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PHENOTYPIC CHANGES IN THE MEGAKARYOCYTE-PLATELET LINEAGE

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Abstract

Morphologic and phenotypic changes occur during the maturation of megakaryocytes (MK) from the pluripotent stem cell to platelets. As the MK acquires organelles, it also acquires membrane glycoproteins and granule contents. Platelet membrane GP IIb/IIIa and platelet peroxidase are present from early stages of maturation to the final product of the megakaryocyte, the platelet, while Ia-like antigen appears to be expressed only during early stages of maturation. The MK synthesizes increasing amounts of lysosomal enzymes, GP Ib, and alpha granule proteins as it matures from the megakaryoblast stage to the mature cell. The platelet contains only vestiges of a protein synthetic apparatus; it therefore has acquired most of its contents, except for serotonin, during the maturation of the MK.

KEY WORDS: Megakaryocyte, Platelet, Phenotype, Antigens, Maturation.

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Introduction

The maturation and differentiation of hematopoietic cells occurs in association with specific structural, functional, and antigenic changes. Megakaryocytes mature in the following sequence: stem cell → colony-forming-unit-megakaryocyte (CFU-Meg) → proliferative and non-proliferative precursor → promegakaryoblast → megakaryoblast → immature megakaryocyte → maturing megakaryocyte → mature megakaryocyte → platelets (Table 1). It should be emphasized that this is a continuum of maturation and that overlap may occur between stages. As maturation proceeds, proliferative potential decreases. It is interesting to note that a "blast" cell in other cell lineages usually indicates a cell with proliferative potential whereas "megakaryoblast" indicates a nonproliferative cell. The stages of megakaryocyte maturation have previously been defined in terms of cytoplasmic maturation [27, 58, 60], although more recently, Williams and Levine [84] have introduced nuclear configuration as another descriptive characteristic. Since it has been difficult to purify stem cells or CFU-Meg, the morphology of these cells has been only preliminarily described [77]. Promegakaryoblasts are difficult to identify on purely morphologic criteria. In mammals, immature megakaryocytes are small cells measuring 6 to 24 μm in the long axis [84], containing a large, round, indented, or lobed nucleus [52] with prominent nucleoli, and having a high nuclear:cytoplasmic ratio. These cells contain few cytoplasmic organelles other than polyribosomes and mitochondria; α granules are infrequently seen [4, 5]. Lysosomal enzymes are present in this earliest recognizable cell of the megakaryocytic series, even before the formation of alpha granules and demarcation membranes are observed [4, 5]. Microperoxisomes are also formed early in megakaryocyte differentiation [10]. Maturing megakaryocytes are heterogeneous in size (14 to 30 μm) and in the level of polyploidy (8 to 32N). They contain a large protein synthetic apparatus and variable numbers of demarcation membranes and α granules. Mature megakaryocytes are approximately 20 to 50 μm in diameter [84], with a low nuclear:cytoplasmic ratio, and contain an extensive demarcation membrane system and a reduced protein synthetic apparatus. Studies of megakaryocyte differentiation have reported changes in the phenotypic profile at various stages of maturation.

This paper will describe what is currently known about megakaryocyte/platelet antigens during megakaryocytopoiesis *in vivo* and *in vitro,* and examine the phenotypic characteristics of cells from patients with megakaryoblastic leukemia.

Table 1. Characteristics of Maturational Stages of Megakaryocytes*

Maturational Stage	Size (μm)	Nuclear Morphology	Demarcation Membranes	Granules
Stem Cell	8	?	?	?
CFU-Meg	6-8	?	?	?
proliferative and nonproliferative precursor (sAChE+)**	8-18	?	?	?
Promegakaryoblast	10-15	round, nonsegmented	-	-
Megakaryoblast***	≳14 6-24	compact, lobed	+	(+)
Immature Megakaryocyte	6-24	round, indented, or lobed	+	+
Maturing Megakaryocyte	14-30	horseshoe or multi-lobed	++	++
Mature Megakaryocyte	20-50	compact but multi-lobulated	+++	+++

* This table is derived from a series of studies in rodents and humans and is a synthesis of data from Paulus (Blood 35:298-311, 1970), Ebbe and Stohlman (Blood 26: 20-35, 1965), Odell and Jackson (Blood 32:102-110, 1968), Williams and Levine (Br J Haematol 52:173-180, 1982), Dupont et al. (Biol Cell 49:137-143, 1983), Jackson (Blood 42:413-421, 1973), Tranum-Jensen and Behnke (Biol Int Rep 1:445-452, 1977), Breton-Gorius and Vainchenker (Sem Hematol 23:43-67, 1986), and Spangrude et al. (Science 241:58-62, 1988).

** Small, acetylcholinesterase-positive cell.

*** The definitions for megakaryoblast and immature megakaryocyte probably require additional resolution.

Megakaryocytes In Vivo

Prior to the availability of immunocytochemical markers, histochemical stains were employed for the identification of megakaryocytes. Acetylcholinesterase staining has proved extremely useful in the identification of dog [39], cat [75,76], rat [35,75,76], and murine [51,75,76] platelets and megakaryocytes, although it is not applicable for the identification of human megakaryocytes. Histochemical stains for acid phosphatase and aryl sulfatase identify primary lysosomes of rat and human megakaryocytes [4,5]. A peroxidase activity specific for the megakaryocyte/platelet lineage (PPO), localized to the endoplasmic reticulum and perinuclear envelope of the megakaryocyte and to the dense tubular system of the platelet, has been demonstrated [9] and applied to the identification of megakaryoblastic leukemia cells [11,12]. Breton-Gorius et al. [16] concluded that this PPO was the earliest known marker appearing in immature megakaryocytes, since in bone marrow from a patient with megakaryoblastic leukemia, some promegakaryoblasts (the diploid precursors of megakaryoblasts) expressed both PPO and platelet membrane glycoproteins, while a few promegakaryoblasts identified by PPO did not express these glycoproteins.

Recently, antibodies specific for platelet membrane glycoproteins have been employed in the identification of normal human megakaryocytes *in vivo:* both immature and mature megakaryocytes were labeled utilizing immunofluorescence techniques and antibodies directed against platelet glycoproteins [62,63,79]. A monoclonal antibody to the GP IIb/IIIa complex has been used to purify human megakaryocytes from sternal bone marrow [25]. Immunogold techniques at the ultrastructural level have localized platelet membrane glycoproteins to human [16] and murine [71] bone marrow megakaryocytes (Fig. 1). Levene et al. [42] have shown that monoclonal antibodies which immunoprecipitate platelet glycoprotein IIb/IIIa also recognize the committed progenitor colony-forming-unit-megakaryocyte (CFU-Meg) (vide infra). Fraser et al. [29] have presented evidence that the human pluripotential hematopoietic stem cell expresses platelet lineage-specific markers. Jackson has demonstrated that small rat bone marrow cells expressing AChE activity also were labeled by fluorescent anti-platelet serum [36]. Immunofluorescence techniques also have localized platelet Factor 4 [54,62,67,81], β-thromboglobulin [54], fibrinogen [62,81], thrombospondin [54], FVIII:vWF [62,63,81], fibronectin

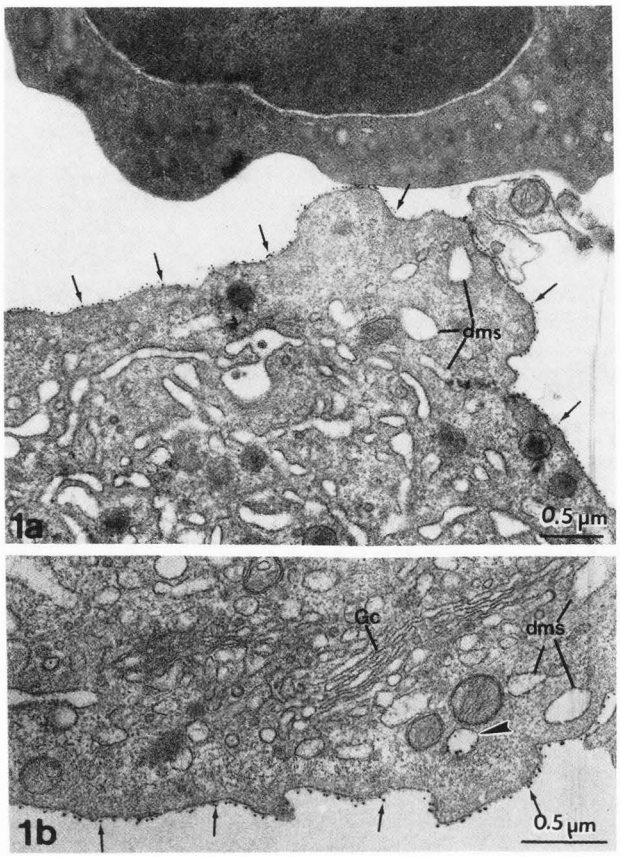


Figure 1. (a) A mature mouse bone marrow megakaryocyte (MK) reacted with platelet antiserum followed by protein A-gold (size, 10 nm) probe. Label is present along the extent of the MK plasma membrane (arrows) and is absent from the plasma membrane of the neighboring cell. (b) A higher magnification of a cell from the same preparation, showing immunogold label along the plasma membrane and within an element of the demarcation membrane system (arrowhead). (Even though the demarcation membrane system has been shown to be in continuity with the outside space, only rare immunogold particles were observed inside these elements because the large size of the particles inhibited their entry into these channels). dms, demarcation membrane system; Gc, Golgi complex.

[63], platelet-derived growth factor [81], and coagulation Factor V [57] to human bone marrow megakaryocytes. Immunoperoxidase techniques at the light microscope level have localized α granule membrane protein 140, thrombospondin, FVIII:vWF, β-thromboglobulin, platelet Factor 4, and fibrinogen in a granular distribution in human bone marrow megakaryocytes [3].

Flow cytometry is a potentially powerful tool for the study of megakaryocyte maturation, since it has the capability of physically separating small subpopulations from a large number of cells and simultaneously measuring multiple parameters. For example, flow cytometric techniques have been successfully used to study the development of erythroid cells and B-lymphocytes in human bone marrow [49, 50]. Loken et al. [50] were able to identify erythroid cells at various stages of maturation with three markers: glycophorin A was expressed on mature erythrocytes but not on earliest precursor cells, the HLe-1 antigen was lost during maturation, and transferrin receptor was expressed at intermediate stages of erythroid development. Similar techniques were used to define stages of B-lymphocyte maturation based on cell surface antigen expression [49]. Although flow cytometric studies have been performed on megakaryocytes [7, 23, 37, 47, 56, 74, 85], collectively these studies have been limited by poor discrimination between different populations of megakaryocytes, and/or histological identification restricted only to mature cells. Further use of this technology should enable the more precise identification of cells of this lineage at various stages of development.

Megakaryocytes In Vitro

Culture techniques are available which support the proliferation and differentiation of the progeny of CFU-Meg, which are defined by their capacity to form megakaryocyte colonies in agar [55], methylcellulose [28], or plasma clot [78], *in vitro*. They have enabled the identification and study of immature megakaryocytes. At least two types of murine megakaryocyte colonies have been recognized in soft agar cultures [19, 44, 45, 48, 82, 83]. One type is typically composed of 10-30 large megakaryocytes which are mature in appearance ("big cell" type). The other ("heterogeneous" type) is composed of more cells (often greater than 100), some of which are small and immature as evaluated in part by staining for acetylcholinesterase, a specific marker for murine megakaryocytes.

Recently, murine megakaryocytes from big cell and heterogeneous colonies grown in soft agar culture have been identified and characterized by labeling for megakaryocyte/platelet antigens [70, 71]. The use of specific markers on megakaryocytes grown in soft agar cultures allows one to examine patterns of protein expression in megakaryocytes at early time points in culture and particularly, to study the small immature cells which partially constitute the heterogeneous megakaryocyte colonies. The investigation of murine heterogeneous colonies is of particular interest, since it is believed that they are composed solely of megakaryocytes and their precursors [44-46] and offer the first opportunity to study by morphological procedures the development of cells of the megakaryocyte lineage starting with diploid (2N) precursors. For example, a recent study utilizing acetylcholinesterase histochemistry at the fine-structural level on murine colonies has allowed the identification of small immature cells as megakaryocytes [71] (Fig. 2).

The use of markers in *in vitro* systems has permitted limited studies of the transitional maturation stages between CFU-Meg and megakaryocytes. For example, it has been demonstrated in the mouse that the small acetylcholinesterase-positive cell represents an intermediary stage between the CFU-Meg and the megakaryocyte [51, 52, 77], and small cells expressing platelet glycoproteins or cytoplasmic factors have been described in humans *in vitro,* in plasma clot cultures [22, 53, 79], and in murine megakaryocyte colonies grown in soft agar cultures [43, 71] (Fig. 3). In a study of human megakaryocytes grown in methylcellulose culture, at days 4 and 5 there was a number of cells 8-10 µm in diameter which expressed the GP IIb/IIIa complex and a smaller number which labeled for GP Ib and the cytoplasmic proteins vWF, PF4, and thrombospondin [80]. By day 7 of culture, 75% of cells were double-labeled with antibodies to membrane and cytoplasmic constituents, and the number of labeled cells

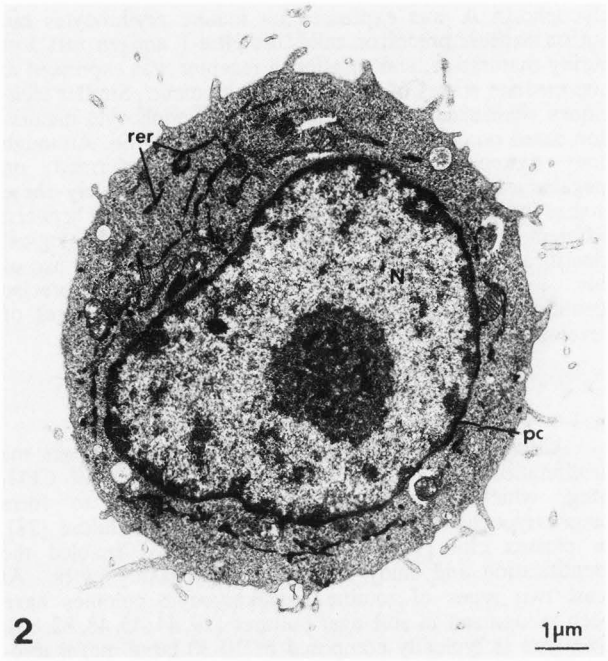


Figure 2. An immature mouse megakaryocyte from a 7-day heterogeneous colony reacted for acetylcholinesterase. Reaction product is confined to the perinuclear cisterna (pc) and the rough endoplasmic reticulum (rer). N, nucleus.

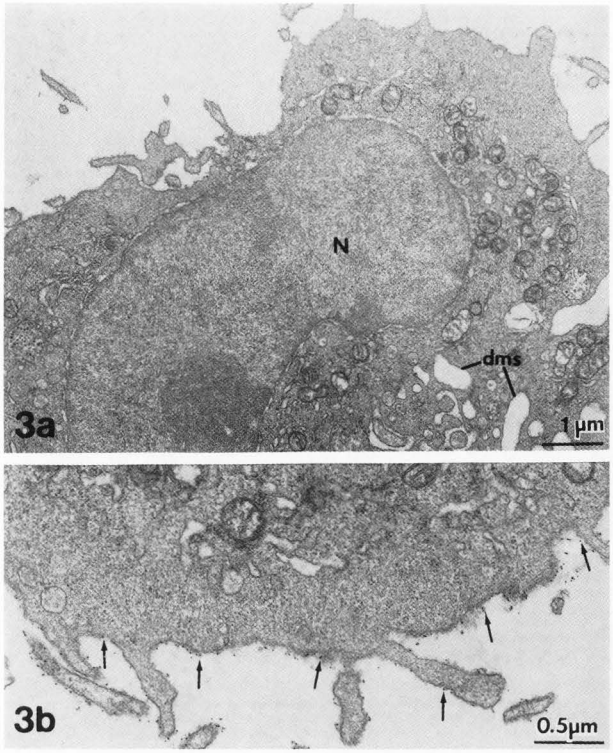


Figure 3. (a) An immature mouse megakaryocyte from a 7-day heterogeneous colony reacted for platelet antiserum followed by protein A-gold (size, 10 nm) probe. (b) At higher magnification of this cell, immunogold label is apparent along the extent of the plasma membrane (arrows). dms, demarcation membrane system; N, nucleus.

increased significantly after 7 days. These investigators concluded that all platelet proteins which they examined are expressed by promegakaryoblasts, but that GP IIb and GP IIIa are present earlier in differentiation than the other proteins [80].

Antigenic Similarities and Differences between Megakaryocytes and Platelets

Numerous studies have been performed to examine the expression of antigens at various times during human or murine megakaryocyte development. Changes in antigenic profiles may be attributed to a) acquisition of new antigens, b) exposure of cryptic antigens, or c) loss of antigenic sites. Most of these studies have been performed using immunofluorescence techniques or ultrastructural immunogold techniques; polyclonal antibodies to GP IIb/IIIa, or monoclonal antibodies to various epitopes of GP IIb, GP IIIa, or the complex GP IIb/IIIa have been used most frequently.

The expression of GP IIb/IIIa throughout the megakaryocyte/platelet lineage is somewhat controversial (Table 2). Polyclonal antibodies to GP IIb/IIIa or monoclonal antibodies to epitopes of these glycoproteins have been used to detect this antigen on stem cells [6,29], CFU-Meg [6,42], immature megakaryocytes [3,6,14,25,29,31,41,42,62,63,73,81], mature megakaryocytes [3,6,14,25,29,31,34,41,42,62,63,73,81], and platelets [6,14,25,29,31,34,42,63,73]. Although GP IIb/IIIa has been reported to be expressed on human pluripotent stem cells and CFU-Meg using absorbed heteroantisera [6], this complex was visualized by immunofluorescence only on day 4 or 5 in cultures of human CFU-Meg [80]. These discrepant results may be due to technical differences between the spleen colony inhibition assay and immunofluorescence techniques used in cultures of CFU-Meg. Interestingly, an immunocytochemical study of murine megakaryocytes *in vitro* reported that all cells in megakaryocyte colonies grown in soft agar culture, regardless of time in culture or extent of differentiation, exhibited a similar density of immunogold label along the plasma membrane with platelet antiserum or anti-GP IIIa serum [71]. It should be noted that GP IIIa has been reported on the surfaces of endothelial cells and some osteoclasts [3]. Therefore, several markers should be used simultaneously to determine cell lineage specificity.

Studies which have compared the distributions of GP Ib and GP IIb/IIIa on differentiating megakaryocytes have concluded that GP Ib is expressed early in megakaryocyte maturation, but that there is a greater variability in expression of GP Ib on platelet and mature megakaryocyte plasma membranes than there is for GP IIb/IIIa [14,34,63,81] (Table 3). Hyde et al. [34] reported that only 24% of megakaryocytes reacted with anti-GP Ib antibodies whereas other investigators have reported that all megakaryocytes and their precursors stained with monoclonal antibodies to GP Ib [14]. These conflicting results may be due to technical problems in detecting label on small mononuclear cells which are the megakaryocyte precursors. Since there are fewer antigenic sites on megakaryocyte plasma membranes for GP Ib than there are for GP IIb/IIIa [14], it is important that other markers be utilized in conjunction with

Table 2. Expression of GP IIb/IIIa During Megakaryocyte Maturation

		Megakaryocyte Maturational Stage							
Antibody	Specificity	Pluripotent Stem Cell	CFU-Meg	ProMKblast MKblast Immature MK	Mature MK	Platelet	Species	Detection Method	Reference
α-IIIa(P)	IIIa	ND	ND	+	+	ND	H	IF	62
α-IIb(P)	IIb	ND	ND	+	+	+	H	IF	63
α-IIIa(P)	IIIa	ND	ND	+	+	+			
PBM6.4(M)	IIb/IIIa	ND	ND	+	+	+	H	IF	25
J15(M)	IIb/IIIa	ND	ND	+	+	+	H	IF,IG	14
J15(M)	IIb/IIIa	ND	ND	+	+	ND	H	IF,IG	81
α-IIb/IIIa(P)	IIb/IIIa	ND	ND	+	+	+	H	IF,IG,EIP	31
α-HuP1-m1(M)	IIb & IIIa	ND	ND	+	+	+	H	IF	73
PC-1(M) PC-3(M) PC-4(M)	IIb/IIIa	ND	+	+	+	+	H	IF,C'-MC, ELISA	42
APS(P)	PLT MB	+	+	+	+	+	M	IF,SCIA	6
J15(M)	IIb/IIIa	+	+	+	+	+			
A5.15(M)	IIb/IIIa	+	+	+	+	+			
AIIbs(P)	IIb	+	ND	ND	ND	+	H	IF,C'-DCA	29
AIIIas(P)	IIIa	+	ND	ND	ND	+			
APS(P)	PLT MB	+	ND	+	+	+			
TP80(M) PLT-2(M)	IIb/IIIa	ND	ND	+	+	ND	H	FACS,IAP	41
10E5(M)	IIb/IIIa	ND	ND	ND	+	+	H	IF,IG	34
α-IIIa(P) Tab(M) T10(M)	IIIa IIb IIb/IIIa	ND	ND	+	+	ND	H	LIP	3

ND=not described
P=polyclonal antibody
M=monoclonal antibody
MK=megakaryocyte
PLT MB=platelet membrane
H=human
M=mouse

Detection Method:

IF=Immunofluorescence
IG=Immunogold
EIP=Electron microscope immunoperoxidase techniques
LIP=Light microscope immunoperoxidase techniques
C'-MC=Complement-mediated cytolysis
ELISA=Enzyme-linked immunosorbent assay
SCIA=Spleen colony inhibition assay
C'-DCA=Complement-dependent cytotoxic assay against CFU-mix
IAP=Immunoalkaline phosphatase techniques
FACS=Fluorescence-activated cell cytometry

antibodies to GP Ib for diagnostic purposes (vide infra).

Several groups of investigators have reported changes in the expression of Ia-like antigens as megakaryocytes develop from the immature stage to the platelet [8,25,41,62,63,73,81] (Table 3). Damiani et al. [25] reported the expression of HLA-DR or Ia-like antigen using the monoclonal antibodies DR and DC1 on the surface of mature human megakaryocytes, but not on immature megakaryocytes or platelets. In contrast, Koike et al. [41] reported the presence of HLA-DR antigen on immature human megakaryocytes but not on mature megakaryocytes; platelets were not examined. Bodger et al. [8] noted the presence of HLA-DR or Ia-like antigens on human pluripotent stem cells and CFU-Meg, although further stages of

Table 3. Expression of Cell-surface Antigens Other than GP IIb/IIIa During Megakaryocyte Maturation

		Megakaryocyte Maturational Stage							
Antibody	Specificity	Pluripotent Stem Cell	CFU-Meg	ProMKblast MKblast Immature MK	Mature MK	Platelet	Species	Detection Method	Reference
Glycoprotein Ib									
α-GP Ib(P)	GP Ib	ND	ND	*	+	ND	H	IF	62
α-GP Ib(P)	GP Ib	ND	ND	+	+	+	H	IF	63
AN51(M)	GP Ib	ND	ND	+	+	+	H	IF,IG,IFe	14
AN51(M)	GP Ib	ND	ND	+	+	ND	H	IF,IG	81
6D1(M)	GP Ib	ND	ND	ND	+ (25%)	+	H	IF,IG	34
Ia Antigen									
α-Ia Ag(P)	Ia-antigen	ND	ND	*	+ (16%)	-	H	IF	62
α-Ia Ag(P)	Ia-antigen	ND	ND	-	+	ND	H	IF	63
RFB-HLA-DR(M)	Ia-antigen	+	+	ND	ND	ND	H	FACS,C'-MC	8
PTF 29.12(M)	I-E/C-like Ag	ND	ND	ND	+ (34-40%)	-	H	IF	25
BT 3.4(M)	Ia-antigen	ND	ND	ND	+ (16-24%)	-	H	IF	25
α-HLA-Dm1(M)	Ia-antigen	ND	ND	-	-	-	H	IF	73
1 35(M)	HLA-DR	ND	ND	+	-	-	H	IF,IG	81
(M)	HLA-DR	ND	ND	+	-	ND	H	FACS	41
Other									
α-FcR(P)	Fc-receptor	ND	ND	*	+ (95%)	+ (85%)	H	Rosette or IF assay	62
α-CR1(P)	CR1(C4b-C3b receptor)	ND	ND	*	+ (20-40%)	+ (3%)	M	C'-RA	62
α-FcR(P)	Fc-receptor	ND	ND	+	+	ND	H	IF	63
Ovalb-α-ovalb(P)	Fc-receptor	ND	ND	+ (30%)	+ (30%)	+ (1%)	H	IF	64
KLH-α-KLH(P)	Fc-receptor	ND	ND	+ (97%)	+ (97%)	+ (100%)	H	IF	64
α-type 2H(M)	2H Ag of ABO system	ND	+ (10-20%)	+ (10-20%)	+ (10-20%)	ND	H	IF	26
α-PL^{A1}(P)	PL^{A1}	ND	ND	ND	+	+	H	IF,IG	34
α-PL^{A1}(P)	PL^{A1}	ND	+	+	+	ND	H	IF	26
1C10(M)	unknown	+	ND	ND	ND	+	M	SCIA	65
13C6(M)	unknown	+	ND	ND	ND	-	M	SCIA	65
RFB-1(M)	**	+	+	ND	ND	ND	H	FACS,C'-MC	8
"Group A"(6 M)	unknown	ND	+	+	-	-	H	IF,ELISA	42
MKB-1(M)	unknown	ND	(+)	+	+	-	H	C'-MC	42
MY10(M)	myeloid Ag	ND	ND	+	-	ND	H	FACS	41
MY9(M)	myeloid Ag	ND	ND	+	-	ND	H	FACS	41

* Did not distinguish between immature and mature megakaryocytes

** Reacts with CFU-GM and immature lymphoid cells in bone marrow

ND=not described, P=polyclonal antibody, M=monoclonal antibody, MK=megakaryocyte, H=human, M=mouse, IF=Immunofluorescence, IG=Immunogold, ELISA=Enzyme-linked immunosorbent assay, C'-MC=Complement-mediated cytolysis, SCIA=Spleen colony inhibition assay, FACS=Fluorescence activated flow cytometry, C'-RA=Complement receptor assay, IFe=Immunoferritin

maturation were not studied. Another study reported the presence of Ia-like antigen on a majority of mouse platelets, on 16% of mouse megakaryocytes, and on 0% of mouse megakaryocyte precursors [62], while Thurlow et al. [73]

reported human platelets and megakaryocytes to be negative for Ia-like antigen. A study of human megakaryocytes grown in plasma clot cultures reported that HLA-DR was expressed in promegakaryoblasts but not on mature megakaryocytes or platelets [81]. Therefore, the majority of studies have reported the presence of Ia-like antigen on early megakaryocytes and the absence of this antigen on megakaryocytes at later stages of maturation. Clearly, more data will be required to clarify the distribution of these antigens on differentiating megakaryocytes.

In addition, an early study reported that 30% of mouse megakaryocytes expressed CR_1 (immunoadherence (C4b-C3b) receptor) together with a proportion of blood platelets, suggesting that this receptor may be acquired at a late stage of development [62]; human megakaryocytes did not express this receptor, but did express the Fc-receptor [62, 64]. Koike et al. [41] reported that antigens identified by monoclonal antibodies MY10 and MY9 (which are used to identify myeloid cells) were present on immature human megakaryocytes but not on mature megakaryocytes. The 2H antigens of the ABO blood group system were reported to be heterogeneously expressed in human CFU-Meg using indirect immunofluorescence techniques [26].

It would be interesting to determine whether the CFU-Meg compartment is phenotypically heterogeneous. Enriched samples of murine CFU-Meg, which can be obtained with a one-step procedure [59], provide the basis for a useful system with which to study this question. Jenkins et al. [38] have shown that megakaryocytes derived from human CFU-Meg synthesize GP IIb and IIIa, and Levene et al. [42] demonstrated expression of the GP IIb/IIIa complex on human CFU-Meg using a panel of monoclonal antibodies in conjunction with immunofluorescence, an enzyme-linked immunosorbent assay, and complement-mediated cytolysis. Monoclonal antibodies which bind to subpopulations of bone marrow stem cells may be useful for stem cell purification [65].

A substance which platelets appear to acquire in their dense granules after they circulate in blood is serotonin (5-hydroxytryptamine); dense granules have generally not been reported in megakaryocyte cytoplasm [61]. However, the precursors of these amine storage organelles in rat megakaryocytes were identified using the uranaffin reaction for adenine nucleotides [24], and this uranaffin reaction and mepacrine labeling have been reported to identify dense bodies in maturing human megakaryocytes [30]. In addition, serotonin uptake has been shown to be a marker for the identification of immature megakaryocytes [69] and megakaryocytes maturing in vitro from CFU-Meg [18].

Phenotypic Profile of Cells from Patients with Megakaryoblastic Leukemia

Cases of megakaryoblastic leukemia are becoming more commonly recognized [1, 12, 33, 40, 41, 64, 66, 68, 72]. In a morphologic study of megakaryoblastic leukemia, Breton-Gorius described these cells as undifferentiated or lymphoid-appearing blasts which did not yet contain granules or demarcation membranes [12]. With the discovery of PPO (platelet peroxidase) as a specific marker for cells of the megakaryocyte-platelet lineage, Breton-Gorius and her co-workers established its presence in the rough endoplasmic reticulum and nuclear envelope of cells from patients with megakaryoblastic leukemia [12, 15]. Even though immunocytochemical markers to GP IIb/IIIa and GP Ib have been utilized successfully to identify some immature cells in these cases as megakaryocytic [33, 68, 72], and PPO has been considered a very sensitive marker of human megakaryocytes *in vivo* and *in vitro* [16, 40, 72, 78, 79], it is unclear which appears earlier in maturation, since the expression of PPO has not been studied yet in stem cells.

Histochemical studies of cases of megakaryoblastic leukemia have demonstrated that cells from some patients are α-naphthyl acetate esterase-positive [15, 40], α-naphthyl butyrate esterase-negative [15, 33, 40], acid phosphatase-positive [33, 40], periodic acid-Schiff-positive [33], and chloroacetate esterase-negative [33], a pattern which is consistent with the enzymatic content of normal megakaryocytes [2, 3]. However, although these markers are useful for a differential diagnosis, they are not specific for the megakaryocyte/platelet lineage.

Some investigators have suggested that the myelofibrosis present in many cases of megakaryoblastic leukemia results from the abnormal presence of intracytoplasmic megakaryocyte components in the bone marrow interstitial space, leading to changes in the rate of collagen secretion and inhibition of collagenase by megakaryocyte-derived growth factor and platelet Factor 4, respectively [1, 13, 15, 20, 21]. An immunohistochemical study comparing 4 cases of acute myelofibrosis with 3 cases of acute megakaryoblastic leukemia concluded that the blast cells of acute myelofibrosis are capable of differentiating along various cell lines and are not limited to megakaryocyte differentiation, suggesting that myelofibrosis and megakaryoblastic leukemia are not identical [32]. Recently, a study by Breton-Gorius et al. [17] has emphasized the need for the simultaneous use of several lineage restricted markers in the diagnosis of leukemia, since they detected PPO activity in most cases of early erythroid leukemia, and carbonic anhydrase and ferritin (erythroid markers) in some leukemic platelets and micromegakaryocytes.

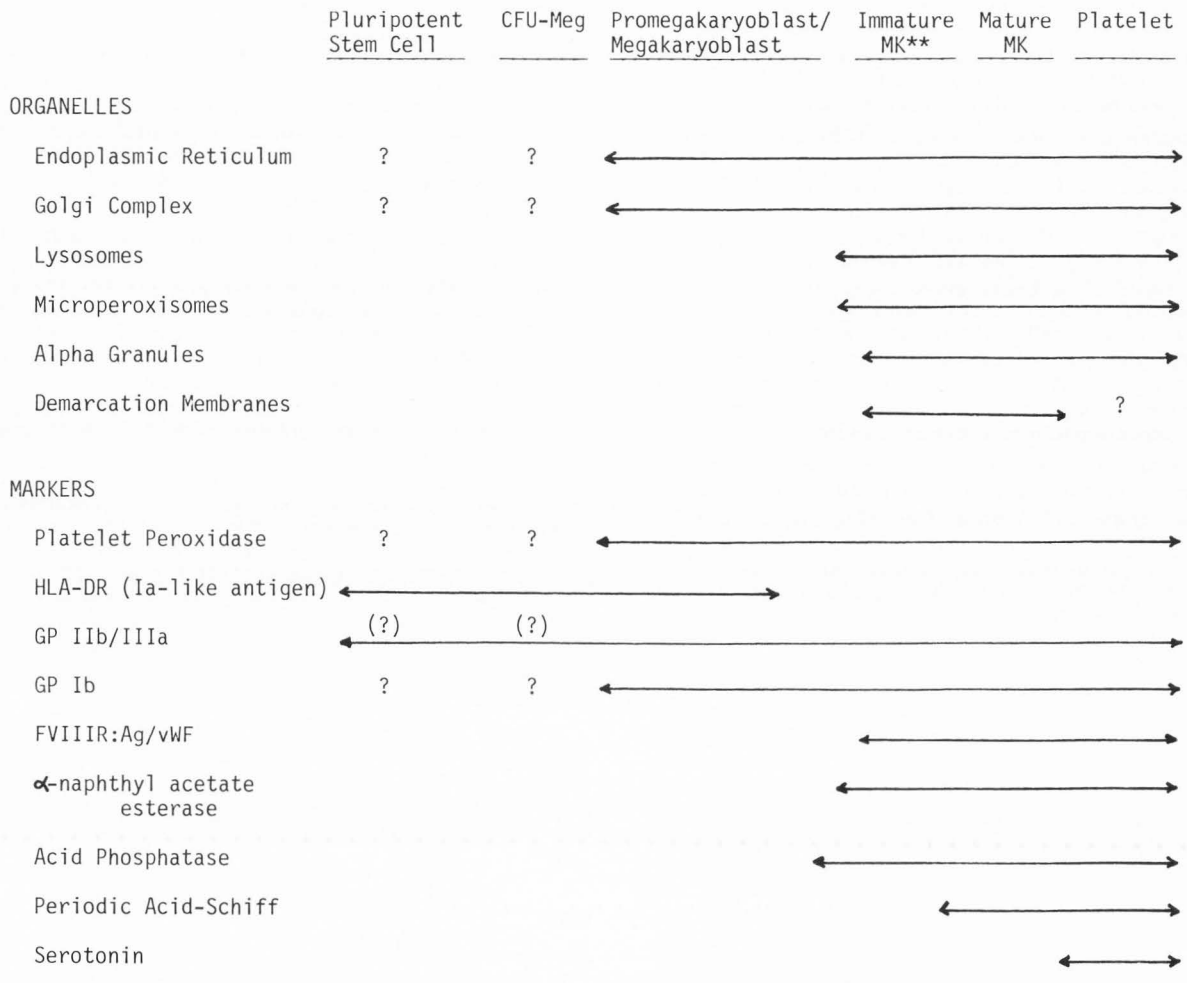
Based upon data obtained from studies of normal megakaryocytes and megakaryocytes from leukemic patients, a scheme for the appearance of various morphologic compartments and antigens during the maturation of this cell lineage is proposed (Table 4).

Summary

As megakaryocytes mature and differentiate, they acquire increasing amounts of certain organelles and contain decreasing amounts of others. Immature megakaryocytes contain abundant rough endoplasmic reticulum and Golgi complex, but few granules and demarcation membranes. In contrast, mature megakaryocytes contain decreasing amounts of rough endoplasmic reticulum and Golgi complex, and increasing amounts of granules and demarcation membranes.

Similarly, megakaryocytes lose and acquire various markers throughout their maturational stages. At one end of the megakaryocyte maturational spectrum is Ia-like antigen, which appears to be present only in immature megakaryocytes. At the other end of the spectrum is serotonin, which is generally observed only in platelets. GP IIb/IIIa and platelet peroxidase are expressed extremely early in differentiation. GP Ib, lysosomal enzymes, and α granule components appear somewhat later in maturation than GP IIb/IIIa and platelet peroxidase. Recent studies point to the necessity of using several different markers simultaneously to accurately diagnose leukemia. The purification of pluripotent stem cells and CFU-Meg will facilitate further studies on the phenotypic profile(s) of megakaryocytes at the earliest stages of development.

Table 4. Appearance of Organelles and Markers During the Maturation and Differentiation of the Megakaryocyte/Platelet Lineage*



* Modified from Koike (Blood 64:683, 1984) and Koike et al. (Blood 69:957, 1987). This table is based, in part, on data obtained from leukemic megakaryocyte precursors and it cannot be excluded that normal maturation is slightly different.

** MK=megakaryocyte

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no Discussion with Reviewers.