Platelet Structure and Function
Role of Prostaglandins

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ABSTRACT

A long way has been travelled since platelets were likened to sponges in 1961. At that time research on thrombotic mechanisms was mainly concentrated on blood coagulation. Since then, a shift of emphasis toward the study of platelets has dramatically evolved. How to prevent platelets from becoming sticky at sites of injury has been the main concern for platelet researchers over the past decade. Following adherence of platelets to a damaged vessel wall, prostaglandin synthesis is triggered leading to the formation of thromboxane A_2, the most potent platelet activating agent so far discovered. By an "autocatalytic" process, thromboxane A_2 together with the released ADP are responsible for the growth of the platelet thrombus. Among the substances released by the α-granules is the mitogenic factor, which, by stimulating the proliferation of smooth muscle cells from the media to intimal layers in arteries, is instrumental in the generation of atherosclerotic plaques.

The narrowing of the vessel wall lumen can be further aggravated by the formation of a thrombus over the plaque, thereby occluding the vessel, and leading to cardiovascular diseases or stroke depending on the location of the lesion. An all-out effort to find a means for preventing platelet stickiness is currently under way. The recent discovery of prostacyclin has been the cornerstone for most of the research carried out so far in this field. The presently available antiplatelet drugs should be used with caution. Indeed, whereas a dramatic thrombosis may occur with full platelet activation, a catastrophic hemorrhage may follow the "neutralization" of platelets. Eskimos who are fed with eicosapentaenoic acid, the precursor of a potent antiplatelet agent, may indeed be immune against thrombotic disorders; however, they have an increased tendency to bleed.

Introduction

As early as 1820, Thackrah recognized the importance of vascular integrity in hemostasis. In 1842, Donné provided the first description of platelets; 40 years later Bizzozero identified them as a separate entity and Wright recognized their origin from megakaryocytes. Although by 1888, Ebert and Schimmelbusch had described the main function of platelet
aggregation in the event of vascular damage, it was not until 70 years later that platelets were recognized to be more important than the better known blood coagulation mechanisms in initiating thrombosis and atherosclerosis.

Apart from the well-known platelet functions crucial for a normal hemostasis, such as adhesion to damaged blood vessels and aggregation at the site of injury, platelets have locomotion features characterized by random migration and response to chemotactic agents such as collagen or cyclic-adenosine monophosphate (cAMP).

These features may contribute to the role of platelets in wound healing and inflammation. They are also scavengers; the platelet membrane is endowed with both pinocytosis and phagocytosis properties, and indeed platelets play an important role in particle clearance from the bloodstream. Since more than 50 percent of the platelet proteins are contractile in nature, all these various functions may be related to contractile phenomena.

The highlights of recent research on platelet function will be briefly summarized and references will be limited to the most relevant papers. For an accurate account of the vast literature published in the last decade on various aspects of platelet function, the reader is referred to some recent reviews.

**Platelet Structure**

Sections of platelets prepared for transmission electron microscopy appear either ellipsoid or circular, depending as to whether the plane of section is transversal (1 in figure 1) or equatorial (2 in figure 1). The plasma membrane communicates with the interior of the cell by a complex canalicular network, the surface-connected canalicular system (SCCS) which provides the route for egress of secreted substances during the "release" reaction. The plasma membrane contains several specific peripheral and integral proteins, among which are glycoprotein receptors that react with appropriate agonists, initiating the series of transformations characteristic of the activated platelet. Internal membrane structures are present in platelets, the most prominent being the dense tubular vesicles (or DTS, dense tubular system) which has a Ca^{2+}-reservoir role and is associated with the SCCS. The disc shape of the platelets appears to be mainly owing to the circumferential band of microtubules located at the rim of the flat disc. A possible role of platelet microfilaments, mainly composed of contractile proteins, for the maintenance of the disc shape cannot be ruled out. The platelet cytoplasm contains mitochondria, glycogen and several types of storage organelles among which are dense granules and α-granules. Active glycolytic and Krebs cycles, as well as oxidative phosphorylation, insure a
steady-state level of ATP which maintains a high-energy state necessary for the functional integrity of platelets.

Platelet activating agents such as adenosine diphosphate (ADP), collagen, thrombin and immune complexes produce a series of morphological transformations. There is a collapse of the marginal band of microtubules associated with an active interaction of de novo polymerized actin and myosin filaments, which leads to a disc-sphere transformation accompanied by pseudopod formation (figure 2).

**Platelet Function**

The processes involved in in vivo platelet activation, usually in the following sequence, include: platelet adhesion to a damaged vessel wall, shape change involving pseudopod formation and disc-sphere transformation, release reaction, platelet aggregation and, ultimately, clot retraction if fibrin is present. The role of contractile filaments and microtubules will be evaluated in each of these processes.

**Platelet Adhesion to an Injured Vessel Wall**

At least one platelet surface receptor, glycoprotein I, and a plasmatic factor, the von Willebrand factor, seem to be involved in this process. As yet, there is no evidence implicating a contractile activity in this event, since most platelets are discoid during the initial stages of adhesion.

**FIGURE 1.** Model of a non-activated platelet. Transversal-elliptical (1) or circular-equatorial cross-sections (2) show the microtubule bundle (MT) at the rim of the flat disc, the dense tubular system vesicles (DTS) associated with the surface-connected canalicular system (SCCS), contractile filaments (CF), glycogen particles (G), mitochondria (M), dense granules (DG) and α-granules (α-G).

**FIGURE 2.** Platelet activation. (a) Intact discoid platelet (equatorial cross-section), same symbols as in figure 1; SO, storage organelles (dense and α-granules). (b) Shape change involving sphering of the platelet and extension of pseudopodia, resulting from active actin-myosin interaction. The marginal bundle of microtubule collapses; microtubular structures and actin filaments present in pseudopodia. At this stage, the process is reversible, and the discoid shape can be recovered. (c) Release reaction resulting from fusion of storage organelles to the SCCS; larger scale drawn. At this stage, the platelet shape is irreversible. For the sake of clarity, only few structures are shown in this drawing.
to subendothelial surfaces. Progress has been made in this field in the past few years thanks to the technique developed by Baumgartner. The method involves denuding the rabbit aorta of its endothelial layer by means of a "balloon" catheter, then inverting a section of the aorta on a rod which is inserted into a perfusion chamber. The aortic segment is perfused with either native or citrated whole blood at various shear rates, and the extent of platelet adhesion and thrombus formation are quantitated by a morphometric procedure. When normal blood is perfused at physiological shear rates, a layer of platelets soon covers the subendothelium and, in many areas, aggregates of platelets are observed. Under pathologic conditions, abnormalities of both adhesion and aggregation may be present.

**Shape Change**

The protrusion of pseudopodia and the disc-sphere transformation seem to be the result of an actin-myosin interaction. In a model recently proposed by us, actin filaments anchor to the plasma membrane, and, during interaction with myosin, exert a tangential force, pulling from the cell surface towards the platelet center. The increased cytoplasmic pressure produced may exert a tension in rigid areas of the membrane. Zones of decreased resistance would foster the development of a fluid area in the surface which could flow outward to form a pseudopod. The "spherical" shape of the contracting actomysin mesh would compress the microtubule loop towards the center of the cell, and would break the tubules in several sites, leaving only remnants (figure 2). The free end of the compressed coil would be elongated and could enter a pseudopod. As pressure at the rim of the flat disc increases simultaneously as a consequence of a more efficient actin-myosin interaction, the cell would reduce its size in the equatorial plane and deform into the only possible shape, that of a sphere.

Ca\(^{2+}\) plays a central role in the shape change processes. The SCCS, originating from invaginations of the plasma membrane, and the Ca\(^{2+}\)-loaded membrane vesicles of the DTS are the respective counterparts of the skeletal muscle T-system membrane and the closed vesicles of the sarcoplasmic reticulum. The platelet stimulation leads to a polymerization of monomeric actin molecules into typical actin filaments and to an intracellular release of Ca\(^{2+}\) from the DTS. Ca\(^{2+}\) would trigger an actin-myosin interaction. A few years ago it was shown by us that platelet actin-myosin interaction is Ca\(^{2+}\)-sensitive.

The study of Ca\(^{2+}\)-requirement in cellular systems has greatly progressed because of the introduction of ionophores, which selectively transport Ca\(^{2+}\) across cell membranes or redistribute it from intracellular storage sites. The effects of ionophores can indeed mimic the actions of physiological platelet stimulating agents.

**Release Reaction**

When platelets are activated, they undergo shape change and "release" various substances in the surrounding medium. There is to-date no strong evidence that contractile events *per se* mediate the release reaction. An actin-myosin interaction might well bring the storage granules and the SCCS membranes into close proximity. This "fusion" would be followed by the extrusion of the granule content via the SCCS. There are at least three types of granules: (1) the dense granules, so-called owing to their density to the electron beam, believed to be the sites of storage of ADP, serotonin, Ca\(^{2+}\) and pyrophosphate; (2) the \(\alpha\)-granules, storage sites for fibrinogen, the heparin-neutralizing platelet factor 4, and mitogenic factor(s); and (3) the lysosomal granules. Some agents, such as ADP, are only able to cause secretion from the dense granules. Others, such as thrombin and collagen, cause secretion from both the dense granules and the \(\alpha\)-granules. The release of \(\alpha\)-granule components requires lower concentration.
of agonists. Of interest is the release of a mitogenic factor from α-granules which has been postulated to be the agent responsible for the proliferation of smooth muscle cells from the media layers of arteries into the intimal layers, forming fibrous plaques characteristic of atherosclerosis.

Two pools of adenine nucleotides are present in platelets: a cytoplasmic metabolic or energy pool with an ATP:ADP ratio of 7.7 and a storage pool in the dense granules with an ATP:ADP ratio of 0.7. All the processes occurring in platelet activation are energy-consuming with the release reaction requiring most of the energy available and converting ATP, through a series of intermediates, into hypoxanthine.

**PLATELET AGGREGATION**

Platelet aggregation refers to the property of activated platelets to become sticky and attach to one another. It requires external calcium, unlike the other processes described. Stickiness probably represents a change in the external platelet proteins or lipids, causing a change in the physical properties of the membrane. The aggregation process in platelet-rich plasma is easily evaluated photometrically at 37°C and with constant stirring. Changes in light transmission are recorded continuously. When ADP is used as an aggregating agent, a small decrease in light transmission, owing to platelet shape change, occurs immediately after the addition of ADP; this is followed by a gradual increase of light transmission as the plasma becomes less turbid owing to the formation of solitary aggregates of platelets. At intermediate concentrations of ADP (2 μ), there is biphasic aggregation with the second phase usually associated with the release reaction (figure 3).

**CLOT RETRACTION**

In the presence of polymerizing fibrin, in vitro, the activation of platelets by thrombin leads to the clot retraction phenomenon. This is a Ca²⁺-sensitive process requiring platelets to adhere to one another. It was possible to quantitate clot retraction by measuring the isometric tension of platelet-fibrin clot. Changes in tension were Ca²⁺-dependent and several cycles of contraction-relaxation were obtained by varying Ca²⁺ concentration above or below 10⁻⁶ M. Recently, a zipper model for clot retraction was proposed by us in which the long motile spikes of pseudopodia, by increasing the effective volume of the platelet, could reach out for pseudopodia of neighboring platelets. Polymerizing fibrin probably sticks to the migrating platelets. The creeping platelets pull along the adherent fibrin fibers and may compress the mesh of other pre-formed fibrin fibers. The physiological role of clot retraction is still not understood. Although the hemostatic platelet-fibrin plug cannot be identified with a clot contracting in vitro, the interaction of platelets with fibrin in vivo is well established, and may be crucial for the consolidation of the hemostatic plug.

**Platelet Procoagulant Activities**

Apart from the several coagulant factors that platelet contain (fibrinogen, factors V and XIII), platelets provide a phospholipid entity, factor 3, which is usually made available on the membrane following activation and is crucial for the coagulation cascade.
Prostaglandin Synthesis in Platelets and Endothelial Cells

Whereas prostaglandins are not present in unstimulated platelets, their synthesis and release does occur when platelets are traumatized or exposed to thrombin, collagen, epinephrine or ADP. These agents either directly activate phospholipases A$_2$ and C or/and lead to the intracellular release of membranal Ca$^{2+}$, which is required for the stimulation of these phospholipases. The activated phospholipases C and A$_2$ catalyze the release of arachidonic acid from phosphatidylinositol and phosphatidylycholine, respectively (figure 4). A cyclooxygenase then catalyzes the formation of cyclic endoperoxides PGG$_2$ and PGH$_2$ from arachidonic acid.

In platelets, PGH$_2$ serves as a substrate for thromboxane synthetase which catalyzes the formation of thromboxane A$_2$ (TXA$_2$), the most potent platelet activating agent known. On the other hand, in endothelial cells, PGH$_2$ serves as a substrate for prostacyclin synthetase which catalyzes the formation of prostacyclin (or PGI$_2$), the most potent inhibitor of platelet activation yet discovered. The cyclooxygenase from platelets seems to be more sensitive to acetylation by aspirin than its counterpart from endothelial cells. In fact, the endothelial cell cyclooxygenase is as sensitive to aspirin as the enzyme in platelets. Whereas platelets are unable to synthesize a new cyclooxygenase, the turnover of this enzyme in endothelial cells is very high and therefore its acetylated form is rapidly replaced. Experimental studies in rabbits have demonstrated that low doses of aspirin (10 mg per kg i.v.) inhibited only the platelet cyclooxygenase, whereas doses of 150 mg per kg i.v., also inhibited the formation of PGI$_2$. The rapid resynthesis of cyclooxygenase in endothelial cells lessens the chances that aspirin used in clinical doses promotes thrombosis by inhibiting the enzyme activity.

Dipyridamole, an inhibitor of phosphodiesterase activity, potentiates the effect of PGI$_2$ by slowing the breakdown of cAMP. Since PGI$_2$ is rapidly degraded to the inactive 6-oxo-PGF$_1\alpha$ in vitro, the inhibitory effects of dipyridamole are not demonstrated by in vitro systems. Another drug which impairs platelet function by inhibiting cyclooxygenase is sulfinpyrazone. The results of clinical
trials using the various platelet drugs have so-far produced variable results and are still inconclusive. The therapeutic use of PGI₂ may provide the long-awaited panacea.²⁶

A dietary approach has been recently proposed, based on "loading" the organism with eicosapentaenoic acid, another precursor of prostaglandin synthesis. Unlike eicosotetraenoic acid (arachidonic acid, four double bonds), which is a precursor for both TXA₂ and PGI₂, the fatty acid eicosapentaenoic acid (with five double bonds) is a precursor for the non-aggregating TXA₃ in platelets³⁰ and for the potent antiaggregating agent PGI₃ in endothelial cells (figure 5). And indeed, a very low incidence of cardiovascular diseases is found in Eskimos whose diet is rich in eicosapentaenoic acid and low in arachidonate.¹²

Abnormalities of Platelet Function

The hemostatic disorders associated with qualitative platelet anomalies shall be mentioned very briefly. Von Willebrand disease is characterized by defective platelet adhesion to subendothelial surfaces which is due to a lack of a plasmatic factor (von Willebrand factor) rather than to a platelet defect per se. Platelets from the Bernard Soulier (giant-platelet) syndrome are also characterized by defective adhesion to the subendothelium; however, the deficiency seems to be due to two deficient membrane glycoproteins.²⁷ Thrombasthenia is mainly characterized by an impairment of platelet aggregation with normal adhesion to subendothelium, prostaglandin synthesis, shape change and release. The defect in thrombasthenic platelets may be associated with a decrease in two membrane glycoproteins. Storage-pool disease is characterized by a diminished number of dense granules (dense bodies) leading to the deficient release of the dense granule contents such as ADP and serotonin; the platelet ATP:ADP ratio is consequently greatly increased. α-Granules may also be deficient. Isolated defects in platelet factor—3 availability are rare; these are usually associated with defects in platelet aggregation.

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References


