Analytical Review

Multiple Differentiation in Megakaryocytes and Platelets

By Jean-Michel Paulus

N speaking of megakaryocytes Rhodin has stated: "Rarely does one find any single cell type that displays the great assortment of cell organelles and cell inclusions which the study of many cell types can bring together. In this regard the megakaryocyte is a unique case." This statement can actually be applied to the thrombocytic series as a whole and is true not only at the submicroscopic level, but also at all levels of cellular organization. The properties of megakaryocytes and platelets might be considered to result mainly from a summation of the physiological activities of five cellular types: (1) a polyploid cell, (2) a glandular cell, (3) an enucleate element, (4) an endocytic cell, and (5) a muscular cell.

The Megakaryocyte as a Polyploid Cell

The differentiation of a blast cell into a mature cell and the act of mitosis are closely related phenomena. Whereas it is in the few hours following a division that the intestinal crypt cell makes the crucial decision to reproduce itself exactly or to specialize,2 the megakaryoblast seems to make this choice in the course of mitosis itself. Kinosita has found that in the case of the megakaryoblast a chromosome duplication without definitive cytodieresis indicates that the cell is engaged in differentiation.3 This process is characterized by an inhibition of purely reproductive mitosis, and by a succession of differentiation mitoses, which can geometrically increase the ploidy up to the 32 N value4 or 64 N value5,6.

Certain other phenomena may also qualitatively demonstrate that a given precursor has started to differentiate into a thrombopoietic cell. Some early changes have been noted: morphologic characteristics in phase contrast5,7 and electron microscopy,8 the presence of acetylcholinesterase (for some animal species),9 or the presence of some platelet antigens,10 but the first recognizable megakaryocyte precursors seem to be already polyploid.11,12 None of these criteria has the quantitative accuracy of the determination of the ploidy. Moreover, the latter could have a special significance. It is known that polyploidy, common in plants, is normally associated both with differentiation and inhibition of reproductive mitosis.13,14

Polyploidy in megakaryocytes may have many additional consequences. Some of these consequences are morphologic: for example, the large size of
both nucleus and cell can be explained by polyploidy. More important, however, may be the metabolic and genetic perturbations associated with a high chromosome number. As pointed out by Fankhauser, the associated increase in cell size could influence the relationships between cytoplasm and nucleus and between cytoplasm and environment. Similarly, the increased amount of carotenoid pigment per gene, in the tetraploid yellow corn as well as the abnormalities of development in triploid humans show the possibility of a cumulative effect of excess genes. Finally, polyploid cells are more susceptible to viral accumulation, a feature also demonstrated by megakaryocytes.

However, the opposite possibility has also to be considered. It is conceivable that cytoplasmic differentiation could be the limiting factor in megakaryocyte polyploidisation. In muscle, DNA synthesis and contractile proteins synthesis are mutually exclusive events. In view of the contractile behavior of platelets, it is quite possible that, as in myogenesis, the appearance of some specialized protein (as thrombosthenin) stops the process of genome multiplication. In fact, not more than a small part of the cytoplasmic maturation of megakaryocytes is accompanied by DNA synthesis. Various degrees of polyploidy could then coincide with any degree of cytoplasmic maturation, and genome number would only regulate cytoplasmic volume and the number of platelets produced per cell.

THE MEGAKARYOCYTE AS A GLANDULAR CELL

The well-described process of partition and fragmentation of the megakaryocytic cytoplasm has no other equivalent in the mammalian organism. This true apocrine secretion, as called by Porter and Bonneville, results in the birth of the fully autonomous, metabolically active platelets. It can be clearly distinguished from the five types of glandular secretion described by Kurosuni, in which are extruded only secretory products lacking cellular organization.

Cells which synthesize proteins have been divided into "retaining" and "secreting" cells. Ultrastructurally, the megakaryocyte belongs to the second category. Indeed, in the process of thrombopoiesis, the endoplasmic reticulum plays a major role. Whereas a part of the membranous cytoplasmic system is unstained by phosphotungstic acid and seems not to be involved in the demarcation of platelet territories, other components of this system which are stained by phosphotungstic acid intervene in thrombopoiesis. They also may be considered endoplasmic reticulum. The coalescence of some of the phosphotungstic acid-stained vesicles gives rise to a network of "demarcation membranes" and prepares the liberation of the enclosed platelet territories. This specialized part of the endoplasmic reticulum thus permits the segregation and the elimination of the secretory product. In the pancreas merocrine secretion, whereas the nature and destiny of the secretory product are quite different, the endoplasmic reticulum has a similar function.

Reactions of the thrombocytic series to platelet depletion in the blood may be multiple. In dogs, the first reaction is a small but precocious rise in cir-
MULTIPLE DIFFERENTIATION IN MEGAKARYOCYTES AND PLATELETS

Circulating platelets, which appears about 8 hours after exsanguinotransfusion. In rats, a decrease in cytoplasmic mass and a loss of cytoplasmic demarcation membranes has been observed immediately after bleeding. This suggests that cytoplasmic production can be stimulated by bleeding and that platelet liberation might be a much more active process than was previously believed. In this regard, the following may be relevant: An ATPase enzyme has been found in the platelet membrane. Such an activity is present in many other cell membranes, resembling the nucleoside triphosphatase activity of actomyosin. It is also present in cell organelles associated with contraction—for instance, the mitotic spindle. Furthermore, a contractile, actomyosin-like protein has been isolated from platelets. The platelet shedding could thus be a contractile phenomenon resulting in the separation of the partitioned platelet territories. Some in vivo observations of thrombopoiesis are in agreement with this hypothesis.

The final destiny of the megakaryocytes has been questioned. Some authors have observed that the nucleus of the megakaryocyte becomes pycnotic as thrombopoiesis occurs, a fact which could suggest the death of these cells at this moment. Others consider the liberation of platelets as a cyclic phenomenon not necessarily fatal to the megakaryocytes. The possibility of obtaining 100 per cent viable megakaryocytes from the rat bone marrow, after digestion by collagenase, should be taken in account in this problem.

THE PLATELET AS AN ENUCLEATE ELEMENT

The main cytologic feature of the product secreted by the megakaryocyte—the blood platelet—is the absence of a nucleus. Among all mammalian "cell-like" elements this characteristic property is shared only by the reticulocyte and the erythrocyte. These cells, however, are highly specialized for a single protein, hemoglobin, and quickly lose their mitochondria. Thus the behavior of the platelet might best be compared to that of other enucleate cells, such as surgically sectionned amebae.

How much the absence of a nucleus may explain platelet metabolism as compared with the metabolism of a nucleate cell is difficult to estimate. One might hope to determine this relationship by studying platelets during their aging process, as has been done for the enucleated amebae. However, in vivo separated platelet cohorts of different ages have not been extensively used for such studies. In vitro studies of the senescence effects are possible, but one must be careful in interpreting them, since isotopic and electron microscopic experiments have proved that the use of chelating agents (essential to their preservation) is harmful to platelets.

Bearing in mind these reservations, one may compare the main features of platelet metabolism with those of the enucleated amebae. (1) Unlike enucleated amebae, the circulating thrombocyte degrades glucose mainly by glycolysis with 45 per cent of the metabolized glucose being transformed into lactic acid. However, this metabolic sequence is highly susceptible to aging in an ACD medium. Furthermore, the glycolytic enzymes and ATP are protected by the addition of glutathione, nicotinamide, and inosine. These facts
strongly suggest an analogy between the glycolytic lesion occurring in senescent, enucleated amebae and in senescent, in vitro preserved platelets. (2) Platelets contain respiratory enzymes and their Krebs cycle is operating. Over a 21-day period their respiratory rate is unaffected by senescence. Enucleated amebae display similar behavior. (3) As for enucleated amebae, the incorporation of inorganic phosphate in energy-rich compounds is markedly time dependant, and platelet $P_{32}$ label falls to zero after a few days. Again, because of the possible influence of the EDTA plasma medium, the absence of a nuclear structure in the platelet cannot with certainty be considered responsible for the observed dysfunction. (4) As a consequence of being anucleate, the platelet has no DNA. (5) Circulating platelets contain a low level of RNA although young platelets are more basophilic than older ones, and reportedly contain ribosomes. These facts suggest an age-dependent decrease in RNA as in enucleated fragments. However, because of the progressive decrease in basophilia and in RNA synthesis during the megakaryocytic maturation, the nuclear deprivation cannot be considered as the sole factor for progressive depletion of RNA in platelets.

It may be concluded from this comparison that the biological importance of the absence of a nucleus in platelets cannot be clearly established.

**The Platelet and Megakaryocyte as Endocytic Elements**

The well-known interaction of platelets with injected particles or microorganisms is dependent on two apparently distinct properties: (1) their adhesiveness and (2) their endocytic property. In adhesion, where a surface phenomenon plays the major role, loading of the platelets with particles or microorganisms leads to their agglutination. The platelets disappear from the circulating blood and are sequestrated in the reticuloendothelial system from which they may subsequently be released. Another aspect of the adhesiveness of platelets is their ability to concentrate many of the coagulation factors in a so-called “atmosphère péri-plaquettaire.” Because of this property, they have been compared to sponges. Secondly, the possibility of true endocytosis is clearly established by electron microscopic pictures of “phagocytosis” as well as by phase contrast studies of pinocytosis. Endocytic intervention may induce in vitro the liberation of ADP and the loss of the platelets granules and in vivo a more prolonged thrombocytopenia.

That these two different activities are in fact different steps of the same chain of reactions was already shown in 1927 by Roskam, who believed that platelet adhesion was similar to the first phase of phagocytosis and that it depended on the modification of the microorganism and platelet surfaces by the medium. The fact that it is possible to selectively inhibit these different steps—adherence to the particle, phagocytosis, release of ADP with platelet agglutination—strengthens this interpretation.

Since the lysosomes are the cytoplasmic organelles involved in the process of digestion of exogenous material, their intervention in many of the aspects of platelet physiology and pathology has been suggested. Granules have many characteristics of lysosomes. They contain acid phosphatase, $\beta$-glucuronid-
dase, and cathepsin. They play a major role in platelet autolysis in EDTA and their dissolution inside the platelet during viscous metamorphosis, suggested both by electron microscopic and in vivo fluorescence microscopic studies (ref. 69; see discussion in ref. 70) bears some resemblance to an externally induced autolysis, which could be similar to streptolysin-induced degranulation and lysis of phagocytes. Moreover, granules disappear during phagocytosis of particulate matter. They originate in the Golgi complex as do granulocyte granules and other lysosomes.

In addition to lysosomes, the "intracellular digestive tract" includes vacuoles at various stages of their evolution. In platelets some originate at the membrane, while others are considered, in accordance with the ideas of Novikoff, to be formed in the Golgi complex. They too intervene in autolysis in EDTA.

THE PLATELET AS A CONTRACTILE ELEMENT

The metabolic behavior of the circulating platelet is very similar to that of muscle. The high activities of the glycolytic enzymes, contrasting with the low activity of the hexosephosphate shunt, of the Krebs cycle and of the respiratory chain suggested this comparison to Waller et al. It was further supported by Bettes-Galland and Lücher's discovery of a contractile protein in platelets, similar to actomyosin and endowed with an ATPase activity. The latter calcium- and magnesium-dependent activity differs from the sodium-dependent, potassium-stimulated pump ATPase activity which is also present in the platelet membranes and in the membranes of platelet granules. Electron microscopic evidence of submembraneous myofibrils, surrounding an equatorial cytoskeleton of microtubules, provides a morphologic basis for the contractile ability of platelets. Moreover, it can be shown that during viscous metamorphosis or the qualitatively equivalent phenomenon of clot retraction, and during muscular contraction, the same sequence of reactions occurs. According to Grette, a modification of the platelet permeability barrier could allow, as in muscle, a subsequent mobilization of Ca ions which initiates the contraction. In both systems, a protein endowed with ATPase activity then contracts. During this process the consumption of ATP is great, and the intracellular level of ADP and phosphate have a tendency to rise. In anaerobic conditions such as those in which agglutinated platelets are found, this phenomenon stimulates glycolysis.

In spite of the similarities between the two contractile systems, two important differences in behavior exist: (1) In hemostasis, platelets first adhere to the collagen fibers of the conjunctive tissue of the ruptured vessel. The platelet-collagen interaction, together with the subsequently intervening thrombin, results in the destruction of platelet organelles and release of ADP into the medium. Whereas in muscle an increase in ADP is prevented by the presence of the "high-energy phosphate buffering system" which regenerates the ATP, in platelets, this system is not so efficient. Perhaps the lack of phosphocreatin and the small concentration of creatin kinase are among the reasons why a fast accumulation of ADP can occur. This accumulation can
be prevented by addition to a platelet suspension of phosphoenolpyruvate and pyruvate kinase which play the role of the missing "high energy phosphate buffering system." ADP acts as the major thrombocyte agglutinating factor. Thus a triple biochemical specialization in platelets—i.e., their reaction with collagen and thrombin, their lack of an efficient ADP phosphorylating system, and their aggregation ability under the influence of ADP—allows the assembly of a complex contractile system. In contrast for muscle the contractile system is already completely preassembled. (2) Contrary to the muscular fiber, the contracted platelet aggregate is unable to relax. It is immobilized by the fibrin network and the platelets become altered. By coagulating fibrinogen, thrombin contributes to this immobilization. (In certain conditions, the clot will eventually be dissolved.) Thrombin, like trypsin is a powerful proteolytic enzyme which alters the permeability of platelets and releases not only ADP but also ATP. In the case of muscle at least, ATP is an essential requirement for relaxation, and trypsin is known to inhibit the relaxation action of muscle vesicles. These facts suggest that the inability of the platelet aggregate to relax may depend upon the permeability—and coagulative—action of thrombin.

CONCLUSION

The properties of the megakaryocyte and the platelet may be briefly summarized as follows: a polyploid cell secretes, by an apocrine type mechanism, an anucleate element, endowed with strong autolytic and endocytic properties, and capable of performing contractile work. The latter element, specialized in this way, is prepared to play a major role in hemostatic reactions.

The relationships between the five properties considered for these elements are still largely unknown. A certain degree of polyploidy may conceivably be necessary for the megakaryocyte to secrete platelets, but the importance of polyploidy in its cytoplasmic maturation has recently been questioned. The influence of the absence of a nucleus on platelet metabolism, as well as on the endocytic properties of cells, is still a matter of controversy. Finally, there remains to be established the possible role of contraction in the release of platelet material, and molecular material degraded by lysosome action.

SUMMARIO IN INTERLINGUA

Le proprietates del megacaryocyto e del plachetta pote esser summarisate brevemente in le sequente maniera: Un cellula polyploide secerne, per un mechanismo a typo apocrinic, un elemento anucleate que possede forte proprietates autolytic e endocytic e es capace de effectuar un action contrahitori. Iste ultime elemento, assi specialisate, es preste a prender un rolo major in reactiones hemostatic.

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MULTIPLE DIFFERENTIATION IN MEGAKARYOCYTES AND PLATELETS

continua esser controverse. Finalmente, remane a establir le rolo possibile de contraction in le liberation de material plachettal e de material molecular decomponite per un action lysosomatic.

ADDENDUM

Further information in this subject may be found in Marcus and Zucker’s recent comprehensive review of platelet physiology.7

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REFERENCES


416

JEAN-MICHEL PAULUS


Analytical Review: Multiple Differentiation in Megakaryocytes and Platelets

JEAN-MICHEL PAULUS