Dysplastic Platelets and Circulating Megakaryocytes in Chronic Myeloproliferative Diseases. I. The Platelets: Ultrastructure and Peroxidase Reaction

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A light and electron microscopic study was made of the circulating platelets from five patients with variants of the chronic myeloproliferative diseases (agnogenic myeloid metaplasia, chronic granulocytic leukemia, and "megakaryocytic leukemia"). Morphologic abnormalities were found in at least some of the platelets in all of the patients. Presence of giant forms, paucity or absence of granulation, disorganization and scarcity of the microtubules, and haphazard distribution and hypertrophy of the dense tubular and open canalicular systems constituted the main abnormalities. Additionally, there were features of platelet immaturity, including abundant rough endoplasmic reticulum and ribosomes and presence of Golgi profiles, centrioles, and nuclear remnants. The relationship, if any, of these morphologic abnormalities to the hemostatic defects in myeloproliferative diseases remains to be elucidated.

IN A LIGHT and electron microscopic study of the platelets in five patients with variants of the chronic myeloproliferative diseases, clear-cut abnormalities were found. These structural abnormalities may be significant in regard to the platelet functional defects and the hemostatic disturbances that are commonly observed in the myeloproliferative syndromes.

MATERIAL AND METHODS

Wright-stained smears were used for evaluation of platelet morphology. Platelet size was measured with a movable eyepiece and a standard stage micrometer. The widest diameter of 100 platelets was measured to provide a range of diameter values. Platelet-rich plasma (PRP) was prepared from heparinized blood. Samples of the upper two-thirds and lower one-third of the PRP were processed separately. For electron microscopy, the platelets were fixed in the PRP. The initial fixation was with 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 5-10 min; then a platelet pellet was obtained and fixed in 3% glutaraldehyde for 1 hr.

For the peroxidase reaction, the techniques of Breton-Gorius and Guichard and White were used. Control samples were processed without H2O2 or diaminobenzidine.

The samples were postfixed with 2% osmic acid in phosphate buffer and embedded in epoxy resin. The sections were double stained with lead citrate and uranyl acetate. For the peroxidase
reaction, sections were viewed unstained, lightly stained with lead citrate, and double stained. A Hitachi HU-12 electron microscope was used.

The chromosomes were studied using either the direct bone marrow method of Tjio and Whang as modified by Lam-Po-Tang or 72-hr PHA-stimulated peripheral blood cultures utilizing the Difco TC kit method.

CASE REPORTS

Case 1

In December 1965, this 39-yr-old white laborer was given a diagnosis of myelofibrosis with agnogenic myeloid metaplasia. The hemoglobin level was 11.9 g/dl, the leukocyte count was 7900/cu mm with 2% blasts and 1.5% megakaryocytes. The platelet count was 356,000/cu mm, and giant forms were observed. He was treated with splenic irradiation and busulfan (Myleran).

Coagulation studies in December 1970, July 1971, and December 1971 revealed qualitative platelet abnormalities including prolonged Ivy bleeding time; defective aggregation with ADP (no second wave and subsequent disaggregation), with epinephrine (absent), and with collagen (delay in onset); abnormal platelet adhesiveness; and an abnormal prothrombin consumption test. The platelet factor 3 (PF-3) content was decreased. During the latter part of 1971 the platelet count ranged from 1,000,000 to 1,800,000/cu mm.

In December 1971, splenectomy was performed. Postoperatively, the platelet count increased to 2,577,000/cu mm, with more bizarre platelet morphology. The number of circulating megakaryocytes also increased. He received nitrogen mustard, chlorambucil (Leukeran), and busulfan (Myleran).

In March 1972, when the samples for electron microscopy were obtained, the leukocyte count was 172,000/cu mm with 6.5% neutrophils, 2% lymphocytes, 1.5% metamyelocytes, 9% myelocytes, 1% progranulocytes, 16.5% blasts, and 63.5% megakaryocytes, many of them being young, mononuclear, and of atypical (lymphoblastoid) morphology. There were four normoblasts per 100 leukocytes. The platelet count was 198,000/cu mm. A bone marrow aspirate showed abundant blasts and megakaryocytes and was interpreted as representing leukemic transformation.

In a direct chromosome study from the buffy coat of the peripheral blood, 25 evaluable metaphases were karyotyped. In three metaphases there was loss of the Y chromosome. No Ph chromosome was seen.

The patient died in May 1972 from gastrointestinal bleeding and bronchopneumonia. An autopsy revealed infiltration of liver, kidneys, and lymph nodes by immature myeloid cells including megakaryocytic precursors. The bone marrow was sclerotic.

Comment. This patient appears to represent the evolution of agnogenic myeloid metaplasia to megakaryocytic leukemia. No polyploid lines were demonstrated. The loss of the Y chromosome is interpreted as an age-related phenomenon. Prominent throughout his illness was a bleeding tendency.

Case 2

A 65-yr-old white farmer was admitted in November 1971 for evaluation of agnogenic myeloid metaplasia diagnosed elsewhere in February 1971.

At the time of diagnosis, the hemoglobin level was 8.5 g/dl, and the leukocyte count was 40,000/cu mm with granulocytic immaturity to the blast stage (14%). The platelet count was 248,000/cu mm. The marrow showed an increase in megakaryocytes and fibrous tissue. He was treated with busulfan and prednisone.

In November 1971, when the samples for electron microscopy were obtained, the leukocyte count was 28,700/cu mm with 30.5% neutrophils, 10.5% lymphocytes, 4.5% monocytes, 4% eosinophils, 2.5% basophils, 1% metamyelocytes, 4% myelocytes, 7% progranulocytes, 16% blasts, and 20% megakaryocytes. The platelet count was 344,000/cu mm. The platelets were large and of abnormal shape.

A marrow biopsy showed hypercellularity, megakaryocytic hyperplasia, and moderate fibrosis.

On a direct chromosome preparation from the buffy coat, a typical Ph1 chromosome was found in all intact metaphases. There was Y chromosome loss which, as in case 1, was interpreted as an age-related phenomenon. No polyploid lines were demonstrated.
DYSPLASTIC PLATELETS AND CIRCULATING MEGAKARYOCYTES.

The Ivy bleeding times were 7½ and 10 min, the platelet factor 3 (PF-3) activity was decreased, and the prothrombin consumption test was abnormal. Platelet aggregation studies with ADP, epinephrine, and collagen were abnormal. Platelet retention on glass beads was normal.

The patient received roentgen radiation to the spleen and busulfan. He died in March 1972. No autopsy was performed.

Comment. This patient had an atypical myeloproliferative disease. There were features of myeloid metaplasia and of chronic granulocytic leukemia (primarily the presence of the Ph¹ chromosome). The high number of circulating megakaryocytes was unusual.

Case 3

A 60-yr-old laborer was first admitted in July 1968 for evaluation of anemia of 2 yr duration. He had the somatic features of Klinefelter's syndrome. The tip of the spleen was palpable. The hemoglobin level was 11.3 g/dl, and the leukocyte count was 5500/cu mm with granulocytic immaturity to the blast stage (0.5%). The platelet count was 568,000/cu mm. Large and atypical platelets were noted. The bone marrow showed increased fibrous tissue. A buccal smear was positive for the Barr body. Two direct bone marrow chromosome preparations and a peripheral blood culture were done. All studies showed a 47,XXY karyotype in all metaphases. The Ph¹ chromosome was not present.

Progressive enlargement of the spleen and liver was observed. Recurrent splenic infarcts occurred. In September 1970, he received roentgen radiation to the spleen, and testosterone enanthate (Delatestryl) therapy was begun. The bone marrow was densely fibrotic.

In February 1972, when the samples for electron microscopy were obtained, the hemoglobin value was 11.7 g/dl. The leukocyte count was 24,600/cu mm with 70% neutrophils, 4% lymphocytes, 1% monocytes, 1% eosinophils, 2% basophils, 7% metamyelocytes, 13% myelocytes, and 2% megakaryocytes. The platelet count was 536,000/cu mm. The platelet population was mixed, with normal and giant platelets, often devoid of granulation ("blue platelets"), being present. Coagulation studies revealed abnormalities of platelet function [decreased retention on glass beads (3%) and abnormal aggregation with epinephrine and ADP].

In July 1972, another splenic infarct occurred, and he died shortly afterward. Autopsy revealed massive infarction and hemorrhage of the spleen, thrombosis of the common carotid arteries, and multiple cerebral infarcts.

Comment. This patient had agnogenic myeloid metaplasia and myelofibrosis. A thrombotic diathesis was prominent.

Case 4

A 24-yr-old housewife was admitted in August 1971 for evaluation of splenomegaly and weight loss.

The hemoglobin level was 10.1 g/dl, and the leukocyte count was 350,000/cu mm with granulocytic immaturity to the blast stage (8.5%) and 1.5% circulating megakaryocytes. The platelet count was 954,000/cu mm with frequent large and atypical forms. The bone marrow showed marked increase in granulopoiesis, a maturation bulge at the myelocyte level, and basophilia. A chromosome study of the bone marrow aspirate revealed a typical Ph¹ chromosome in all metaphases. She was treated with radiation, to the spleen, and busulfan.

In February 1972, when the sample for electron microscopy was obtained, the patient had fever and multiple retinal hemorrhagic infiltrates. The leukocyte count was 22,300/cu mm with 7.5% lymphocytes, 4.5% monocytes, 6.5% eosinophils, 39.5% basophils, 24.5% neutrophils, 1.5% metamyelocytes, 8% myelocytes, 2.5% progranulocytes, 4.5% blasts, and 1% megakaryocytes. The platelet count was 71,000/cu mm, and the platelets were large and of abnormal shape.

Subsequently, massive enlargement of the liver and spleen occurred, and she died in May 1972. No autopsy was performed.

Comment. This patient had Ph¹-positive, but clinically severe, chronic granulocytic leukemia.

Case 5

This 55-yr-old housewife was first seen in September 1968; there was splenic enlargement. The hemoglobin value was 12.8 g/dl, and the leukocyte count was 12,600/cu mm with granulocytic...
immaturity and 0.5% megakaryocytes. The platelet count was 844,000/cu mm, and the platelets were large and atypical in shape. A biopsy of the bone marrow showed both cellular and fibrotic areas and an increased number of megakaryocytes.

She returned in 1969, 1970, and October 1972. Except for gradual increase in the leukocyte count to 70,000/cu mm, no significant change occurred.

In March 1973, when the samples for electron microscopy were taken, the leukocyte count was 146,000/cu mm with 6.5% lymphocytes, 2.5% monocytes, 2.5% eosinophils, 3% basophils, 44.5% neutrophils, 5% metamyelocytes, 30% myelocytes, 4% progranulocytes, and 2% blasts. The platelet count was 225,000/cu mm. Morphologic abnormalities of the platelets persisted. The bone marrow was fibrotic.

Comment. In this patient, most of the clinical features are in keeping with a diagnosis of agnogenic myeloid metaplasia, but the final phase with very high leukocyte count is unusual.

RESULTS OF SPECIAL STUDIES

Light Microscopy

In the Wright-stained smears, particularly in cases 2, 3, 4, and 5, there was a duality of the platelet population, with normal and abnormal forms being present. The abnormal platelets were prominent and could be classified into two types: (1) the predominant one characterized by a clear blue, agranular cytoplasm ("blue platelets"), often with extensive pseudopods at the periphery (Fig. 1, A–D), and (2) giant granular forms.

Most of the blue platelets were large and ranged from 3.0 to 13.0 μ in diameter. Few small blue platelets were seen. The diameter range of the normal

![Fig. 1. Peripheral blood smears; all are stained with Wright's stain. (Upper left) Large, round, "blue platelet" with scanty granulation. (x 350). (Upper right) Giant "blue platelet" with no discernible granulation; a small dystrophic platelet is also seen. (x 350). (Lower left) Large, agranular platelet, showing a central condensation which superficially resembles a nucleus (pseudonucleus). (x 350). (Lower right) Giant platelets with central cytoplasmic condensation; note particularly the prominent pseudopod formation. (x 350).](image-url)
platelets was 0.64-3.42 µ. The blue platelets often appeared isolated and at times showed condensation of a darker blue color toward the center of the cell, giving the appearance of a pseudonucleus (Fig. 1 C).

When viewed under phase contrast or with interference Nomarski optics, the glutaraldehyde-fixed platelets frequently appeared round (suggesting that they were probably spherical), in contrast to normal platelets that often appear discoid.

**Electron Microscopy**

The percentage of abnormal forms varied but reached essentially 100% in case 1. The abnormalities related to size, shape, number and distribution of the various organelles, and the presence of features of immaturity. In general,

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**Fig. 2.** (A) Normal discoid platelet sectioned in longitudinal plane. Note microtubules (MT) at poles. Adjacent to microtubules are profiles of dense tubular system (DTS), also noted on close inspection elsewhere. Granules (G) and mitochondria (M) are present as well as vacuoles of open canalicular system (OCS). (B) Dystrophic platelet from patient with chronic myeloproliferative disorder. This discoid shape was rarely seen but is presented to contrast with the normal appearance shown in (A). Note absence of granulation and proliferation of interwoven membranes, both of open canalicular (OCS) and dense tubular systems. Microtubules (MT) are present.
Fig. 3. Large, round, hypogranular, dystrophic platelets. (A) Showing several sections of dilated or open canalicular system. (B) Showing one microtubule (arrow) and canalicularae of both open and dense tubular systems.
the largest and most dystrophic platelets were observed in the samples taken from the lower third of the platelet-rich plasma.

The morphologically abnormal platelets were larger than the normal elements, and diameters of 10 μ were not infrequent. The majority of these platelets were round. The typical discoid shape of the normal platelet (Fig. 2A) was only occasionally seen, and, when observed, other abnormal features were present (Fig. 2B).

The distribution of the cell organelles (granules, mitochondria, and open canalicular and dense tubular systems) was altered, but there was no single pattern of abnormality. In some platelets the most prominent change was paucity or absence of granulation (Fig. 3). In certain instances, all cell organelles were markedly decreased, and the platelets were simply cytoplasmic fragments only recognizable as platelets because of one or two characteristic features. These elements should be differentiated from cytoplasmic fragments of leukocytes. The mitochondria were sometimes abundant. Masses of glycogen were prominent in some platelets.

A clear-cut circumferential band of microtubules was not demonstrated in the dystrophic platelets. Either disorganized or short segments of microtubules were present (Fig. 3B). Membranous hypertrophy was a conspicuous feature. Some of the membranes were part of the rough endoplasmic reticulum with ribosomal granules (Fig. 4). Other membranes, also forming tubules, canaliculi, or vacuoles, depending on the plane of section, were smooth or agranular (Fig. 5).

In several instances we were able to demonstrate unquestionable continuity of the rough or ribosomal endoplasmic reticulum with the smooth dense tubular system (Fig. 4). This finding supports the concept that the dense tubular system is derived from the rough endoplasmic reticulum by ribosomal loss.6

In many cells the membranous system formed intricate structures of tubular elements (Fig. 6). It has been demonstrated that the rough endoplasmic reticulum and the dense tubular system are positive for peroxidase, in contrast to the tubular elements of the Golgi apparatus and open canalicular or surface-connecting system, which are negative in this reaction.5'6 We found that the system most frequently hypertrophic was the dense tubular system (Fig. 5B). In some elements there was a close admixture of the dense tubular and the open canalicular systems, while in others the rough endoplasmic reticulum predominated, and in some the Golgi apparatus was prominent or multiple. Also, there were free ribosomes and polysomes. Some giant platelets showed profiles of demarcation membranes (Fig. 7). Nucleated forms (giant platelets with nuclear remnants and circulating megakaryocytes) were also seen, particularly in cases 1 and 2. The morphology of these cells will be discussed in a separate report.9 Mitotic figures were not infrequent.

The electron microscopic study showed that the central condensation noted on light microscopy ("pseudonucleus") resulted from central clustering of the organelles (granules, mitochondria, and membranes), leaving a clear, organelle-free, peripheral cytoplasm (Fig. 8).
Fig. 4. (A) Section of whole dysplastic platelet, showing scarce granulation but abundant rough endoplasmic reticulum and dense tubular system. In center is prominent Golgi apparatus (G). (B) Enlargement of square in (A). At several sites (arrows) there is continuity of granular or rough endoplasmic reticulum with a smooth membranous system with morphologic features of dense tubular system.
Fig. 5. Dysplastic platelets, showing paucity of granulation and proliferation of dense tubular system. Few sections of open canalicular system are present. (A) Processed in a routine manner. (B) Specimen incubated for peroxidase reaction. Both double stained with uranyl acetate and lead citrate.
DISCUSSION

Newer and better methods of tissue processing for electron microscopy have permitted more accurate assessment of platelet ultrastructure. Giant and hypogranular platelets have been described in a variety of familial or constitutional disorders. The ultrastructure of these platelets resembles that of the “blue platelets” seen in our patients who have an acquired thrombocytopenia incidental to a myeloproliferative disorder (both of the type reported here and in variants of myelomonocytic leukemia).

In myeloproliferative diseases, but particularly in agnogenic myeloid metaplasia, the presence of giant, bizarre, hypogranular or agranular platelets has been reported. Only two significant ultrastructural studies of the platelet line in myeloproliferative diseases have been published. In both studies, osmic acid was the only fixative, and fixation was carried out in the cold. Hattori found giant and hypogranular forms as well as cells with features of immaturity and hypertrophy of the membranous systems. Among 40 patients
studied by Haguenau and co-workers, there were five with chronic granulocytic leukemia and five with myeloid metaplasia. They remarked on the “hyperplasia of the vesicular-vacuolar system” and the considerable variation in size and shape of the platelets.

The presence, in peripheral blood, of dysplastic platelets could result from their release from abnormal megakaryocytes, or from a disturbance in the splenic or marrow microenvironment. Abnormalities of the marrow megakaryocytes in myelofibrosis have been described. In chronic granulocytic leukemia, the megakaryocytes have been found to contain the Ph chromosome. Megakaryocytes of small size and with low ploidy values (4N and 8N), as well as ultrastructural abnormalities, have been reported in chronic granulocytic leukemia. It seems logical to conclude that, in the myeloproliferative diseases, the platelet abnormality probably reflects primarily a defect in the bone marrow precursor, perhaps in the common stem cell.

The presence of a significant number of circulating “immature” platelets, including nucleated forms, would add to the leukoerythroblastic picture frequent in myeloproliferative diseases.

The morphologic platelet abnormalities that we are reporting, while definite, cannot be considered specific. We think, however, that they clearly establish
Fig. 8. Dysplastic platelet, showing clustering of organelles that probably corresponds to the pseudonucleus observed by light microscopy (Fig. 1C.) At high magnification of area marked by arrow, numerous disorganized microfilaments were discerned.

the involvement of the platelet line in this group of disorders. It is tempting to relate the morphologic changes to the hemostatic abnormalities,2,4 but such a relationship cannot be concluded from our study.

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