}$ antiserum and lanes 5–8 with rabbit anti-GP IIIa antiserum. Sample 1 is identical with sample 5, 2 with 6, 3 with 7 and 4 with 8. (+) and (−) indicate $Pl^{A1}$-positive and -negative platelets, respectively. Intact and CHT refer to untreated and chymotrypsin-treated platelets, respectively. For other explanations, refer to Fig. 2.

were decreased, whereas that of GP IIb was increased. (The apparent molecular weight of the 60 kDa component in a reduced system corresponds to 66000 and we refer to the component in the text as the 66 kDa component.)

In order to directly identify the antigen reacting with the antibody, immunoblotting experiments were carried out. First, purified GP IIb, GP IIIa and the 66 kDa component were analyzed. Fig. 2 shows gels stained with Coomassie blue and the nitrocellulose transfers of these gels stained with anti-$Pl^{A1}$ IgG. Both GP IIIa and 66 kDa component contained the $Pl^{A1}$ antigen, while GP IIb did not. The GP IIb was transferred onto the nitrocellulose paper since it was detected by Amido Black and by rabbit anti-GP IIb antiserum (data not shown). Moreover, when samples of $^{125}$I-labeled platelet membranes were analyzed, all of the counts corresponding to $^{125}$I-GP IIb (and $^{125}$I-GP IIIa) were recovered on the nitrocellulose transfers of the gel (results not shown). However, GP IIb could not be detected by the anti-$Pl^{A1}$ antiserum.

Fig. 3 shows that immunoblotting with anti-GP IIIa antiserum or anti-$Pl^{A1}$ antiserum of Triton X-114 extracts of intact or chymotrypsin-treated platelets obtained from a $Pl^{A1}$-positive subject, we detected the presence of both GP IIIa and the 66 kDa component. However, only by means of the anti-GP IIIa serum were these components detectable on the platelet surface of $Pl^{A1}$-negative individuals; anti-$Pl^{A1}$ antiserum did not recognize either of these components. In extracts of chymotrypsin-treated platelets, both anti-$Pl^{A1}$ antiserum and rabbit anti-GP IIIa antiserum also recognized a fragment smaller than 66 kDa component, which was presumably also derived from GP IIIa (Band 4). In addition, rabbit anti-GP IIIa antiserum recognized a low molecular weight fragment which was not reactive with anti-$Pl^{A1}$ antibody.

## Discussion

The main objective of this study was to map the $Pl^{A1}$ antigen on proteolytic products of GP IIIa obtained when human platelets are treated with chymotrypsin. The results of these experiments demonstrate that the $Pl^{A1}$ antigen is present on a 66 kDa membrane component, produced by the action of chymotrypsin on the platelet surface. These experiments provide definitive evidence that the 66 kDa component is a product of proteolytic digestion of GP IIIa. The parallel between the reactivity of rabbit anti-membrane serum and anti-$Pl^{A1}$ IgG demonstrates the predominant immunogenicity of GP IIIa among platelet membrane glycoprotein. In agreement with other reports [23], we determined that the GP IIb molecule did not contain $Pl^{A1}$ alloantigen.

Fig. 3 shows the presence of two chymotryptic fragments that had lower molecular weight than 66 kDa component. One of these fragments (Band 4; Fig. 3) could correspond to a radioactive band ($M_r$ 55 000) detected by Peerschke and Coller [24] with chymotrypsin. In our own experimental system, using platelets digested with chymotrypsin for 45 min, this band was not observed by immunoprecipitation followed by SDS-polyacrylamide gel electrophoresis and autoradiography. At present, it is difficult to explain the discrepancy obtained by the two methods.

Our data demonstrate that $Pl^{A1}$ antigen resides on a 66 kDa fragment of GP IIIa. Kunicki and Aster [5] have demonstrated previously the stability of $Pl^{A1}$ to chymotrypsin; however, the chymotrypsin digestion fragments of GP IIIa were not isolated by these authors. Deglycosylation of human platelet GP IIIa was also found to have no effect on the $Pl^{A1}$ alloantigen (Newman, Martin and Kahn; quoted in Ref. 25). It can be speculated that $Pl^{A1}$ deficiency may represent a variance in the specific amino acid sequence in GP IIIa of $Pl^{A1}$-negative individuals. The GP IIIa present on $Pl^{A1}$-negative platelets showed the same pattern of chymotrypsin digestion as did GP IIIa present on $Pl^{A1}$-positive platelets. The presence of $Pl^{A1}$ antigen did not affect the reactivity of GP IIIa degradation products with anti-GP IIIa antibody raised in rabbits. Complete characaterization of the $Pl^{A1}$ antigen domain can be achieved by further proteolytic digestion of the 66 kDa molecule and characterization of its fragments. While our studies were in progress, Kieffer et al. [23] reported that a component that migrated with an apparent molecular weight of 58 kDa in non-reduced system reacted in an immunoblot procedure with anti-$Pl^{A1}$ antiserum. This 58 kDa component was absent in normal platelets but it was present in platelets pretreated with chymotrypsin. It is possible that this fragment corresponds to that described by us [13].

In our previous work [13], we demonstrated that a 66 kDa protein was present on the surface of chymotrypsin-treated platelets, but not on the surface of intact (unstimulated) platelets. As we increased the concentration of chymotrypsin in the pre-incubatin mixture, the appearance of the 66 kDa component correlated with the enhanced fibrinogen-induced aggregation of chymotrypsin-treated platelets. We hypothesized at that time, but did not prove, that the 66 kDa protein may contain a fibrinogen binding site. This issue is still unclear for the following reasons: monoclonal antibodies named 123 [26] and $A_2A_9$ [27], both of which inhibit the fibrinogen-induced aggregation of ADP-stimulated intact and chymotrypsin-treated platelets, do not immunoprecipitate the 66 kDa protein from digests of SDS-denatured, chymotrypsin-treated platelets. It is possible that these monoclonal antibodies may not recognize the 66 kDa protein in a SDS-denatured state. Since these monoclonal antibodies bind identically to intact ((unstimulated) and to ADP-stimulated platelets [27,28] it may be that they bind to sites near, but not directly at, the fibrinogen binding site. Our most recent findings show that antibodies (antisera and IgG) developed in rabbits against the human 66 kDa component purified from chymotrypsin-treated platelets completely inhibited the fibrinogen-induced aggregation of ADP-stimulated platelets [29].

The data regarding the effect of anti-$Pl^{A1}$ antibody on the function of human platelets are inconsistent. Schreiber et al. [30] described complement-mediated induction of platelet release reaction induced by anti-$Pl^{A1}$ antibody. On the other hand, Van Leeuwen et al. [31] observed that anti-$Zw^a$ antibodies (which appear to be identical with anti-$Pl^{A1}$ antibodies) were able to inhibit the ADP- and

collagen-induced platelet aggregation and $^{125}$I-fibrinogen binding to ADP-stimulated platelets. Our data shows that the $Pl^{A1}$ antigenic site is located in the 66 kDa derivative of the GP IIIa molecule. Further investigation is necessary to demonstrate conclusively whether this domain of GP IIIa is also involved directly in the binding of fibrinogen.

## Acknowledgments

This study was supported in part by NIH Grants HL15226, HL28149, HL14217, Grant 1538 from Council for Tobacco Research and by Training Grant HL05976. E.K. is a recipient of a New Investigator Research Award (HL 32594) from the National Heart, Lung and Blood Institute. We wish to thank Dr. Yigal H. Ehrlich for helpful discussions and Mrs. Mary LaCasse for the preparation of this manuscript.

## References

1 Van Loghem, J.J., Dorfmeijer, H. and Van der Hart, M. (1959) Vox Sang. 4, 161–169
2 Shulman, N.R., Aster, R.H., Leitner, A. and Hiller, M.C. (1961) J. Clin. Invest. 40, 1597–1620
3 Shulman, N.R., Marder, V.J., Hiller, M.C. and Collier, E.M. (1964) Prog. Hematol. 4, 222–304
4 Aster, R.H. (1977) in Hematology (Williams, W.J., Beutler, E., Erslev, A.J. and Rundles, R.W., eds.), pp. 1326–1360, McGraw-Hill Book Co., New York
5 Kunicki, T.J. and Aster, R.H. (1969) Mol. Immunol. 16, 353–360
6 McMillan, R., Mason, D., Tani, P. and Schmidt, G.M. (1982) Br. J. Haematol. 51, 297–304
7 Lane, J., Brown, M., Bernstein, I., Wilcox, P.K., Slichter, S.J. and Nowinski, R.C. (1982) Br. J. Haematol. 50, 351–359
8 Bennett, J.S., Vilaire, G. and Cines, D.B. (1982) J. Biol. Chem. 257, 8049–4054
9 Kunicki, T.J. and Aster, R.H. (1978) J. Clin. Invest. 61, 1225–1231
10 Kunicki, T.J., Pidard, D., Çazenave, J.P., Nurden, A.T. and Caen, J.P. (1981) J. Clin. Invest. 67, 717–724
11 Van Leeuwen, E.F., Von dem Borne, A.G.K., Von Riesz, L.E., Nijenhuis, L.E. and Engelfriet, C.P. (1981) Blood 57, 49–54
12 Niewiarowski, S., Budzynski, A.Z., Morinelli, A., Brudzynski, T.M. and Stewart, G.J. (1981) J. Biol. Chem. 256, 917–925
13 Kornecki, E., Tuszynski, G.P. and Niewiarowski, S. (1983) J. Biol. Chem. 258, 9349–9356
14 Mustard, J.F., Perry, D.W., Ardlie, N.G. and Packham, M.A. (1972) Br. J. Haematol. 22, 193–204
15 Tuszynski, G.P., Knight, L.C., Kornecki, E. and Srivastava, C. (1983) Anal. Biochem. 130, 166–170
16 Laemmli, U.K. (1970) Nature 227, 680–685
17 Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354
18 Kessler, S.W. (1976) J. Immunol. 117, 1482–1489
19 Kornecki, E., Tuszynski, G.P. and Niewiarowski, S. (1984) Haematologia 17, 387–398
20 Barber, A.J. and Jamieson, G.A. (1970) J. Biol. Chem. 245, 6357–6365
21 Newman, P.J., Knipp, M.A. and Kahn, R.A. (1982) Thromb. Res. 27, 221–224
22 Tuszynski, G.P., Damsky, C.H., Fuhrer, S.P. and Waren, L. (1977) Anal. Biochem. 83, 119–129
23 Kieffer, N., Boizard, B., Didry, D., Wautier, Y.-L. and Nurden, A.T. (1984) Blood 64, 1212–1219
24 Peerschke, E.I.B. and Coller, B.S. (1984) Blood 64, 59–63
25 Miller, W.W. and Harmon, J.A. (1984) Advances in Immunobiology, Blood Cell Antigens and Bone Marrow Transplantation, pp. 79–101, Alan R. Lis Inc., New York
26 Kornecki, E., Lee, H., Merlin, F., Hershock, D., Tuszynski, G.P. and Niewiarowski, S. (1984) Thromb. Res. 34, 35–49
27 Niewiarowski, S., Kornecki, E., Hershock, D., Tuszynski, G.P., Bennett, J.S., Soria, C., Soria, J., Dunn, F., Pidard, D., Kieffer, N. and Nurden, A. (1985) J. Lab. Clin. Med., in the press
28 Bennett, J.S., Hoxie, J.A., Leitman, S.F., Vilaire, G. and Cines, D.b. (1983) Proc. Natl. Acad. Sci. USA 80, 2417–2421
29 Chung, S.Y., Hershock, D., Rucinski, B., Tuszynski, G.P. and Niewiarowski, S. (1985) Thromb. Haemostasis 54, 173, abstr.
30 Schreiber, A.D., Cines, P.D., Zmijewski, C. and Colman, R.W. (1979) Blood 53, 578–587
31 Van Leeuwen, E.F., Leeksma, O.C., Van Mourik, J.A., Engelfriet, C.P., Von dem Borne, A.E.G. (1984) Vox Sang. 47, 280–189