Ultrastructural alterations of megakaryocytes in thrombocytopenia: A review of 43 cases

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Abstract
Thrombocytopenia is a frequent occurrence in a variety of hematopoietic diseases; however, the details of the mechanism leading to low platelet count remain elusive. Megakaryocytes are a series of progenitor cells responsible for the production of platelets. Alterations in megakaryocytes in the bone marrow are a causative factor resulting in thrombocytopenia in varied diseases. Based on ultrastructural analysis of incidentally encountered megakaryocytes in 43 patients with blood diseases marked by low platelet counts, electron micrographs demonstrated that aberrant megakaryocytes predominated in idiopathic thrombocytopenic purpura, aplastic anemia, and myelodysplastic syndrome; autophagy, apoptosis, and cellular damage in megakaryocytes were a prominent feature in aplastic anemia. On the other hand, poorly differentiated megakaryocytes predominated in acute megakaryoblastic leukemia (AMKL) although damaged megakaryocytes were seen in non-AMKL acute leukemia. This paper documents the ultrastructural alterations of megakaryocytes associated with thrombocytopenia and reveals distinctive features for particular blood diseases. A comment is made on future avenues of research emphasizing membrane fusion proteins.

Keywords: Blood disease, Megakaryocyte, Platelet, Thrombocytopenia, Ultrastructure

1. INTRODUCTION

Thrombocytopenia – marked primarily by low platelet counts – is regarded as a comparatively rare condition, affecting some 200,000 patients a year worldwide (data from the National Organization for Rare Diseases) and yet the patients who have this condition suffer unpleasant symptoms such as hemorrhage, rash, headache, hematuria, heavy menstruation in the case of women, malaise and fatigue, and may need medication such as autoimmune suppressants. The megakaryocyte – the cell of origin for platelets as mature elements in the blood – is clearly the focus of attention in the investigation of the mechanism of thrombocytopenia, and understanding its normal and abnormal development would be key to helping to understand thrombocytopenia.

The normal megakaryocyte undergoes a development in which 4 stages are observed morphologically. Stage I cells are characterized by blast-cell features—small size and a lack of marker structures; stage II cells have small or modest numbers of dense-core granules and α-granules, as well as early development of the demarcation membrane system (DMS) and the open canalicular system (OCS), structures which are increasingly well developed in stages III and IV; in stage IV, the granules, DMS, OCS, and pro-platelets are fully developed.1,2 By contrast, megakaryocytes in pathologic conditions—for example, idiopathic thrombocytopenic purpura (ITP), aplastic anemia, and myelodysplastic syndrome (MDS) – show abnormal structures.3

In the present study, we have studied megakaryocytes observed as incidental findings in the bone marrows of 43 patients with various blood diseases complicated by thrombocytopenia, including 5 cases of ITP, 18 of aplastic anemia, 6 of MDS, 7 of acute megakaryocytic leukemia (AMKL), and 7 of acute leukemia not of AMKL type (Table 1). All patients had low platelet counts and were diagnosed at the Institute of Hematology and Blood Diseases Hospital, Tianjin using standard procedures. The bone marrow aspirates were processed for transmission electron microscopy as previously described.4

2. RESULTS

We summarize the abnormal features of megakaryocytes to provide data which, in combination with other techniques, might in due course, point to possible mechanisms of thrombocytopenia.

2.1. Idiopathic thrombocytopenic purpura

ITP, also known as immune thrombocytopenia, is always complicated by a low platelet count and serious bleeding episodes in children.5 ITP is marked by increased platelet destruction in the circulation and decreased platelet production in the bone marrow.6 Quite apart from the destruction of platelets by autoantibodies, abnormalities of megakaryocytes, which could influence platelet production, have been noted recently.7,8 In our
material, megakaryocytes exhibited an abnormally high accumulation of glycogen and increased numbers of mitochondria and DMS in 4 out of 5 cases. One case showed abundant glycogen particles around the nuclei of megakaryocytes; abnormally abundant glycogen was present in platelets too (Fig. 1). These changes are consistent with observations in morphologic reviews of ITP as well as in human subjects treated with prednisone. It was thought that glycogen accumulation in megakaryocytes and platelets resulted from the stress of autoimmune platelet destruction in ITP.9,10 One megakaryocyte was full of mitochondria but had few megakaryocytic-specific organelles—namely, granules, DSM, and OCS (Fig. 2A, B). Another megakaryocyte included massive DMS that failed to segregate cytoplasm into individual pro-platelets (Fig. 2). In addition, 1 of 5 cases showed a badly damaged megakaryocyte which featured a condensed nucleus and necrotic cytoplasm (Fig. 2).

Although the causative factors behind the development of these abnormalities remain to be identified, the final result is the abnormal accumulation of glycogen and mitochondria, and the abnormal distribution of DMS and OCS which interfere with platelet-production in megakaryocytes in ITP.

### 2.2. Aplastic anemia

Low platelet counts are a significant sign associated with the prognosis of aplastic anemia.11 Some clinical reports have indicated that alterations in megakaryocytes are associated with decreased platelets and progression in aplastic anemia.12–15 It is usually thought that thrombocytopenia results from the loss of megakaryocytes in bone marrow, although this has not been demonstrated morphologically in aplastic anemia.16

In the present study, all megakaryocytes showed pathologic changes indicating aberrant developmental features. In addition to other cytologic damage, autophagosomes, and apoptotic bodies were seen in 18 cases. Autophagy and aberrant development were predominant in 12 cases; here, megakaryocytes exhibited inclusions such as autophagosomes, autolysosomes, and lipofuscin bodies. Some of the autophagic cells were accompanied by accumulations of glycogen, lipid droplets, and vacuoles (Fig. 3).

Apopotic megakaryocytes were characterized by condensed nuclei, peripheral vesicles, and abnormal pro-platelets, the latter forming mainly in the peripheral cytoplasm. Apoptosis is a normal end-stage physiological feature in the process by which megakaryocytes shed platelets into the circulation,17 but most apoptotic megakaryocytes in aplastic anemia contained prominent rough endoplasmic reticulum (rER), lysosomes, and amorphous materials instead of pro-platelets (Fig. 4A, B). This suggests that in aplastic anemia these apoptotic megakaryocytes

| Table 1 | Alterations of megakaryocytes in thrombocytopenia-related diseases |
|---------|---------------------------------------------------------------|
|         | ITP | AA | MDS | AMKL | non-AMKL |
| Cases   | 5   | 18 | 6   | 7    | 7        |
| Aberrant features | 4  | 6  | 5   | 2    | 2        |
| Autophagy | 0  | 0  | 0   | 0    | 0        |
| Apoptosis | 0  | 2  | 0   | 0    | 0        |
| Cellular damage | 1  | 4  | 0   | 0    | 5        |
| Poor differentiation | 1  | 7  |     |      |          |

AA = aplastic anemia, AMKL = acute megakaryocytic leukemia, ITP = idiopathic thrombocytopenic purpura, MDS = myelodysplastic syndrome, non-AMKL = other than AMKL.
were experiencing pathologic change closely related to a decrease in platelets. Additionally, in 1 case of aplastic anemia, a megakaryocyte containing 3 necrotic neutrophils was observed, showing small and disruptive pro-platelets in the cytoplasm (Fig. 4C, D). We cannot explain this emperipolesis of necrotic neutrophils in megakaryocytes, and more cases need to be examined before formulating an explanation.

In summary, it is reasonable to assume that excessive autophagy, apoptosis, and cellular damage in megakaryocytes have some, as yet undefined, role in low platelet production, but nevertheless constitute an ultrastructural characteristic of thrombocytopenia in aplastic anemia.

2.3. Myelodysplastic syndrome

While formerly considered a form of pre-leukemia, MDS is regarded as a malignant myeloid hematopoietic condition often characterized by certain genetic mutations and morphologic alterations. Most patients have the complications of low platelet count in the circulation and abnormalities of megakaryocytes in the bone marrow\(^2\),\(^3\),\(^19\) and these 2 features are associated with the progression and prognosis of the disease.\(^2\),\(^2\),\(^21\) Some recent case reports have reported morphologic alterations of megakaryocytes in bone marrow from MDS patients.\(^2\),\(^2\),\(^2\)

In the present study, megakaryocytes from MDS patients were characterized by an asymmetric development of nucleus and cytoplasm, and aberrant distribution of cytoplasmic organelles in 5 out of 6 cases; in the 1 remaining case, megakaryocytes showed poorly differentiated features.

Most of the asymmetrically developed megakaryocytes had a small diameter of 10 to 15 \(\mu\)m, but showed mature characteristics including a small nucleus, \(\alpha\)-granules, dense core-granules, DMS, and OCS, but fewer pro-platelets in the cytoplasm. Some of them enclosed activated lymphocytes in a process resembling emperipolesis (Fig. 5). The small size of the megakaryocytes was usually thought to result from a failure of chromosomal polyploidization and synchronous cytoplasmic expansion.\(^2\),\(^4\),\(^2\)

Another alteration of megakaryocytes is the uneven distribution of DMS and OCS in the cytoplasm as a result of which pro-platelets were not demarcated properly. Some DMS and OCS were stacked focally and presented with structural abnormalities, instead of segregating cytoplasm into pro-platelets as in normal megakaryocytes (Fig. 6A, B). It may be a causative factor in the megakaryocyte failure to produce platelets in the bone marrow in MDS patients. Additionally, megakaryocytes exhibited undifferentiated and damaged features in 1 of 6 cases (Fig. 6C, D).

Therefore, megakaryocytes in MDS presented with diverse characteristics; the significance of these features in relation to thrombocytopenia needs the further study of a larger number of cases.

2.4. Acute megakaryocytic leukemia

AMKL often occurs in children and young patients and is characterized by a proliferation of malignant progenitors of megakaryocytic lineage in the bone marrow. Most cases are complicated by low platelet count, chromosomal alterations, and abnormalities of transcriptional regulators.\(^2\),\(^6\)

In the present study, all cases of AMKL contained stage I and stage II megakaryoblasts although in varying numbers. They were 10 to 30 \(\mu\)m in diameter and in 7 cases were characterized by a prominent nucleolus, a high nucleus-cytoplasmic ratio, and few specific organelles (Fig. 7). The 2 of 7 cases contained immature megakaryocytes at different stages, some of them containing DMS, OCS, and \(\alpha\)-granules in the cytoplasm.
Interestingly, the small megakaryocytes and aberrant megakaryocytes with stacked DMS which were occasionally found in AMKL were similar to those in MDS (Fig. 8). This result is consistent with other morphologic observations, suggesting that megakaryocytes of AMKL were poorly differentiated and shared some characteristics with those of MDS patients.

2.5. Acute leukemia of non-AMKL type

Low platelet counts are also seen in most acute leukemias of non-AMKL type, acute myeloid leukemia, and lymphoid leukemia, which are often complicated by the clinical feature of acquired amegakaryocytic thrombocytopenia. It is usually thought the low platelet count results from the disruption of megakaryocytic differentiation and defective thrombopoiesis in the leukemic microenvironment.

In our study, in 7 cases, most megakaryocytes exhibited a damaged cell structure, such as vacuolization, autophagosomes, secondary lysosomes, and necrosis (Fig. 9). These images demonstrate that damaged megakaryocytes are incapacitated with regard to platelet production in bone marrow in acute leukemia of non-AMKL type, although the mechanism and especially the early steps of the process remain to be elucidated.

3. SUMMARY AND FUTURE RESEARCH

The alterations seen in megakaryocytes in thrombocytopenia are distinctive for different hematopoietic diseases. Aberrant megakaryocytes predominated in ITP, in aplastic anemia, and in...
pro-platelets, megakaryoblast including aberrant DMS on 1 side of the cell but few isolated peroxidase in a well-differentiated megakaryoblast, dense core granules (arrows) in (C), cytoplasm in a case of ALL, autophagosomes, secondary lysosomes, and a lytic nucleus in a case of ALL, instead of pro-platelets in the cytoplasm in a case of AML-M2, megakaryocyte surrounded by promyelocytes and containing many vesicles in the case of AML-M5.

Figure 8. Megakaryocytes in megakaryocytic leukemia. (A) A well-developed megakaryoblast 12 μm in diameter containing a small nucleus, undeveloped DMS, and few pro-platelets in the cytoplasm, ×5k. (B) The reactivity of platelet peroxidase in a well-differentiated megakaryoblast, ×5k. (C) A well-developed megakaryoblast including aberrant DMS on 1 side of the cell but few isolated pro-platelets, ×5k. (D) Higher magnification view showing aberrant DMS and dense core granules (arrows) in (C), ×20k.

Figure 9. Megakaryocytic damage in acute leukemia of non-AMKL type. (A) A megakaryocyte surrounded by promyelocytes and containing many vesicles instead of pro-platelets in the cytoplasm in a case of AML-M0, ×3k. (B) Damaged megakaryocyte includes expanded vesicles and a monoblast (arrow) in the case of AML-M0, ×3k. (C) A necrotic megakaryocyte including many autophagosomes, secondary lysosomes, and a lytic nucleus in a case of ALL, ×3k. (D) A megakaryocyte showing the uneven distribution of DMS and lytic cytoplasm in a case of ALL, ×3k.

MDS; autophagy, apoptosis, and necrosis of megakaryocytes were dominant features in aplastic anemia, while low platelet counts were associated with poorly differentiated megakaryocytes in AMKL, but with damaged megakaryocytes in acute leukemia of non-AMKL type.

Some of the ultrastructural abnormalities described here probably represent the late stages of cellular pathology in these megakaryocytes; as always in the investigation of human disease, the very earliest stages are the most difficult to investigate because they are associated with patients without overt clinical symptoms and such patients tend not to be investigated and sampled for investigation. In themselves, therefore, these ultrastructural findings cannot yet be used to construct an understanding of the underlying pathologic mechanisms which lead to these changes and which would help us to understand platelet disorders.

If we are able to develop tests to identify patients at risk of thrombocytopenia or in the very early stages of thrombocytopenia, these ultrastructural findings could form the basis of information which might in due course be integrated into the results of other investigational modalities; this offers the promise of a deeper understanding of the thrombocytopenias and hence appropriate therapy.

One line of investigation for understanding the fundamental mechanism of thrombocytopenia would be to understand the molecular and cytologic mechanism by which the DMS and the OCS drive the formation of pro-platelets and mature platelets. This is a process in which membrane is synthesized and membrane fusion occurs to produce the channels which will lead to platelet formation. SNARE proteins (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) are becoming recognized as important in membrane fusion and these proteins have been demonstrated in a megakaryocyte cell line. Such work, where electron microscopy can be expected to play a part, offers possibilities for the detailed analysis in pathologic situations of the membrane fusion required for pro-platelet formation.

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