Dysplastic Platelets and Circulating Megakaryocytes in Chronic Myeloproliferative Diseases. II. Ultrastructure of Circulating Megakaryocytes

By Jorge E. Maldonado

Small circulating megakaryocytes were identified in four patients with chronic myeloproliferative disorders, and their morphologic features by light and electron microscopy are described. The morphologic and cytogenetic studies suggest that at least some of these cells are truly monoclonal. The circulating megakaryocytes were associated with the presence of dysplastic platelets. Both findings are thought to represent involvement of the megakaryocytic-platelet line, probably as an expression of stem cell disease.

Small numbers of megakaryocytes are found in the peripheral blood in humans and in animals; they have been demonstrated primarily by concentration of buffy coat preparations, and they are filtered out in the lungs. Under conditions of stress and in certain pathologic states, larger numbers of megakaryocytes are present in the circulating blood. This paper reports observations made by light and electron microscopy on small megakaryocytes present in the peripheral blood of patients with variants of the chronic myeloproliferative diseases.

MATERIAL AND METHODS

The clinical summaries of the cases, including the cytogenetic studies, were presented in the accompanying paper (cases 1, 2, 3, and 4). Blood smears obtained by finger puncture were stained with Wright's stain and used for the differential leukocyte counts and for the morphologic study of the cells presumably identified as circulating megakaryocytes. For electron microscopy, samples were taken from the buffy coat obtained either by differential centrifugation or by spontaneous sedimentation at a 45° angle after addition of dextran. The processing method used for electron microscopy was as previously described. Initial fixation was with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3).

RESULTS

Light Microscopy

In the differential leukocyte count at the time that samples were obtained for electron microscopy, performed by an observer unaware of our study, the megakaryocytes ranged from 1% in case 4 to 63.5% in case 1. Some of the cells had nuclear and cytoplasmic characteristics that clearly indicated a relationship...
Peripheral blood smears and stained with Wright's stain. These illustrations show cells classified as circulating megakaryocytes. These cells show a single and immature nuclear profile and a lymphoblastoid appearance. Note pseudopods of clear blue cytoplasm, reminiscent of the "blue platelets," present in some preparations and illustrated in upper right. (Upper left, x 155; upper right, x 500; lower left, x 350; and lower right, x 250.)

to the megakaryocytic line. On the other hand, in all of the preparations but particularly in cases 1 and 2, there were nucleated elements that are best described as atypical lymphoblastoid cells; on close analysis, these were placed in the megakaryocytic series. These cells had a light blue cytoplasm, frequently exhibited peripheral pseudopods, and were at times vacuolated (Fig. 1). Release of cytoplasmic fragments with the features of "blue platelets" was seen rather frequently. Cytoplasmic granulation was sparse. The nuclei were centrally or eccentrically located, the chromatin was finely dispersed, and nucleoli were frequent. In the smear preparations, the diameters of the cells identified as megakaryocytes ranged from 8 to 25 μm.

Electron Microscopy

By electron microscopy the identification of the circulating megakaryocytes was easy and accurate. Even though the cells differed from the normal marrow megakaryocytes, they had characteristic cytoplasmic features of megakaryocytes such as demarcation membranes, typical "bull's-eye" or alpha granules, and vacuoles of the open canalicular system.

The circulating megakaryocytes ranged in diameter from 5 to 12 μ. Although some of this variation may reflect the level of the section, it appeared that very small cells (5 μ) with cytoplasmic features of platelets but with a nucleus ("nucleated platelets") and very small megakaryocytes were present (Fig. 2).

By and large, the cells had an oval configuration and a round or oval single
Fig. 2. Cells 1, 2, 3, and 4 are circulating megakaryocytes of different sizes and stages of development. Dystrophic agranular platelets (P) are present. Also, a mitotic figure (M) is apparent. Cell 1 could be described as a "nucleated platelet." G in cell 2, Golgi apparatus; N, nucleus.
Fig. 3. (A) Circulating megakaryocyte, showing single nuclear profile (N), Golgi membranes (G), membranes of the demarcation system (DMS), rough endoplasmic reticulum (RER), and open canalicular system (OCS) as well as typical "bull's eye" granules and mitochondria. (B) Higher magnification of area marked in (A), showing granules (G), mitochondria (M), and microtubules (arrows) in cytoplasm of circulating megakaryocyte. N, nucleus.
nuclear profile. The chromatin was fine, and nucleoli often were present. The cytoplasm was relatively abundant, but in the younger cells it was scanty, and the nucleocytoplasmic ratio was low. Not infrequently, one or more profiles of Golgi apparatus were seen in the paranuclear region (Figs. 2 and 3). Sections of microtubules were conspicuous (Fig. 3).

Three types of membranous systems predominated in the cytoplasm: (1) demarcation membrane system, (2) rough endoplasmic reticulum, and (3) open canalicular system (Fig. 3).

The demarcation membrane system of the circulating megakaryocyte had features of the demarcation membrane system of the marrow megakaryocyte. It was formed by paired smooth membranes separated by a variably distended space, at times filled with a moderately electron-dense material. Although the membranes crossed the cytoplasm, no clear-cut segmentation of platelets or evidence of their release was demonstrated. The open canalicular system appeared in the sections as distended empty sacs, sometimes with definite communication with the surface of the cell or in continuity with the demarcation membrane system (Fig. 3). These distended sacs probably correspond to the cytoplasmic vacuoles seen by light microscopy.

Short but well-defined lamellae of rough endoplasmic reticulum and free ribosomes and polysomes were seen in many cells, but particularly in the more immature ones. Lamellae of smooth membranous system, possessing the features of the dense tubular system, also were observed.

Besides the membranous systems, the cytoplasm showed granules, mitochondria, and, occasionally, accumulations of glycogen. Typical very dense bodies, so-called serotonin granules, were seen only rarely. Most of the granules were of the type described as “bull’s-eye” or alpha type—a moderately dense structure with an eccentric and denser round core. No significant abnormalities of the mitochondria were clearly discernible, although some were larger and more elongated than in normal platelets or normal marrow megakaryocytes.

Some of the circulating megakaryocytes had a regular, relatively smooth surface, while other cells exhibited pseudopods (Figs. 4 and 5) composed of essentially organelle-free cytoplasm.

A phenomenon of great interest was the close cell-to-cell interaction between the circulating megakaryocytes and the adjacent platelets and megakaryocytes (Figs. 4 and 5). In the interacting cells, cytoplasmic changes similar to those observed during the early stages of platelet aggregation were seen. The most remarkable change consisted of the central and perinuclear migration of the various organelles, with clearing and pseudopod formation of the peripheral cytoplasm.

DISCUSSION

In 1922 Minot called attention to the occurrence of circulating megakaryocytes in myeloproliferative states. Since then, circulating megakaryocytes and megakaryocytic fragments have been reported in agnogenic myeloid metaplasia, in chronic granulocytic leukemia, and in primary thrombocytopenia. In some patients, as in case 1 of our series, the number of circulating megakaryocytes and the degree of marrow infiltration by megakaryocytic
Fig. 4. Section of pellet of aggregated cells, showing close interaction of circulating megakaryocyte at lower part of the illustration and platelets above and below it. Note clear peripheral cytoplasm and pseudopod formation as well as central migration and clustering of the organelles, which remain discernible.

precursors are of such magnitude that the term "megakaryocytic leukemia" would be appropriate.\textsuperscript{14,19}

Differentiation between circulating megakaryocyte and nucleated platelet is difficult. We reserve the use of the term "circulating megakaryocyte" for those elements with megakaryocytic cytoplasmic features and possessing a relatively large or prominent nucleus.

At least some of the circulating megakaryocytes observed in our patients seem to be true mononuclear cells. At least morphologically, by light as well as by electron microscopy, they appear to have a single nucleus, and no polyploid lines were demonstrated in the cytogenetic studies.\textsuperscript{8} The earliest megakaryocyte recognizable by light microscopy is a 4N cell,\textsuperscript{16,17} and the demonstration of a
diploid megakaryocytic precursor would constitute a major advance in establishing a link between the uncommitted stem cell and the more differentiated and clearly recognizable, but polyploid, megakaryocytic precursors.18,19

Trautmann20 and Franzén et al.21 recognized small megakaryocytes in the bone marrow of patients with chronic granulocytic leukemia. Undritz and Nusselt-Bohaumilitzky22 proposed that the presence of small megakaryocytes in
the myeloproliferative disorders represented a "regression" toward a diploid megakaryocytic line. This hypothesis has received support recently. Queisser et al.\textsuperscript{23} indeed found 2N megakaryocytes in a patient with preleukemia that evolved to acute myelomonocytic leukemia with megakaryocytes of abnormal morphology.

Breton-Gorius et al.\textsuperscript{24-26} studied the circulating megakaryocytes in five patients with variants of the myeloproliferative disorders including myelofibrosis. The ultrastructural examination revealed small and dystrophic circulating mononuclear megakaryocytes, similar to those found in this study. The bone marrow megakaryocytes also were morphologically abnormal. We have made similar observations in patients with myelomonocytic leukemia and refractory anemia.\textsuperscript{27} Breton-Gorius et al.\textsuperscript{24-26} have used the peroxidase reaction for identification of megakaryocytes. We do not think the technique is suitable for application to routine diagnostic hematology.

Popescu\textsuperscript{28} also found circulating megakaryocytes in 14 patients who had myeloproliferative diseases and proposed that these cells release platelets intravascularly.

In our patients, the presence of circulating megakaryocytes was associated with morphologically abnormal platelets.\textsuperscript{8} A disturbed marrow or splenic microenvironment may lead to the circulation of such abnormal megakaryocytes.

As in the normal megakaryocyte,\textsuperscript{29-33} in our patients typical alpha granules and demarcation membranes were more abundant in the more mature elements. Although demarcation membranes were present, there was no evidence of platelet segmentation or release. As in the normal rat,\textsuperscript{34} microtubules were found in all stages of development. And as in normal human,\textsuperscript{35} mouse,\textsuperscript{31} and rat,\textsuperscript{32} rough endoplasmic reticulum and polysomes were present with greater frequency and in larger amounts in the more immature cells.

The cytoplasmic changes of the circulating megakaryocytes seen during cell-to-cell interaction may indicate a certain degree of functional ability.

In patients with myeloproliferative diseases, a certain number of cells frequently are classified by light microscopy as "blasts." Although the majority of these cells are myeloblasts, in certain instances some most likely are megakaryocytic precursors. These cells have been described as "lymphoblastoid," and indeed they resemble atypical immature lymphocytes. In rats, Odell and Jackson\textsuperscript{36} have demonstrated by radioautography that the youngest megakaryocytes have a lymphocytelike appearance. It is difficult to establish with any degree of accuracy the true classification of a given cell by light microscopy. We suspect, however, that the frequency of circulating megakaryocytes in the myeloproliferative diseases very well may be higher than is reflected by the usual leukocyte differential count.

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