

Structure/Function Relationships of Megakaryocytes and Platelets

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In order to understand normal platelet function and the alterations associated with disease, it is crucial to learn how these pieces of cytoplasm are generated and to what extent their structure subserves their physiologic role. Such knowledge has become even more important with the recognition and availability of cytokines, including thrombopoietin, shown to have an effect on the megakaryocyte/platelet lineage. In line with the educational intent of this publication, the author will highlight clear-cut, well-established observations (for more complete reviews see refs. 1-3) rather than dwelling on data that are still subject to controversy. Many of the conclusions drawn from *in vitro* experiments, such as those obtained by culturing the cells in serum-free semi-solid or liquid media, belong in this latter category. It should, however, be appreciated, that such experiments are mandatory if one is to dissect the specific role of various cytokines used singly or in combination.

Development of Megakaryocytes

Megakaryocytes are derived from committed progenitors that are not morphologically distinguishable from other hematopoietic stem cells or even from lymphocytes, unless they are delineated by virtue of their lineage-specific epitopes, such as GpIIb/IIIa. This can be done by light microscopy as well as on the ultrastructural level (Figure 1, Figure 2 and ref. 4).

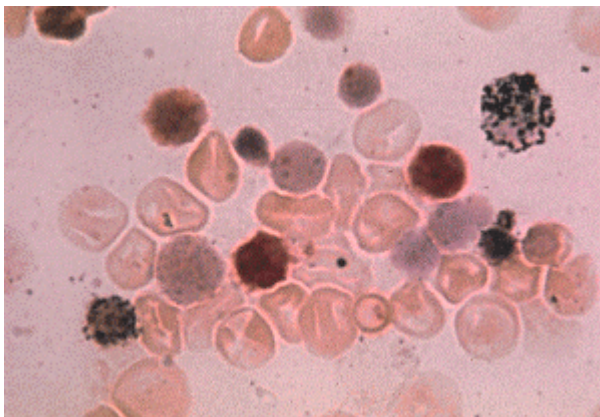


Figure 1. Cord blood cells after Ficoll-Hypaque gradient centrifugation dually stained for GpIIb/IIIa (black silver particles) and a-naphthyl butyrate esterase (ANBE) followed by Giemsa. Note that there is no overlap between ANBE stained monocytes and the anti-GpIIb/IIIa-stained cells. (Reproduced from Zucker-Franklin D, Yang JS, Grusky G. Characterization of GpIIb/IIIa positive cells in human umbilical cord blood; their potential usefulness as megakaryocyte progenitors. *Blood* 79:347, 1992.)

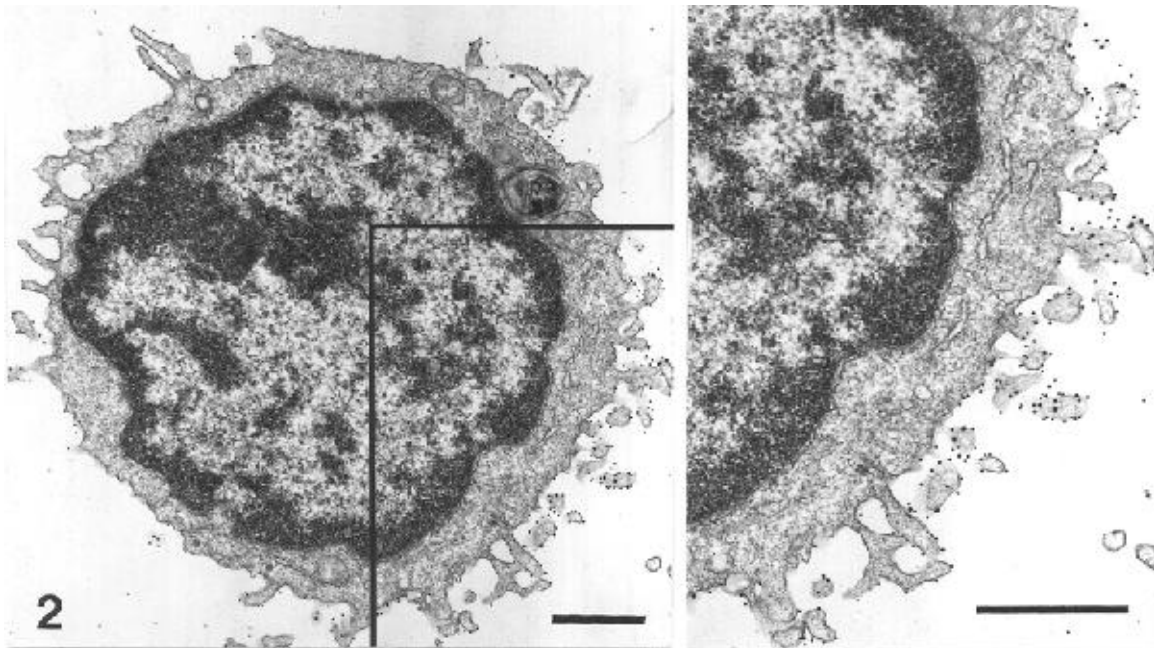


Figure 2. Electron micrograph of a CD34 positive progenitor cell from human cord blood stained by the immunogold technique. The bracketed area is shown at higher magnification on the right. Bar represents 1 micron. (Reproduced from Ref. 4)

The cell then undergoes numerous endomitoses in the process of which the nuclear chromatin reduplicates, but the cytoplasm never divides. The final size of the cell roughly corresponds with its ploidy. As in other cells, the cell-specific proteins are synthesized by ribosomes on the rough endoplasmic reticulum and then packaged via the Golgi zone into granules. Megakaryocyte granules contain b-thromboglobulin, platelet factor 4, thrombospondin, fibronectin, von Willebrand factor (vWF), and P-selectin. In addition, the granules of MK and platelets have been shown to contain several plasma proteins, such as fibrinogen,⁽⁵⁾ Factor V, albumin and immunoglobulin,⁽⁶⁾ which are not synthesized by the cell and must, therefore, be endocytosed. Apart from its polyploidy, another remarkable feature of this cell is manifested in its cytoplasm. Here a system of membranes, referred to as the demarcation membrane system (DMS), appears to be involved in delimiting areas believed to represent platelet territories (Figure 3).

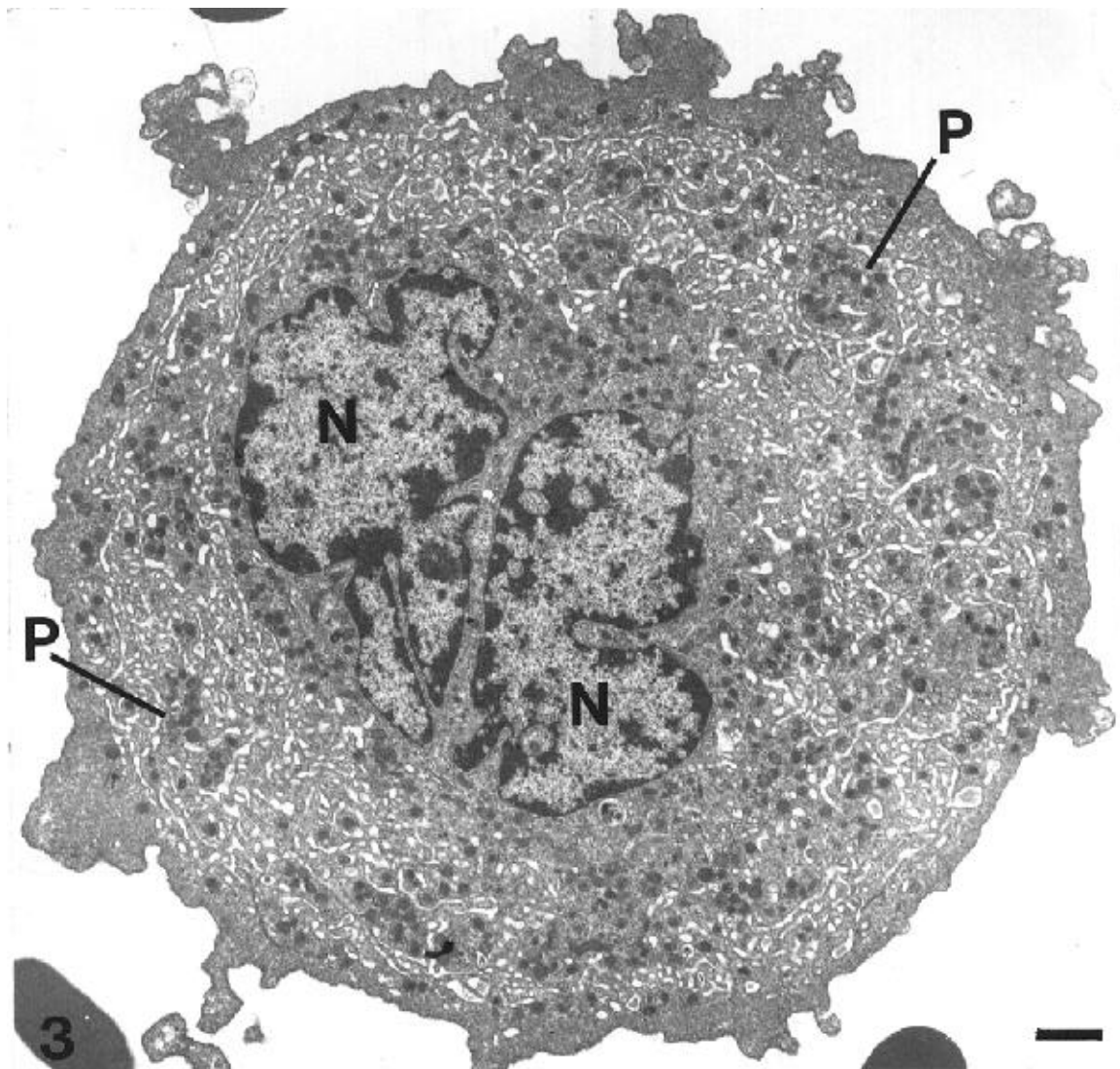


Figure 3. Mature megakaryocyte showing fully granulated platelet territories (P). N, nucleus. Note that the peripheral zone of cytoplasm is devoid of any organelles. Bar represents 1 micron. (Reproduced from Ref. 2)

Particular attention should be drawn to the observation that the DMS originates within the MK cytoplasm⁽⁷⁾ and that, therefore, these membranes need not be antigenically, structurally, or biochemically identical to the surface membrane of the cell (see below).

The question as to where fragmentation of megakaryocytes occurs has not yet been answered definitively. However, there are convincing early⁽⁸⁾ as well as recent⁽⁹⁾ data to support the concept that the majority of MK exit from the medullary compartment intact and that most platelets are released in the capillary bed of the lungs. Thus, in healthy individuals, very few “denuded” MK nuclei are seen in bone marrow biopsy specimens (Figure 4).

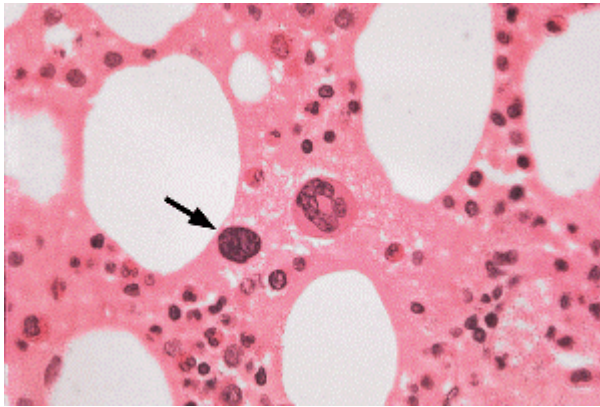


Figure 4. Bone marrow biopsy from HIV-infected person stained with Wright's and Giemsa. Arrow indicates denuded megakaryocyte nucleus adjacent to normal appearing cell.

However, in some pathologic conditions, particularly in HIV-infected patients, the observation of a conspicuously large number of “bare” MK nuclei may have diagnostic significance.⁽¹⁰⁾ This does not negate reports describing the presence of so-called pro-platelets in marrow sinusoids.⁽¹¹⁾ Motile cells, including megakaryocytes, extend long cellular processes during migration. In the case of megakaryocytes, such cytoplasmic extensions or pseudopods would of necessity comprise many platelet territories. In large vessels these are likely to round up and would then be considered as “giant” platelets. The author has introduced the term “compound” platelets for megakaryocyte fragments consisting of more than one platelet territory (see Figures 5-7 for elucidation of this concept). On the basis of these observations, it is likely that large or giant platelets are young. This does not imply that small platelets are necessarily old. For instance, the administration of thrombopoietin to healthy mice with a normal platelet count results in considerable thrombocytosis. However, many of the newly formed platelets are immature, as evidenced by large numbers of ribosomes and dearth of granules and other organelles, while being relatively small (unpublished observations). Whether these “young” platelets function normally is not known. The most definitive method of assessing the percentage of young platelets in the circulation uses flow cytometry employing the dye Thiazole orange, which reacts with RNA.⁽¹²⁾

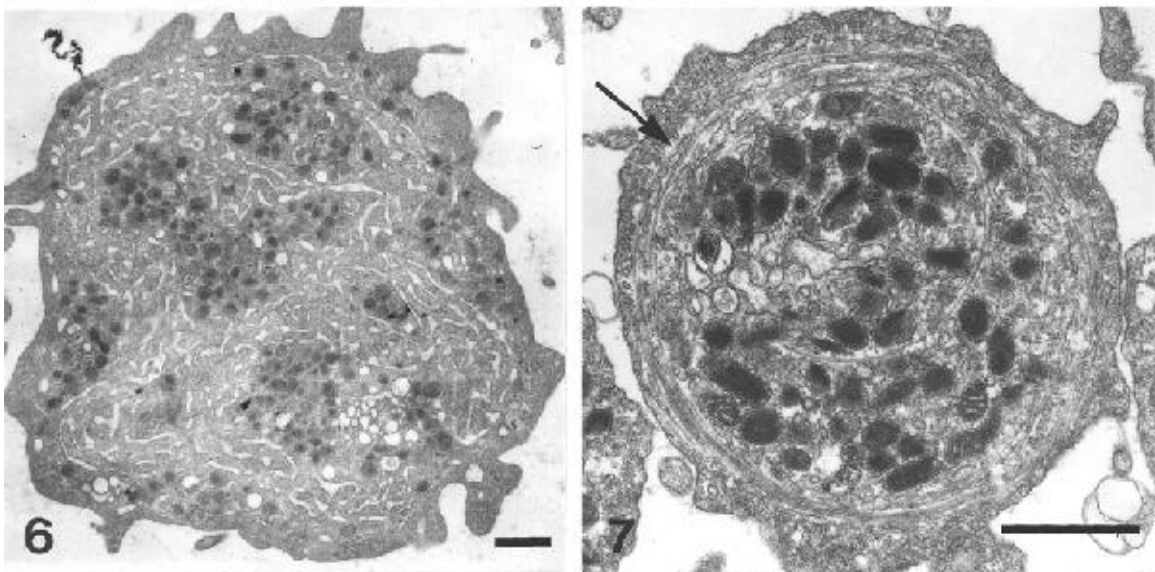
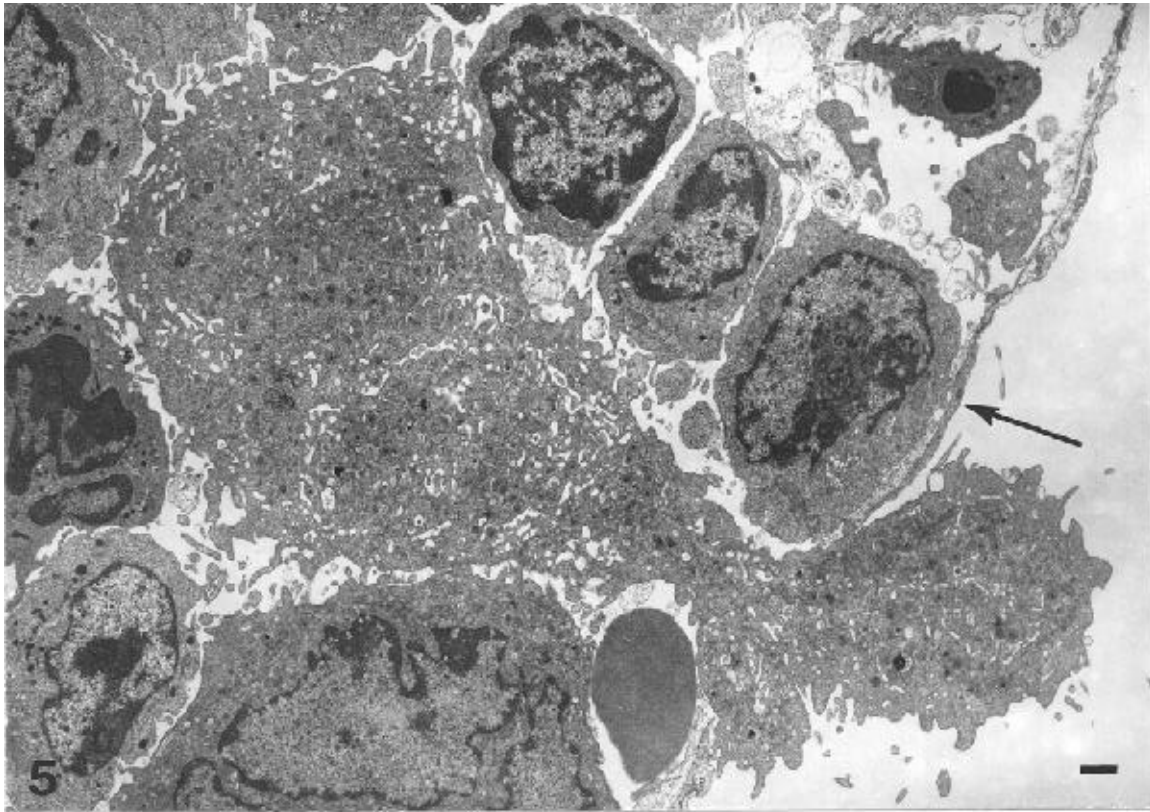


Figure 5. Large megakaryocyte process is seen to protrude into the sinusoidal space via a gap in the endothelium (arrow). The MK nucleus is not in this plane of section. Other hematopoietic cells are still within the medullary space. Bar represents 1 micron.

Figure 6. Giant platelet from the peripheral blood of a patient with idiopathic thrombocytopenic purpura. Note that it consists of several platelet territories which have not yet undergone further fragmentation. Bar = 1 micron.

Figure 7. Normal platelet features a conspicuous band of microtubules (arrow), evenly distributed granules, mitochondria and canaliculi. Bar = 1 micron. Note that the magnification of Figure 7 is 2-1/2 times the magnification of the giant platelet depicted in Figure 6, and 4 times the magnification of Figure 5.

While there is no dispute over the concept that platelets represent megakaryocyte fragments, it is not generally appreciated that the plasma membranes of MK and platelets are not identical. This has been substantiated by a variety of means. Firstly, some antibodies that react with platelets do not react with all MK.⁽¹³⁾ This permits rapid restoration of the platelet count in some acute thrombocytopenias mediated by platelet antibodies. Secondly, freeze-fracture analyses carried out on the plasma membranes of MK and platelets revealed that the partition coefficient of intramembranous particles (IMP), which are believed to represent integral membrane proteins, is the reverse in the two cells.⁽⁷⁾ Thirdly, many enveloped viruses appear to bud exuberantly from the DMS and only rarely from the plasma membrane of the MK, suggesting that the protein destined to constitute part of the viral envelope is differentially inserted in the MK plasma membrane. The platelet surface is believed to derive from the DMS of the MK.⁽¹⁴⁾ These are only a few examples to illustrate that platelet surface membranes probably differ from those of any other circulating blood cells.

The observations cited above are important in considering the effect of various cytokines. For instance, although it has been demonstrated that both MK and platelets have thrombopoietin receptors (Tpo),⁽¹⁵⁾ the number of receptors per unit plasma membrane has not yet been established. Moreover, while there is evidence to suggest that Tpo is endocytosed by platelets,⁽¹⁶⁾ this has as yet to be demonstrated to pertain equally to megakaryocytes. In fact, it would not be remote to postulate that the binding of Tpo to its receptors may transduce the signal for fragmentation or proliferation (depending on the stage of megakaryocyte maturation), whereas interiorization of the Tpo-receptor complex by platelets would serve to render platelets unresponsive to this ligand. It remains to be investigated whether "compound" platelets interiorize thrombopoietin as well and whether this cytokine is involved in further fragmentation of large megakaryocyte cytoplasmic fragments into smaller units.

The Effect of Cytokines

The preceding overview of megakaryocyte/platelet structure should be considered whenever the effects of various cytokines are being evaluated. Only a few exemplary observations can be recorded here. For instance, it is quite clear that in a serum-free liquid culture system initiated with mouse bone marrow cells devoid of recognizable megakaryocytes, neutralization of thrombopoietin with soluble Mpl will prevent differentiation of mature megakaryocytes.^(17,18) Similarly, in a serum-free liquid culture system initiated with purified human CD34 progenitors, only the addition of Tpo results in generation of platelet-producing megakaryocytes.⁽¹⁹⁾ In both experimental situations, IL-3 markedly increased the total number of cells in the culture.

The mechanisms whereby other megakaryocytotropic cytokines function is more difficult to interpret. For instance, stimuli that increase the production of acute phase reactants will concomitantly increase the plasma levels of Tpo, IL-6, IL-3 and several other substances synthesized by the liver. Also, it is generally recognized that the administration of IL-6 to healthy animals will increase the number, size and ploidy of megakaryocytes as well as the peripheral blood platelet count, albeit only transiently.⁽²⁰⁻²²⁾ Continued administration of IL-6 results in pathologically altered megakaryocytes⁽²¹⁾ and sometimes rebound thrombocytopenia.⁽²²⁾ The administration of IL-11 also has a salutary effect on platelet production in vitro⁽²³⁾ and when administered to patients whose bone marrow is suppressed by chemotherapy.⁽²⁴⁾ It is difficult to know whether IL-11 affects MK proliferation directly or whether its effect is mediated indirectly, e.g., by increasing the synthesis of Tpo. However, in the culture systems mentioned before, IL-11 appeared to promote MK fragmentation when Tpo was neutralized.⁽¹⁸⁾ In view of the likelihood that the pharmacologic use of most purified or cloned cytokines will be associated with some adverse effects, the synergism of these agents may allow their simultaneous use at lower dosages. This strategy has proven to be useful in other areas of therapy.

In summary, while there is little doubt that the isolation and cloning of megakaryocytotropic cytokines, especially thrombopoietin, has been a major development in the study of megakaryocyto- and thrombocytopoiesis, these agents are even more exciting as potential treatment modalities for thrombocytopenia. At the same time, the physiologic effects of these growth factors are complex. Therefore, it should be helpful to consider future developments in this area in the context of what is known about the structure/function relationships of these cells during normal homeostasis.

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