Biochem. J. (1983) **214**, 331–337
Printed in Great Britain

Monocytes and platelets share the glycoproteins IIb and IIIa that are absent from both cells in Glanzmann's thrombasthenia type I

G. GOGSTAD, Ø. HETLAND, N. O. SOLUM and H. PRYDZ
Research Institute for Internal Medicine, University of Oslo, Rikshospitalet, Oslo 1, Norway

(*Received 21 January 1983/Accepted 6 May 1983*)

By means of an antiserum specific to the complex of the platelet membrane glycoproteins IIb and IIIa we demonstrate here that monocytes and purified monocyte membranes share these glycoproteins with platelets. The monocyte glycoprotein IIb–IIIa complex showed complete immunological identity with the platelet counterpart and, furthermore, dissociated after EDTA treatment exactly as did the platelet complex. In Glanzmann's thrombasthenia type I, monocytes as well as platelets lack this antigen completely.

The human monocyte/macrophage system is the subject of much current interest. One of the main areas under study is the plasma membrane, its receptors, and other proteins that in many cases have been shown to mediate important external signals related to inflammation, surveillance of foreign and altered cells, coagulation and fibrinolysis, as well as to uptake of metabolites.

We describe here what we believe to be the first documented genetic defect in the human monocyte membrane. By utilizing an antiserum specific to a complex of the platelet membrane glycoproteins IIb and IIIa we show here that this is a prominent antigen in whole monocytes as well as in isolated monocyte membranes from normal subjects, and that the corresponding antigen is absent from the monocytes of a patient with Glanzmann's thrombasthenia type I.

Materials and methods

Chemicals

Chemicals were obtained as follows: Lymphoprep from Nyegaard and Co. A/S, Oslo, Norway; agarose type HSA from Litex, Glostrup, Denmark; RPMI 1640 medium from Gibco Biocult, Paisley, Renfrewshire, Scotland, U.K.; MEM-S and foetal-calf serum from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.; Hepes, sucrose and Triton X-100 from Sigma Chemical Co., St. Louis, MO, U.S.A.; dextran T-500 from Pharmacia, Uppsala, Sweden.

Abbreviations used: GP, glycoprotein; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; MEM-S, minimal essential medium for suspension cultures. The nomenclature for platelet membrane glycoproteins is adopted from Phillips & Poh Agin (1977*a*) with the exception that GP III is referred to as GP IIIa. The definition of G4 is given by Gogstad *et al.* (1982*a*).

Antibodies

Antibodies to whole human platelets were obtained as described by Hagen *et al.* (1979). Antibodies to the human platelet GP IIb–IIIa complex were obtained as described by Gogstad *et al.* (1982*b*). A monoclonal antibody to human monocytes was kindly provided by Dr. G. Gaudernack, University of Tromsø, Tromsø, Norway. This antibody has been described by Kaplan & Gaudernack (1982). Antibodies to human serum albumin were obtained from Behringwerke, Marburg, Germany, and antibodies to human fibrinogen were from Dako, Copenhagen, Denmark.

Blood

Blood was obtained from healthy fasting donors. For the isolation of platelets it was anticoagulated and processed as previously described in detail by Gogstad (1980). Monocytes were isolated essentially as described by Lyberg & Prydz (1981). Samples from individual donors were always processed separately and only pooled for the final analyses. Briefly, blood was drawn into heparin (10–20 units/ml), mixed with dextran T-500 and left for 45–60 min to allow erythrocyte sedimentation. The supernatant was layered over 15 ml of Lymphoprep and spun at 400 ***g*** for 30 min at 20°C. The mononuclear-cell layer was harvested, washed twice in MEM-S with heparin (10 units/ml) and three times in RPMI 1640 medium containing 20 mM-Hepes, pH 7.3, and 20% (v/v) foetal-calf serum,

each washing being followed by centrifugation at 200 *g* for 8–10 min to remove platelets. By this procedure more than 95% of the adherent cells were monocytes as judged by non-specific esterase (Yam *et al.*, 1971) and May–Grünwald–Giemsa staining.

Whole monocyte homogenates were prepared after incubating the cells in RPMI 1640 medium supplemented as described above for 15 h at 37°C, scraping them off with a cell scraper and washing three times in phosphate-buffered saline, pH 7.3 (containing per litre: NaCl, 8 g; KCl, 0.2 g; Na_2HPO_4, 1.15 g; KH_2PO_4, 0.2 g; disodium EDTA, 0.233 g). The final pellet was resuspended in 1 ml of barbital-buffered saline [0.15 M-NaCl/buffer (containing, per litre: sodium diethylbarbiturate, 5.875 g; NaCl, 7.335 g; 0.1 M-HCl to pH 7.3), 9:1, v/v] and homogenized in a Potter–Elvehjem Teflon/glass homogenizer.

Platelet membranes

Platelet membranes were prepared as described by Barber & Jamieson (1970).

Monocyte plasma membranes

Monocyte plasma membranes were prepared at 4°C from isolated monocytes by a modification of the procedures of Segel *et al.* (1979) and Wang *et al.* (1976). Monocytes [$(1–3) \times 10^8$ cells] were suspended in 10 ml of lysis buffer (1 mM-$NaHCO_3$/0.5 mM-$CaCl_2$, pH 7.4) and homogenized with 20 strokes in a Dounce homogenizer. The lysate was diluted to 50 ml and centrifuged at 500 *g* for 20 min. The pellet was resuspended in 10 ml of lysis buffer, homogenized and centrifuged as above. The combined supernatants were centrifuged at 13000 *g* for 20 min and the resulting pellet resuspended in 1.74 ml of lysis buffer and mixed with 5.25 ml of 60% (w/v) sucrose in the same buffer, giving a final sucrose concentration of 45%. This suspension was placed in a centrifuge tube and 5.5 ml of 35% sucrose in lysis buffer was carefully layered above. The resulting discontinuous gradient was centrifuged at 95000 *g* for 120 min in a SW 41 Ti rotor in a Beckman LC-70 ultracentrifuge. The plasma-membrane band was recovered from the gradient just above the 35/45%-sucrose boundary, diluted 5-fold with 0.05 M-Tris/HCl buffer, pH 7.8, containing 0.12 M-NaCl, and centrifuged at 50000 *g* for 1 h. This pellet represented the final membrane fraction.

Purity of the plasma membranes

Purity was monitored by the use of a monoclonal antibody to human monocytes, by transmission electron microscopy and by the activity of 5′-nucleotidase (EC 3.1.3.5). 5′-Nucleotidase was assayed as described by Johnsen *et al.* (1974). Monocyte cultures were exposed to the ^{125}I-labelled monoclonal antibody (4 ng/ml) for 2 h at 4°C. The cells were harvested and washed with RPMI 1640 medium containing 0.5% heat-aggregated immunoglobulin (Lyberg & Prydz, 1982). The plasma membranes were isolated as described above and the yield and specific radioactivity determined and compared with the labelling of the whole cells.

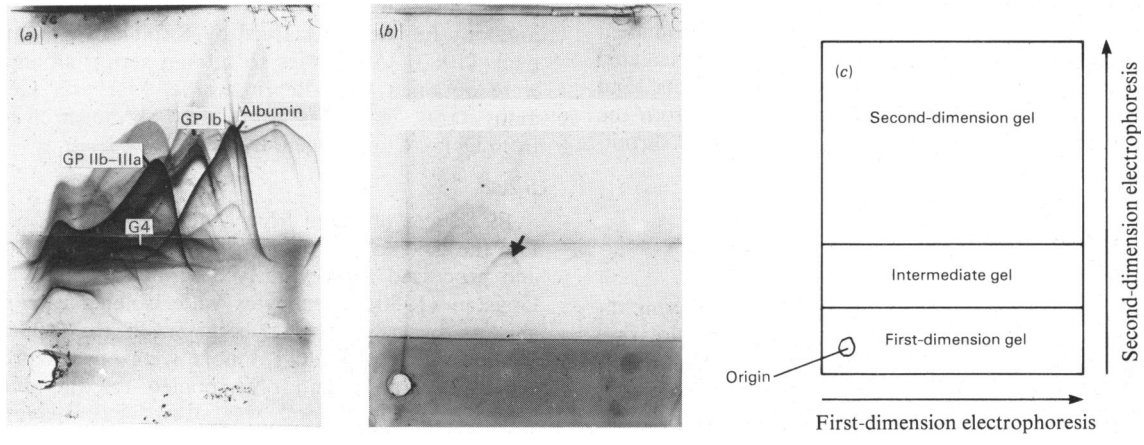

Fig. 1. *Crossed immunoelectrophoresis of (a) platelets (150 μg of protein) and (b) monocytes (750 μg of protein) against antibodies to whole platelets*

The cells were solubilized in 38 mM-Tris/0.1 M-glycine buffer, pH 8.7, containing 1% Triton X-100 and applied to 1% agarose containing the same buffer and 0.5% Triton X-100. The first-dimension electrophoresis was performed at 10 V/cm for 1 h, and electrophoresis in the second dimension was performed at 2 V/cm overnight. An antibody-free intermediate gel was inserted between the first-dimension gel and the second-dimension gel, which contained the antibodies (600 $\mu g/cm^2$). The arrow in (*b*) indicates the GP IIb–IIIa complex. (*c*) Illustrates the principle of crossed immunoelectrophoresis.

Crossed immunoelectrophoresis

This was performed as described by Hagen *et al.* (1979). Platelets and monocytes were solubilized in 38mM-Tris/0.1M-glycine buffer, pH8.7, containing 1% Triton X-100. In a typical experiment, 150μg of platelet protein or 750μg of monocyte protein was applied to the gels. For the analyses of monocyte membranes, 25μg of protein was applied to the gels. Routinely the amount of antibodies used in the crossed immunoelectrophoresis of monocyte-derived material was one-sixth of that used for platelets.

The quantities of GP IIb–IIIa complex in monocytes and platelets were compared by projection of the relevant precipitates to a special homogeneous paper, cutting and weighing. The relative amounts of the GP IIb–IIIa antigen in the various fractions were estimated, taking into consideration the amount of protein applied to crossed immunoelectrophoresis and the concentration of antibodies used. A standard curve was constructed from crossed immunoelectrophoresis of solubilized platelets against variable amounts of GP IIb–IIIa-specific antibodies.

Results

Purity of monocyte preparations

The monocyte preparations used in the present study contained no granulocytes, regularly 1–4% lymphocytes and less than one platelet per nucleated cell. To monitor the presence of platelet antigens in

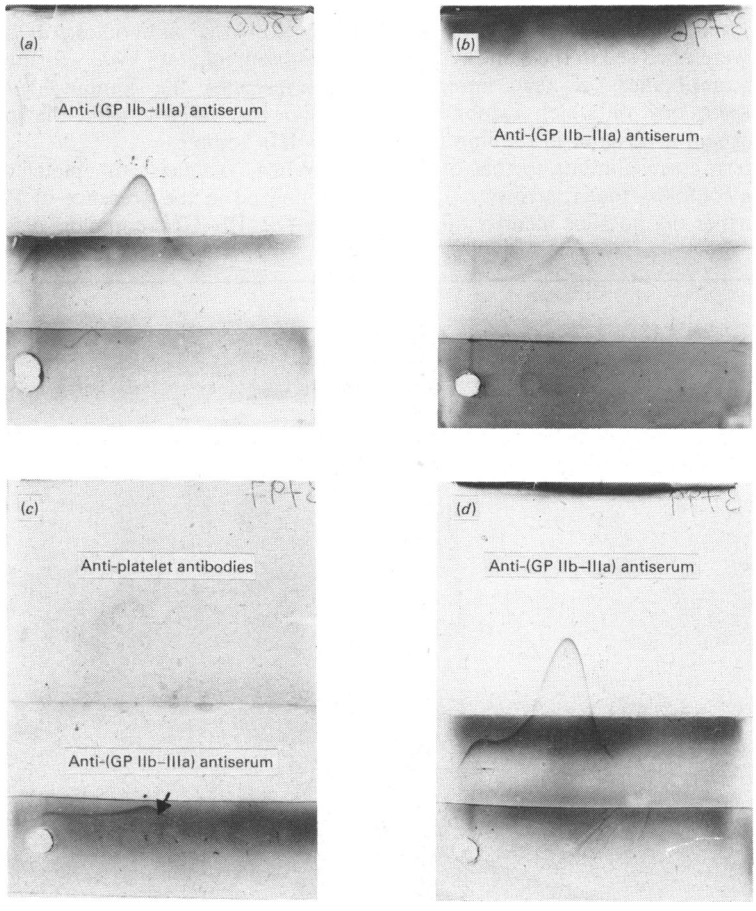

Fig. 2. *Crossed immunoelectrophoresis of platelets and monocytes*

(*a*) Solubilized platelets (150μg of protein) against an antiserum specific to the GP IIb–IIIa complex; no antibodies in intermediate gel. (*b*) Solubilized monocytes (750μg of protein) against an antiserum specific to the GP IIb–IIIa complex; no antibodies in intermediate gel. (*c*) Solubilized monocytes (750μg of protein) against an antiserum to whole platelets with an antiserum specific to the GP IIb–IIIa complex in intermediate gel; the arrow indicates the GP IIb–IIIa complex. (*d*) Solubilized platelets (150μg of protein) plus solubilized monocytes (750μg of protein) against an antiserum specific to the GP IIb–IIIa complex; no antibodies in the intermediate gel. The experimental conditions were as in Fig. 1.

the monocyte homogenates apart from the GP IIb–IIIa complex, antibodies to human albumin and fibrinogen were included in the intermediate gel in crossed-immunoelectrophoresis experiments. The results (not shown) revealed the absence of these two dominant platelet antigens (Hagen *et al.*, 1979), indicating that the platelet contamination of the monocyte preparations was negligible.

Monocytes and platelets share GP IIb–IIIa antigens

Washed platelets solubilized by Triton X-100 and subjected to crossed immunoelectrophoresis against antibodies to whole platelet proteins give rise to a complex pattern of immunoprecipitates (Fig. 1). Some of the previously identified antigens (Hagen *et al.*, 1979) are indicated in this Figure. One of the dominant immunoprecipitates contains a complex between the surface glycoproteins IIb and IIIa (Hagen *et al.*, 1980; Kunicki *et al.*, 1981). When human monocytes were analysed in the same system using anti-platelet antibodies, a few immunoprecipitates were seen, one of which exhibited a pronounced staining and a position in the immunoelectrophoretic pattern corresponding to that of the platelet GP IIb–IIIa complex (Fig. 1, arrow).

To investigate further the possible identity of this monocyte antigen with the platelet glycoprotein complex, an antiserum monospecific to the GP IIb–IIIa complex was used. In crossed immunoelectrophoresis of solubilized platelets, a single immunoprecipitate was formed (Fig. 2*a*). Similarly, a single immunoprecipitate was formed with solubilized monocytes when the same antiserum was used (Fig. 2*b*). When monocytes were subjected to crossed immunoelectrophoresis against antibodies to frozen and thawed platelets with the inclusion of the specific GP IIb–IIIa antibodies in the intermediate gel, the actual immunoprecipitate was found in a lower position in the second-dimension gel than was that in the control (Fig. 2*c*, control in Fig. 1*b*). Thus the monocyte antigen shared determinants with the platelet GP IIb–IIIa complex. When a mixture of solubilized platelets and monocytes was applied to crossed immunoelectrophoresis against the specific GP IIb–IIIa antibodies, a single homogeneous immunoprecipitate was formed (Fig. 2*d*). This demonstrates the immunological identity of the monocyte antigen with the platelet glycoprotein IIb–IIIa complex.

When platelets or platelet membranes were solubilized in the presence of EDTA above pH 8.0, the GP IIb–IIIa complex dissociated on electro-

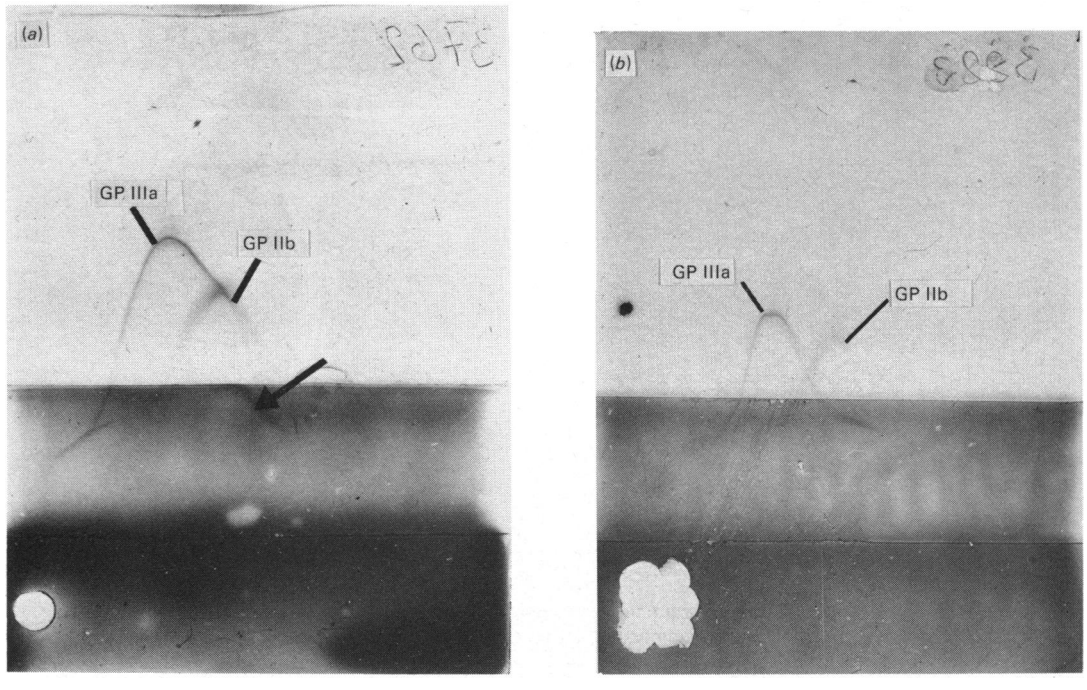

Fig. 3. *Crossed immunoelectrophoresis of (a) platelets and (b) monocytes that have both been solubilized in the presence of 5mM-EDTA*

The antiserum used is monospecific to the GP IIb–IIIa complex. The experimental conditions are as in Fig. 1. The arrow indicates residual GP IIb–IIIa complex.

phoresis (Gogstad *et al.*, 1982*b*). This is seen as a loss of the heavily stained GP IIb–IIIa precipitate with the concomitant appearance of two weakly stained immunoprecipitates in crossed immunoelectrophoresis, representing the separate GP IIb and GP IIIa respectively (Kunicki *et al.*, 1981) (Fig. 3*a*). When monocytes were solubilized in the presence of EDTA, a pattern similar to that of the separate GP IIb and GP IIIa was seen (Fig. 3*b*), showing that EDTA has the same effect on the monocyte antigen as on the platelet GP IIb–IIIa complex.

Isolated monocyte membranes

The purification process was monitored by using a ^{125}I-labelled monoclonal antibody against a monocyte membrane antigen. The yield of purified membranes was 15% on the basis of recovered radioactivity. An increase of about 19-fold in specific radioactivity (c.p.m./mg of protein) was observed when isolated membranes were compared with whole cells. It is noteworthy that the same membrane preparations gave an increase in the specific activity of 5′-nucleotidase of only about 6-fold. The recovery of this enzyme in the purified membrane preparations was, however, only 5–6% of the total activity in the initial homogenate. Electron micrographs showed large vesicular structures with the classical appearance of membranes (Fig. 4), slightly contaminated with mitochondrial structures.

When such isolated membranes were applied to crossed immunoelectrophoresis against anti-platelet antibodies, a single immunoprecipitate representing GP IIb–IIIa was formed (Figs. 5*a* and 5*b*). Thus the

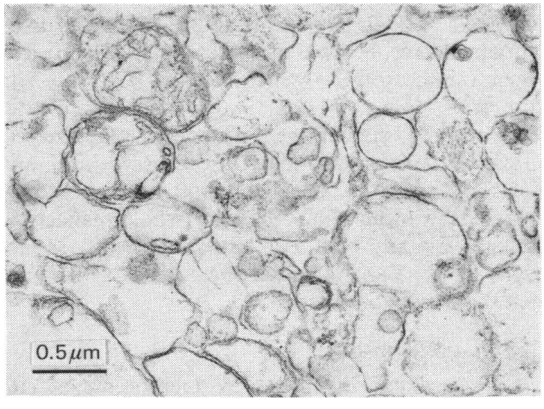

Fig. 4. *Electron micrograph of monocyte membranes fixed in 2.5% (v/v) glutaraldehyde in phosphate buffer, postfixed in 1% OsO_4 in Tyrode solution, dehydrated in alcohol and embedded in Epon*

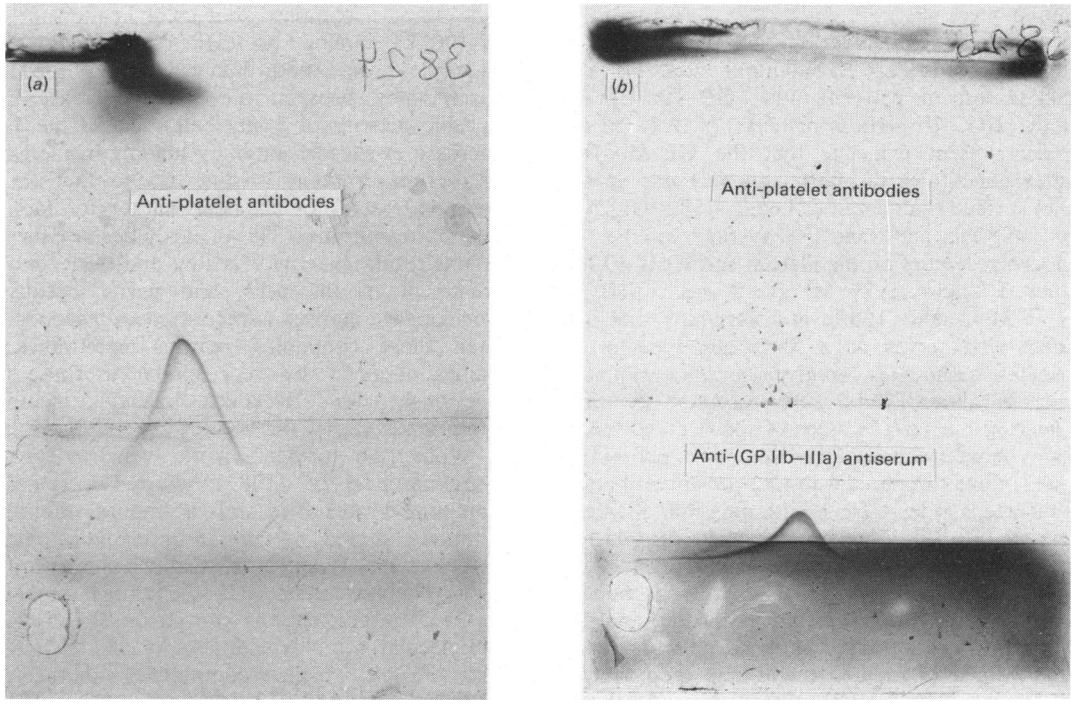

Fig. 5. *Crossed immunoelectrophoresis of solubilized monocyte membranes against antibodies to whole platelets*
(*a*) No antibodies in intermediate gel; (*b*) antiserum specific to the GP IIb–IIIa complex in the intermediate gel; 25 μg of membrane protein was applied to each gel. Other experimental conditions were as in Fig. 1.

monocyte GP IIb–IIIa complex is probably located in the plasma membrane as is its platelet counterpart.

Monocytes in Glanzmann's thrombasthenia

Platelets from patients with Glanzmann's thrombasthenia type I lack the glycoproteins IIb and IIIa (Nurden & Caen, 1974; Phillips & Poh Agin, 1977*b*). Thus a diagnosis of Glanzmann's thrombasthenia type I can be established from crossed immunoelectrophoresis of patient platelet extracts, which show total absence of the GP IIb–IIIa complex (Hagen *et al.*, 1980). When monocytes from such a patient were isolated, solubilized in Triton X-100 and applied to crossed immunoelectrophoresis, no GP IIb–IIIa antigen could be detected by using either the anti-(whole platelet) or the specific anti-(GP IIb–IIIa) antiserum. Monocytes from Glanzmann's disease type I therefore lack these glycoproteins.

Quantitative aspects

On the basis of peak-area measurements and standard curves established with extracts of normal platelets and various antibody dilutions, it is possible to estimate roughly the relative amounts of GP IIb–IIIa in monocytes and platelets. Roughly, platelets contained about 120 times more GP IIb–IIIa antigen per mg of total cell protein than did the monocytes.

If one assumes a similar average protein content per unit volume in platelets and monocytes, this ratio translates into a per-cell ratio (GP IIb–IIIa in monocytes/GP IIb–IIIa in platelets) of 2:1. On the basis of present evidence that the GP IIb–IIIa complex constitutes the fibrinogen receptor on the platelet surface (Nachman & Leung, 1982; Gogstad *et al.*, 1982*c*), and that the average number of fibrinogen receptors on the platelet surface is 40000 (Bennett & Vilaire, 1979; Marguerie *et al.*, 1980*a*,*b*; Plow & Marguerie, 1980), and assuming that this complex also serves as a fibrinogen receptor in monocytes, monocytes can give rise to an estimated number of about 80000 such receptors per cell. Considering the surface areas of the two cell types, the ratio between the GP IIb–IIIa antigen per unit of platelet surface compared with that per unit of monocyte surface is at least 20:1, assuming that all of the monocyte GP IIb–IIIa complex is in the plasma membrane. [These estimations are complicated by the fact that an unknown (but small) proportion of platelet GP IIb–IIIa is located in the α-granules.] However, the comparable ratio between the amount of this antigen in isolated membrane preparations was calculated to be only 3.7:1 (platelet membranes/monocyte membranes, with respect to total protein in each preparation). The discrepancy may be due to a difference in the purity of the membrane preparations, the intracellular distribution of the GP IIb–IIIa complex (Gogstad *et al.*, 1981) (plasma membrane versus internally), the protein/phospholipid ratio of the membranes, or differential losses during the membrane preparations.

Discussion

Our results show the existence of a monocyte plasma-membrane-located antigen that is immunologically indistinguishable from the platelet GP IIb–IIIa complex. In addition, this antigen exhibited the same dissociation into two separate entities after treatment with EDTA as did the platelet GP IIb–IIIa complex (Kunicki *et al.*, 1981; Gogstad *et al.*, 1982*b*). Furthermore, the antigen was absent from the monocytes of a patient with Glanzmann's thrombasthenia type I in which the only presently known major molecular defects are the lack of platelet GP IIb and IIIa and of platelet fibrinogen. Since other major platelet antigens, like fibrinogen and albumin (Hagen *et al.*, 1979), were absent from the monocyte preparations, it is concluded that a possible contamination by platelets cannot account for the presence of the monocyte antigen studied. This was also supported by examination of the monocyte preparations under the microscope. We conclude that GP IIb and GP IIIa are present in the monocyte plasma membrane and that they are present as a complex after solubilization in Triton X-100 by analogy to their platelet counterparts (Hagen *et al.*, 1980; Kunicki *et al.*, 1981). The discrepancy between the 6-fold increase in the specific activity of 5′-nucleotidase and the 19-fold increase in specific antibody binding in the isolated membrane fraction is due to the fact that 5′-nucleotidase is not an ideal marker for monocyte plasma membranes. This is partly because the initial 5′-nucleotidase activity is low and varies with the treatment of the cells, and partly because 5′-nucleotidase activity is recovered in fractions other than those containing plasma membranes. The values obtained are very similar to those found previously for HeLa-cell plasma membranes (Johnsen *et al.*, 1974).

While the present work was in progress, Burckhardt *et al.* (1982) reported a cell-surface component identified by a mouse monoclonal antibody shared by human monocytes, platelets, megakaryocytes and also weakly expressed on the promyelocytic cell line HL-60 and the monocyte line U937. The antigen was carried on a non-covalent bimolecular complex with components of M_r 93000 and 135000, which is compatible with their being GP IIb and IIIa. Gewirtz *et al.* (1982) suggested that the leukaemia cell line K562 (established from a patient in blastic phase of chronic granulocytic leukaemia) contained similarly sized components

reacting with a rabbit antiserum to platelet glycoproteins.

GP IIb and IIIa have been demonstrated to form a non-covalent complex in the presence of bivalent cations (Kunicki *et al.*, 1981; Gogstad *et al.*, 1982*b*; Hagen *et al.*, 1982; Howard *et al.*, 1982; Jennings & Phillips, 1982). This complex appears to be the inducible receptor for fibrinogen (Nachman & Leung, 1982; Gogstad *et al.*, 1982*c*), which is present on the surface of ADP-stimulated platelets (Bennett & Vilaire, 1979; Marguerie *et al.*, 1979). Increasing experimental evidence links this receptor with platelet aggregation, since the binding of fibrinogen to the platelet surface immediately leads to interplatelet adhesiveness. It is as yet unknown whether GP IIb and IIIa are present in the membrane of native monocytes as separate entities or as a complex, and the physiological importance of this potential receptor on monocytes remains to be investigated. Its absence in Glanzmann's thrombasthenia provides a very useful tool for such studies.

This work was supported by grants from the Norwegian Research Council for Science and the Humanities and the Norwegian Council for Cardiovascular Diseases. Dr. Randi Holme kindly did the electron microscopy.

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