Membrane glycoprotein defects in congenital platelet disorders

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The mammalian platelet is a specialized anucleate cell released in large numbers into the bloodstream from its parent cell, the megakaryocyte, in the bone marrow. Its primary functional role is in the arrest of bleeding and the maintenance of haemostasis. Platelets adhere to exposed subendothelial components of the vessel wall, and this is followed by the formation of platelet aggregates or thrombi at the injured site.

We have studied the membrane structure of platelets isolated from patients with hereditary bleeding disorders characterized by defects of different aspects of platelet function. Such disorders are specifically termed 'constitutional thrombocytopathia'. An example is Glanzmann's thrombasthenia (see Hardisty, 1977). Platelets from these patients adhere normally to subendothelium (Baumgartner et al., 1977); however, no subsequent thrombus formation occurs. A characteristic common to the platelets of all thrombasthenic patients is their inability to aggregate after stimulation by all physiological aggregation-inducing agents. As discussed elsewhere (Nurden & Caen, 1979), thrombasthenic-patients' platelets appear unable to form the linking bonds necessary for the final formation of the aggregates.

Nurden & Caen (1974) studied membrane fractions isolated from thrombasthenic-patients' platelets and noted the presence of glycoprotein abnormalities. SDS*/polyacrylamide-gel electrophoresis followed by periodate-Schiff staining revealed marked decreases in the staining intensity normally observed in the then-termed glycoprotein II and III regions of the gel. Molecular differences in the surface composition of thrombasthenic-patients' platelets were confirmed by Phillips et al. (1975) using the lactoperoxidase-catalysed procedure for

* Abbreviation: SDS, sodium dodecyl sulphate.

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PIA was deleted from platelets in thrombasthenia and then proved (Kunicki & Aster, 1979) that the PIA antigenic marker was associated with glycoprotein IIIa on normal human platelets. Hagen et al. (1980) studied Triton-X-100-solubilized normal and thrombasthenic-patients' platelets by crossed immunoelectrophoresis using a polyclonal rabbit antiserum against normal human platelets. A prominent 125I-labelled immunoprecipitate, termed band 16, was significantly diminished in intensity in the patterns obtained for 125I-labelled platelets from thrombasthenic patients. Platelets from nine thrombasthenic patients have now been analysed by crossed immunoelectrophoresis in our laboratory; the results obtained for three patients as reported by Hagen et al. (1980) and those studied subsequently all showed either the absence of band 16 (six patients) or its presence in severely diminished quantities (15%). All other major 125I-labelled precipitates were in their normal positions on the agarose plate. Band 16 was excised from the agarose gel after its precipitation from normal platelet samples by the rabbit anti-human platelet antibody preparation (Hagen et al., 1980) and analysed by SDS/polyacrylamide gel electrophoresis. Glycoproteins IIb and IIIa were identified as the platelet antigens contained within the precipitate.

Like Glanzmann's thrombasthenia, the Bernard-Soulier syndrome is a platelet disorder with an autosomal recessive inheritance. When anticoagulated whole blood from patients and control subjects is circulated over arterial subendothelium under simulated blood-flow conditions, a defective adherence of the platelets of Bernard-Soulier-syndrome patients to the subendothelium is observed (Weiss et al., 1974; Caen et al., 1976). Quantitative morphological studies by Baumgartner et al. (1977) to speculate that the defect occurred during the initial platelet attachment to the vessel wall. Platelet aggregation as induced by ADP is normal in the Bernard-Soulier syndrome, thus clearly distinguishing the platelet-function defect from that observed in thrombasthenia. Furthermore, a diminished binding of 125I-labelled thrombin to Bernard-Soulier-syndrome platelets has been noted and correlated with a decreased aggregation response specifically observed with thrombin (Jamieson & Okumura, 1978), Hardisty (1977) should be consulted for further details of platelet-function studies in the Bernard-Soulier syndrome.

A glycoprotein abnormality in Bernard-Soulier-syndrome platelets was first described by Nurden & Caen (1975), who observed a much decreased carbohydrate staining of the then-termed glycoprotein I after the analysis of isolated platelet membranes by SDS/polyacrylamide-gel electrophoresis. A greater resolution of the platelet surface components has been achieved in subsequent studies, and the affected glycoprotein is now specifically termed glycoprotein Ib (see Nurden & Caen, 1979). Membrane glycoprotein Ib is normally the predominant periodate–Schiff-staining glycoprotein observed after the analysis of whole platelet suspensions by SDS/polyacrylamide-gel electrophoresis. It is the membrane precursor of glycoprotein Ib/IX/V complex (Solum et al., 1980), a high-molecular-weight acidic glycoprotein located in the soluble phase after platelet homogenization in the presence of Ca2+. Glycoprotein Ib has been isolated and characterized (Okumura et al., 1976), having an apparent mol.wt. of 148000 and consisting of 60% (by wt.) carbohydrate. It is rich in sialic acid, and is the most heavily labelled surface component when washed normal platelet suspensions are treated sequentially with neuraminidase, galactose oxidase and NaB3H4 (Phillips & Poh Agin, 1977a). Bernard-Soulier-syndrome platelets are extremely rare, and the defect is accompanied by a low circulating platelet count. A specific defect in the glycoprotein I region of periodate–Schiff-stained gels after SDS/polyacrylamide gel electrophoresis has been confirmed in several studies (Caen et al., 1976; Hagen & Solum, 1978; Kunicki et al., 1978; Jamieson et al., 1979).

Fig. 1 illustrates the analysis of Bernard-Soulier-
syndrome platelets whose surface proteins were labelled with $^{125}$I by the lactoperoxidase-catalysed procedure. A specific absence of radioactivity in the glycoprotein-IIb region of the gel may be observed; the other major membrane glycoproteins are normally labelled. Confirmation of the presence of glycoprotein IIb was provided by Kunicki et al. (1978), who showed that Bernard-Soulier syndrome platelets reacted normally with antialloantigen P1$^{111}$ antibody. Kunicki et al. (1978) observed, however, that Bernard-Soulier-syndrome platelets lacked another platelet-specific antigen marker, the receptor for quinine- and quinidine-dependent antibodies. Recently we have shown that this receptor activity was associated with a glycoprotein-IIb-enriched fraction purified by chromatography of Triton-X-100-solubilized normal platelet membranes on wheat-germ lectin affinity columns (A. T. Nurden, D. Dupuis, D. Picard & T. J. Kunicki, unpublished work). That the Bernard-Soulier-syndrome platelets actually lacked glycoprotein IIb was further suggested by the studies of Hagen et al. (1980), who showed that a specific immunoprecipitate (band 13) was missing when Bernard-Soulier-syndrome platelets were studied by crossed immunoelectrophoresis. Whereas band 13 was precipitated when normal human platelets were analysed against an antiserum prepared against isolated glycoplatin, no precipitate was observed with Bernard-Soulier-syndrome platelets.

In conclusion, we have described evidence to show that two hereditary platelet disorders characterized by defects involving the two major pathways of platelet function in haemostasis, i.e. adhesion and aggregation, are associated with quantitatively different membrane glycoprotein deficiencies. These appear to be platelet-specific abnormalities; analysis of membranes isolated from erythrocyes of patients of both disorders revealed no glycoprotein defects. Elucidation of the mechanisms of platelet adhesion and aggregation is particularly important in view of the possible platelet involvement in the pathological processes leading to atherosclerosis and thrombosis. Studies such as we have described may offer clues to those membrane components which mediate the surface contact interactions vital to the specific functional activities of this remarkable cell.

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Virally mediated changes in cellular permeability

Viruses probably account for some 60% of infectious diseases in man (Thigpen, 1971). In order to elicit a pathological change, viruses have to enter susceptible cells and to multiply within them; usually this is followed by release and infection of cells at other sites in the body. Since entry and release obviously involve the cell surface, viral infections constitute an important class of membrane diseases.

To date, investigations of the cell surface have focused on two aspects: the characterization of binding sites that promote viral entry into cells, and the demonstration that, after multiplication of viral constituents within cells, viral antigens appear at the cell surface (e.g. Poste & Nicolson, 1977). The latter effect underlies not only the action of the immune system in destroying virally infected cells, but may also play a role in the progression towards establishing an auto-immunity towards a particular component of the surface membrane; the outcome of that may be destruction of any cell bearing the component, long after virus has been eliminated from the body. The more immediate basis of viral disease has generally been ascribed to an inflammatory response resulting from the presence of damaged cells, a response that may be augmented by immune recognition of virally damaged cells. The purpose of the present article is to suggest that the surface membrane is involved in viral disease in a third way, and that this contributes not only to the cytopathic effect of viruses, but may underlie the physiological consequences of certain viral infections also. The property of the surface membrane to be discussed is that of controlling cellular permeability.

That the surface membrane becomes damaged in virally infected cells has been recognized for a long time. Infected cells leak protein (Gilbert, 1963; Blackman & Bubel, 1969; Cassetts, 1973; Katzman & Wilson, 1974; Norkin, 1977; Schümperli et al., 1978) and becomes permeable to Trypan Blue; membrane rupture underlies the final stage of cellular disintegration. Such changes are clearly a consequence of intracellular events. More subtle permeability changes, on the other hand, may be the very cause of cytopathic damage. Thus it has been suggested that virally infected cells become permeable to Na$^+$, and that a net entry of Na$^+$ occurs: this is said to shift the balance between host-protein synthesis and viral-protein synthesis in favour of the latter process, and thus to amplify viral multiplication (Carrasco & Smith, 1976; Carrasco, 1978). Moreover, if virally infected cells were to become permeable to ions and low-molecular-weight compounds in general, such that essential constituents such as nucleotides (including cyclic ones) and other phosphorylated intermediates leak out, and potentially toxic ions such as Ca$^{2+}$ (Kaiser & Edelman, 1977; Robbins & Cotran, 1979; Schanne et al., 1979) leak in, this would contribute significantly to the loss of cellular viability. Experiments carried out in our laboratory have shown that such changes do indeed occur.

Initially, we studied paramyxoviruses (e.g. Sendai virus) that are known (Poste, 1972) to enter cells by fusion between viral envelope and surface membrane. During viral entry, phosphorylated compounds such as phosphocholine (Pasternak & Micklem, 1973), sugar phosphates (Poste & Pasternak, 1978)