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Myeloproliferative Disorders and Myelodysplastic Syndrome Accompanied by Myelofibrosis: Histopathology and Immunoelectron Microscopic Study

Keiichi NEMOTO, Keiichi SATOH, Hajime UMEZU, Keiichi HOMMA and Yoshihisa OHNISHI

Second Department of Pathology, Niigata University School of Medicine, Niigata, Asahimachi 1, Niigata 951, Japan

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Summary. **Four patients with myeloproliferative disorders accompanied by myelofibrosis are described. Case 1 was a 49-year-old woman with myelodysplastic syndrome (MDS) terminating in acute leukemia. Case 2 was an 87-year-old man with essential thrombocythemia. Case 3 was a 59-year-old woman with a very unusual clinical record. She was first diagnosed as idiopathic thrombocytopenic purpura. After a splenectomy, her platelet count gradually increased to more than 100×10^4/cmm. Fortytwo months after the splenectomy, bone marrow fibrosis was recognized and further 4 years later acute leukemia was diagnosed. Case 4 was a 38-year-old man with Philadelphia chromosome positive chronic myelogenous leukemia. Blast crisis and bone marrow fibrosis developed 28 months after the onset of the symptoms. In all cases, bone marrow aspirations yielded dry taps or only small amounts of particles. Through histological studies of the bone marrows, panmyelosis with a prominent megakaryocytic proliferation and slight or moderate reticulin fibroses were observed. The blast cells in the peripheral blood were not megakaryocytic but granulo-monocytic lineages, because the cells reacted with MCS-2 and were negative for glycoprotein IIb/IIIa complex and glycophorin A by immunoelectron microscopic study. On the other hand, it was considered that the blast cells in case 3, which showed small lymphoblastoid morphology and reacted with HPCA-1, were more primitive cells.**

INTRODUCTION

Idiopathic myelofibrosis (IMF) is a chronic, progressive myeloproliferative disease that is characterized by bone marrow fibrosis, splenomegaly with extramedullary hematopoiesis and leucoerythroblastosis in the peripheral blood. On the other hand, it is generally accepted that the other myeloproliferative disorders (MPD) including chronic myelogenous leukemia (CML) and polycythemia vera also complicate bone marrow fibrosis with a high incidence.[1] In essential thrombocythemia (ET), however, it is a very rare complication.[2] Recently, many theories on the pathogenesis of bone marrow fibrosis have been established. Castro-Malapina et al.[3] demonstrated that megakaryocyte was a very important source of the growth factor derived from platelets and suggested that megakaryocytes might play a role in the pathogenesis of the marrow fibrosis by stimulating fibroblast proliferation and collagen secretion. In fact, there are many reports of acute megakaryoblastic leukemia which complicated bone marrow fibrosis.[4-8]

In this report we describe four patients with MPD and the allied disease accompanied by myelofibrosis, and we especially will discuss the histopathology of bone marrows, the morphological and immunological findings of blast cells in the peripheral blood.

Report of four cases

Summaries of clinical findings and blood counts at the stage of bone marrow fibrosis of the four patients are described in Table 1 and 2.

Case 1. A 49-year-old woman was first seen in June 1988 for pancytopenia. There was no jaundice, lymphadenopathy or hepatosplenomegaly. Hematological examination of the peripheral blood revealed pancytopenia with 1.5% of blasts (Table 2, Figs. 1, 3). A few

Table 1. Clinical Findings

Patient	Age Years	Sex	Survival (Months) from			Clinical course	Outcome
			Onset of symptoms	Biopsy	Overt leukemia or blast crisis		
1	49	Female	10	10	2	MDS with Myelofibrosis ↓ Overt Leukemia	Dead
2	87	Male	19	19	/	ET with Myelofibrosis	Alive
3	59	Female	162	60	6	Thrombocytopenia ↓ Splenectomy ↓ Thrombocythemia ↓ Myelofibrosis ↓ Overt Leukemia	Alive
4	38	Male	32	4	4	CML ↓ Blast Crisis and Myelofibrosis	Alive

Table 2. Blood Counts at the Stage of Bone Marrow Fibrosis

Patient	1	2	3 *	3 **	4
RBC X10⁴/cmm	306	369	240	248	396
Platelet X10⁴/cmm	4.0	134.0	44.8	2.2	35.6
WBC X10³/cmm	2.8	14.8	5.4	13.1	6.7
Differential count %					
Blast	1.5	2.0	0	68.5	55.0
Myelocyte	0	1.5	0	0	0
Metamyelocyte	0	2.0	0	0	0.5
Band	5.0	6.0	13.0	0.5	5.0
Polymorphonuclear	52.5	72.5	57.0	7.0	25.0
Eosinophil	0.5	1.0	9.0	1.0	0
Basophil	0	2.0	0	0	0
Monocyte	16.5	5.0	4.0	7.0	0.5
Lymphocyte	22.5	8.0	17.0	8.0	11.5
Erythroblast	1.5	0	+	8.0	2.5

*Findings at the time of the initial bone marrow biopsy.
**Findings at the time of the second bone marrow biopsy.

of pseudopelger anomaly neutrophils, anisocytosis and poikilocytosis of erythrocytes, giant platelets and monocytosis were also discovered (Fig. 1). Only a little marrow was obtained from the sternum and the iliac crest. The smear showed a slight increase of blasts (3.5%) and monocytes (9.0%) and dysplastic change of three series of hemopoietic cells including giant neutrophils, giant eosinophils, megaloblastic change of erythroblasts and immature megakaryocyte (Fig. 2). A bone marrow needle biopsy from iliac crest disclosed moderately cellular marrow spaces with proliferation of three series of hemopoietic cells and a slight increase in reticulin fiber density (Figs. 4, 5). Most important, there was a moderate increase in the number of immature megakaryocytes which had a high nucleo-cytoplasmic ratio, without nuclear chromatin condensation and positive reaction to anti-factor VIII-related antigen. A diagnosis of myelodysplastic syndrome (MDS) with myelofibrosis was made. In January 1989, the blasts in the peripheral blood increased gradually to 9.4% and later up to 68%. The patient was treated with granulocyte monocyte colony-stimulating factor (GM-CSF), 100 g/day, and cytosine arabinoside, 12 mg/day. The chest X-ray film disclosed pneumonia. Although antibiotics and antifungus therapy were given, there was no response and the patient died April 5, 10 months after the onset of her illness. Autopsy was not performed.

Case 2. An 87-year-old man was diagnosed as thrombocythemia in 1987. However, he left his hematological abnormality unchecked. In May 1988, the hematological data were as follows: RBC 387×10^4/cmm, hematocrit 38%, hemoglobin 11.4 g/dl, WBC 12.3×10^3/cmm, platelets 98.5×10^4/cmm. The peripheral smear showed 1% blast, 1% promyelocyte, 83% polymorphonuclear leucocytes, 2% basophils, 4% monocytes, 9% lymphocytes and a gross increase in number of platelets with striking anisocytosis. In February

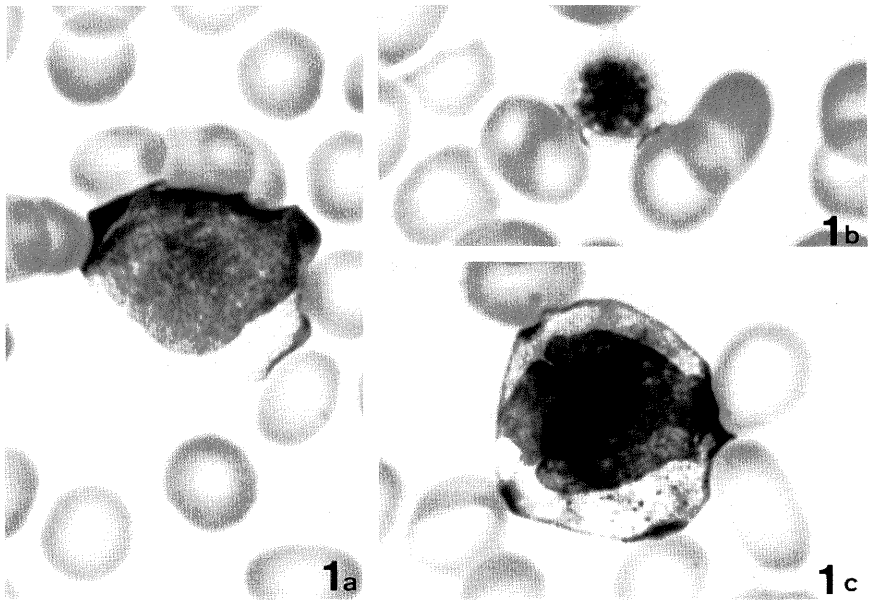

Fig. 1. Peripheral blood from case 1, showing blast (1a), giant platelet (1b) and monocyte (1c) (May-Giemsa, ×1,500)

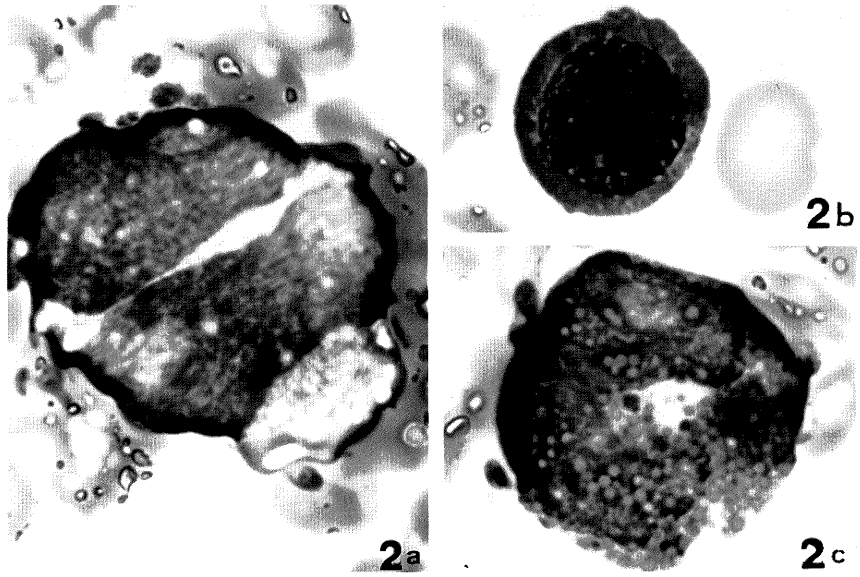

Fig. 2. Case 1. Bone marrow aspiration, demonstrating immature megakaryocyte (2a), megaloblastoid cell (2b) and giant eosinophil (2c) (May-Giemsa, ×1500)

1989, chest X-ray film showed an abnormal shadow in the middle lobe of the right lung. He was admitted to the Prefectural Shibata Hospital because he was suspected to have lung cancer. On admission, platelet count was 134×10^4/cmm and 2% blast was recognized in the peripheral blood (Figs. 6, 7, 8). The spleen was palpable two fingers-breadth beneath the left costal margin. Hepatomegaly and lymphadenopathy were not observed. Although no Philadelphia chromosome (Ph^1) was present, leucocyte alkaline-phosphatase (NAP) was abnormally low at score 89 (control 266), and the rate was 43% (control 88%). Only a small

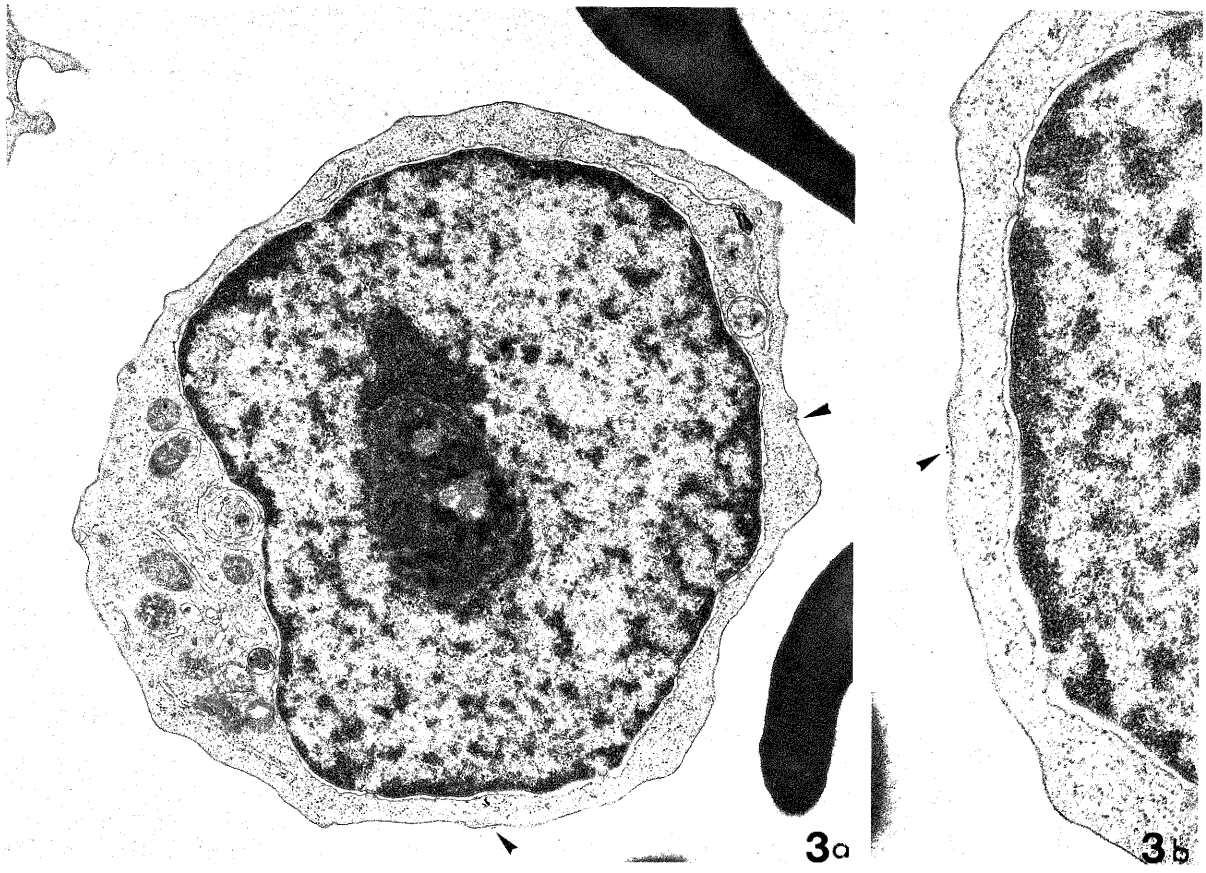

Fig. 3. The blast from case 1, reacts with MCS-2 (arrow) by immunoelectron microscopic study (3a ×11,500, 3b ×24,000).

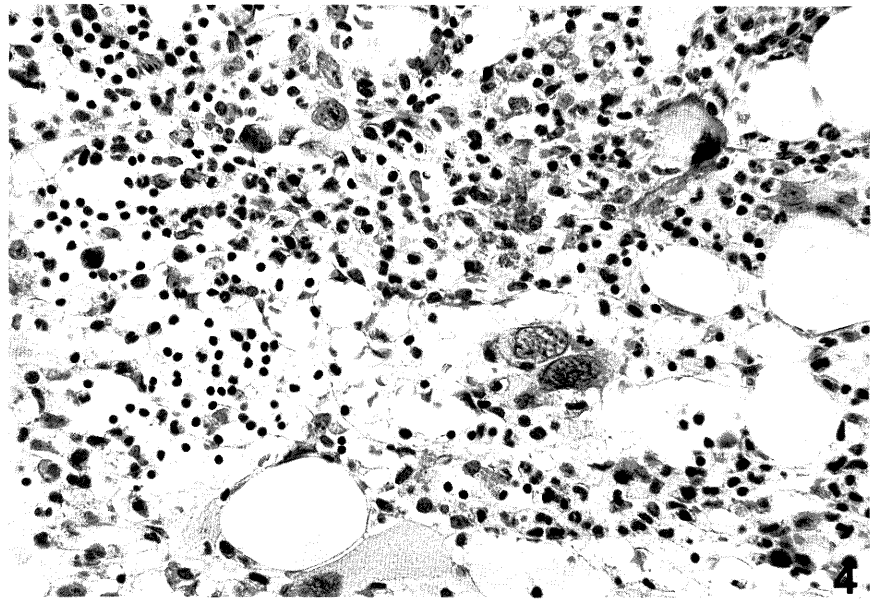

Fig. 4. Bone marrow section from case 1, showing moderately cellular marrow with proliferation of three hemopoietic cells including immature megakaryocytes (H and E, ×300).

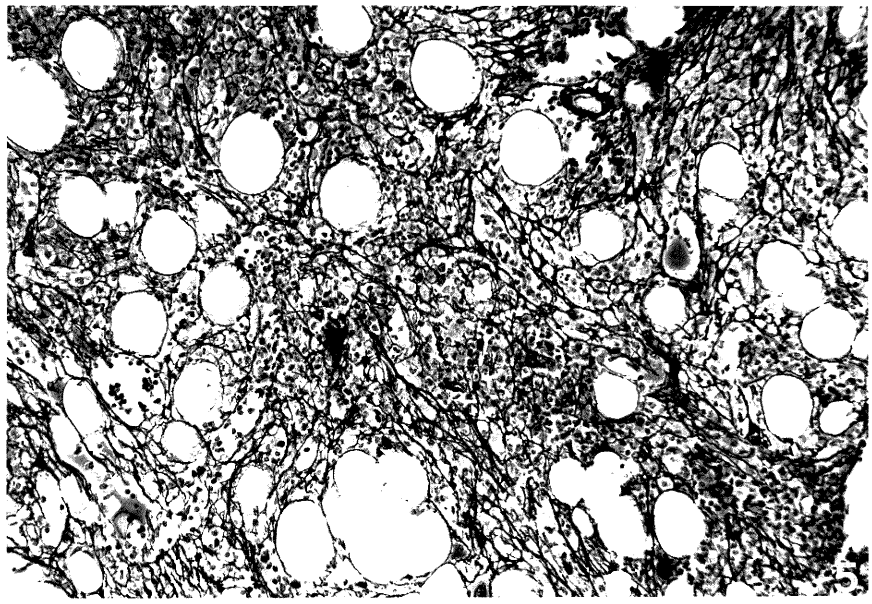

Fig. 5. Silver impregnation staining of the biopsy in Fig. 4 shows irregular increase and thickening of the reticulin fibers (Reticulin, ×150)

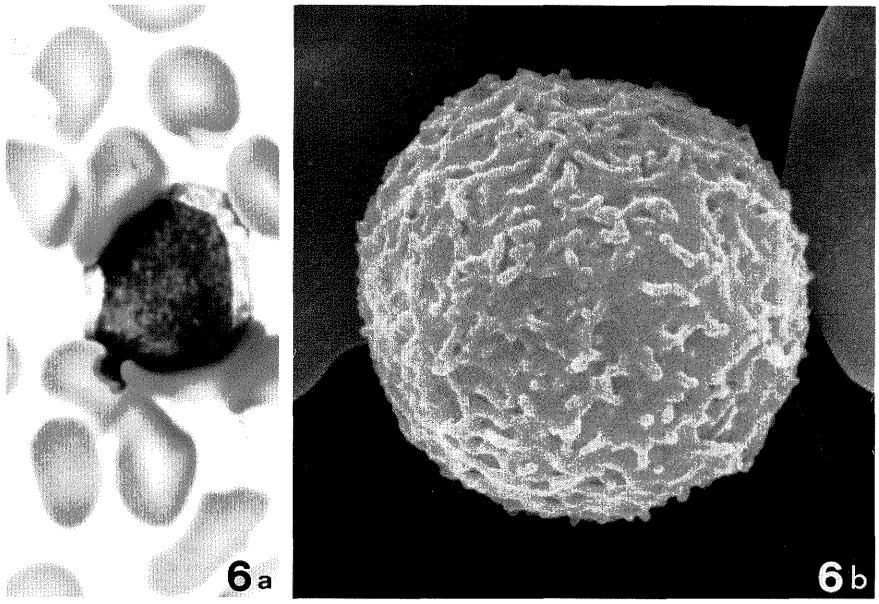

Fig. 6. Case 2. Peripheral blood film showing a blast cells which had a high nucleo-cytoplasmic ratio (6a) (May-Giemsa, ×1,500). Scanning electron micrograph shows numerous microvilli that are diffusely distributed over the hemisphere surface (6b) (×1,600)

amount of bone marrow aspirate could be obtained. The bone marrow needle biopsy showed a cellular marrow composed of all stages in granulopoietic development and a moderate increase in the number of megakaryocyte which had hyperchromatic nuclei and abundant cytoplasm (Fig. 9). The erythroid series had slightly decreased. Silver impregnation demonstrated a marked increase in reticulin fiber density among hemopoietic cells (Fig. 10). He was diagnosed as ET and Busulfan 2 mg/day was administered.

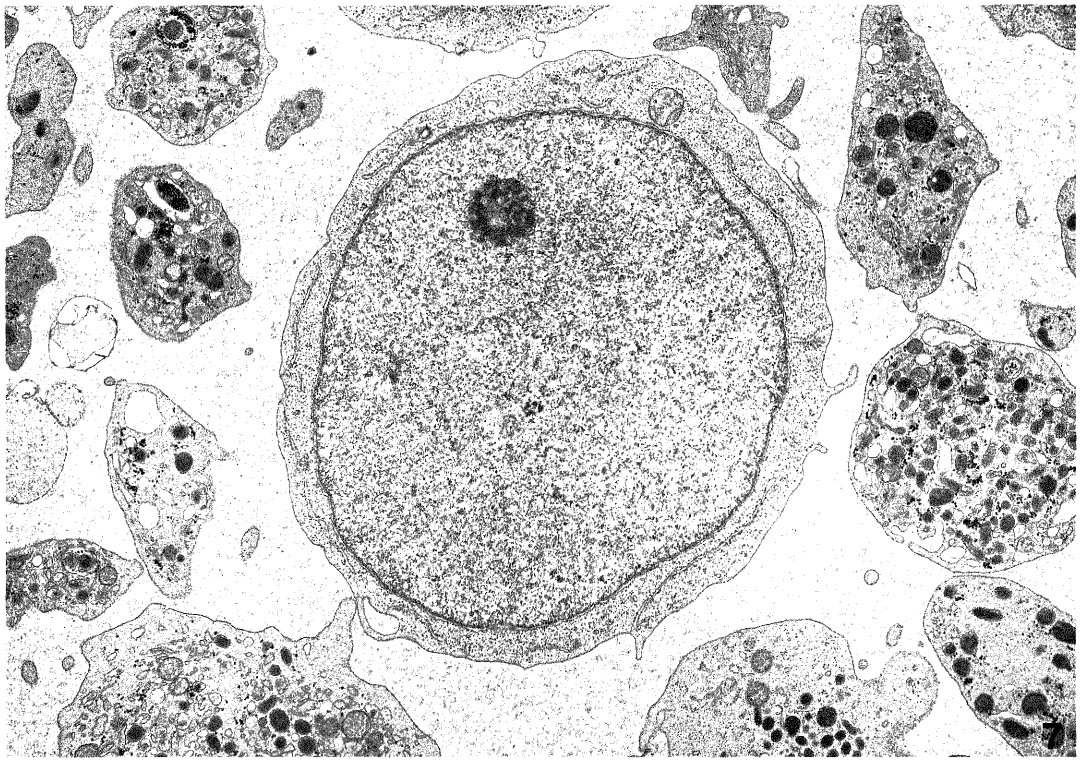

Fig. 7. Transmission electron microscopy of a circulating blast cells and numerous platelets in case 2 (×10,000).

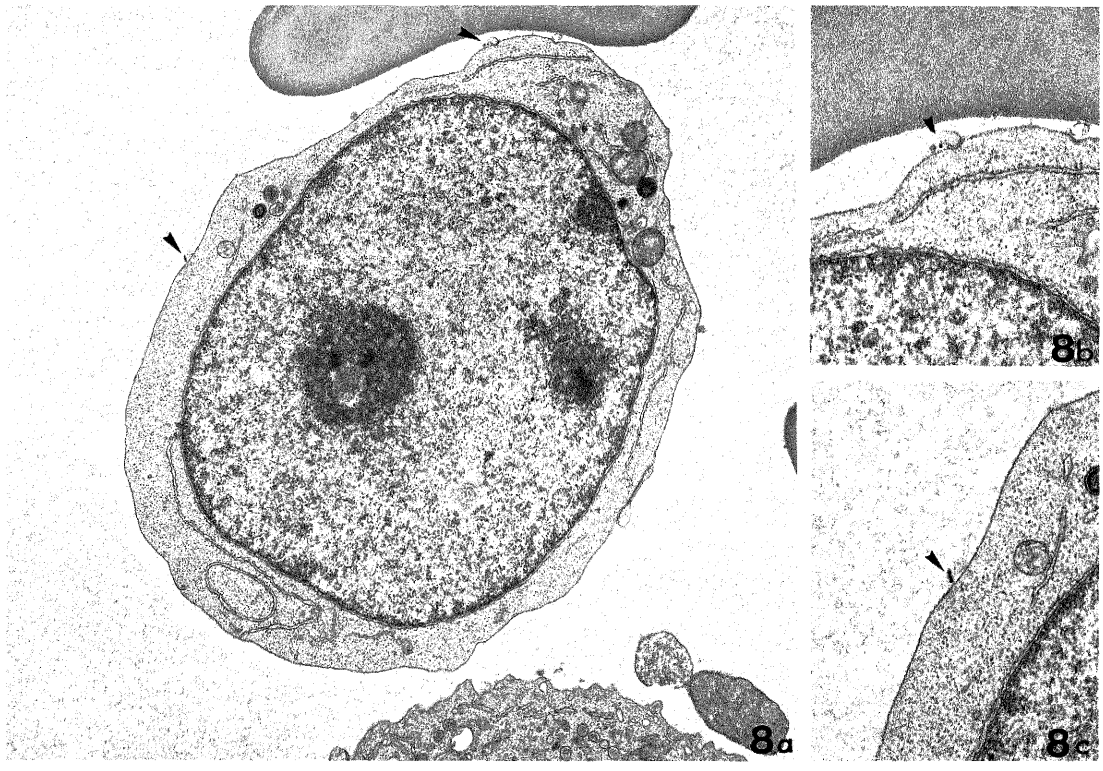

Fig. 8. The blast cell shows activity for Ia (arrow) (8a ×8,500, 8b ×18,000).

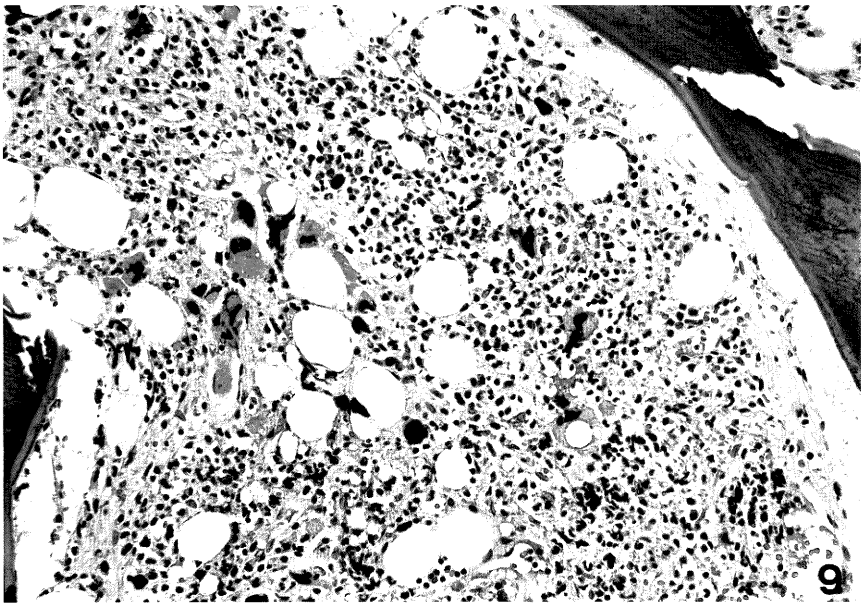

Fig. 9. Case 2. Moderately cellular marrow showing trilineage proliferation with predominance of megakaryocytes and granulocytes (H and E, ×150).

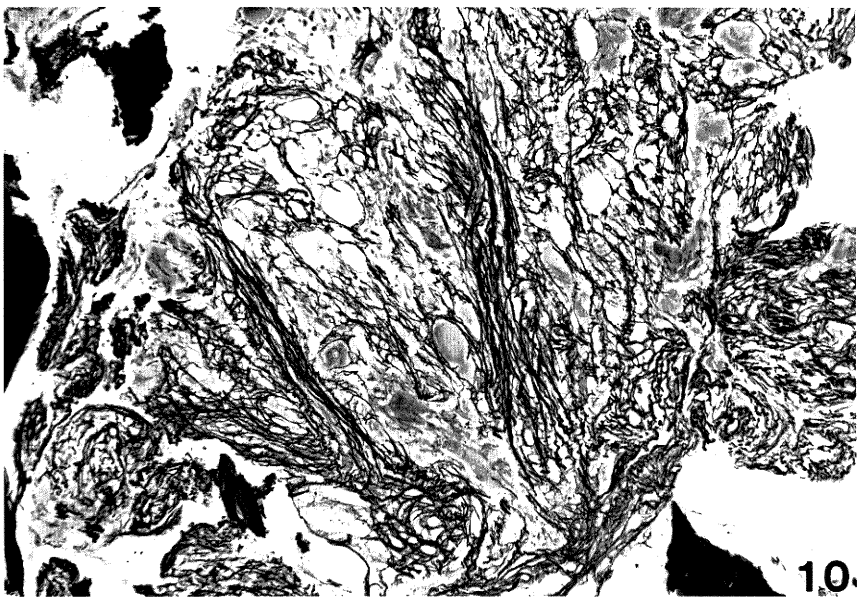

Fig. 10. Case 2. Extensive fibrosis with reticulin fibers (Reticulin, ×150).

Platelet count gradually decreased to 56.3×10^4/cmm. After discharge from the hospital, he has maintained clinical good condition.

Case 3. A 59-year-old woman first noticed nasal bleeding and subcutaneous hemorrhage in 1976. In 1977, prednisolon 20 mg/day was administered under suspicion of idiopathic thrombocytopenic purpura (ITP). The patient was admitted to the Prefectural Cancer Center, Niigata Hospital for the purpose of splenectomy in October 1980. On admission, leucocyte count was 5.7×10^3/cmm and platelet count was 2.2×10^4/cmm. A small number of erythroblasts,

anisocytosis of erythrocytes and large platelets were observed in the peripheral blood. The section of the spleen showed no characteristic feature as ITP. After splenectomy, platelet count increased gradually. In August 1984, the hematological data were as follows: RBC 232×10^4/cmm, WBC 19.1×10^3/cmm, platelet 117×10^4/cmm. NAP was markedly low at score 90 (control 330), and the rate was 19% (control 90%). The bone marrow needle biopsy was performed because it was obviously impossible to obtain particles through bone marrow aspiration. The section revealed panmyelosis including proliferation of immature megakaryocytes (Fig. 11) and a slight increase of reticulin fiber under the cellular regions. Since then, continuous thrombocytosis more than 100×10^4/cmm and anemia were noticed. In January 1988, blast cells (Figs. 12, 13) were observed in the peripheral blood and increased gradually. In March 1989, the hematological findings of her peripheral blood were as follows; RBC 248×10^4/cmm, WBC 13.1×10^3/cmm, platelet 2.2×10^4/cmm. The hemogram revealed 68.5% of blasts (Figs. 12, 13), 0.5% of band, 7.0% of polymorphonuclear leucocytes, 1.0% of eosinophil, 7.0% of monocytes, 8.0% of lymphocytes and 8.0% of erythroblasts. The second needle biopsy 4 years after the first biopsy showed almost complete filling of the intertrabecular space with predominantly lymphoblastoid cells and a few immature megakaryocytes (Fig. 14). She was diagnosed as having overt leukemia and received treatment with 6MP, 30 mg/day and cytosine arabinoside, 20 mg/day. Her clinical condition has remained unchanged.

Case 4. A 38-year-old man suffered from leucocytosis and thrombocytosis in January 1987. As a result of hematological examination he was diagnosed as Ph^1 positive CML. However, he was not admitted to the hospital. In October 1988, abdominal distention was recognized. In February 1989, he was hospitalized to the Yamanashi Medical College Hospital for a transitional phase of blast crisis of CML. The hematological findings revealed RBC 370×10^4 /cmm, WBC 35.8×10^3/cmm and platelet 27.8×10^4/cmm. The differential count of leucocytes were as follows: 3% of blasts, 2% of promyelocytes, 9% of myelocytes, 10% of metamyelocytes, 11% of bands, 45% of polymorphonuclear leucocytes, 4% of eosinophils, 10% of basophils and 6% of lymphocytes. In May 1989, he moved to the Niigata University Hospital for the purpose of bone marrow transplantation. On admission, the spleen was palpable 5 fingers-breadth beneath the left costal margin. The number of blasts in the peripheral blood increased to 28% (Figs. 15, 16). Although bone marrow aspiration was performed, only a small amount of particles was obtained. The smear showed an increase of blast cells to 35%. The biopsy section demonstrated replacement normal intertrabecular tissue near a fibrous connective tissue with scattered foci of hemopoietic cells and prominent dilated venous sinuses including mature megakaryocytes and erythroblast in the lumina (Fig. 17). Although diffuse

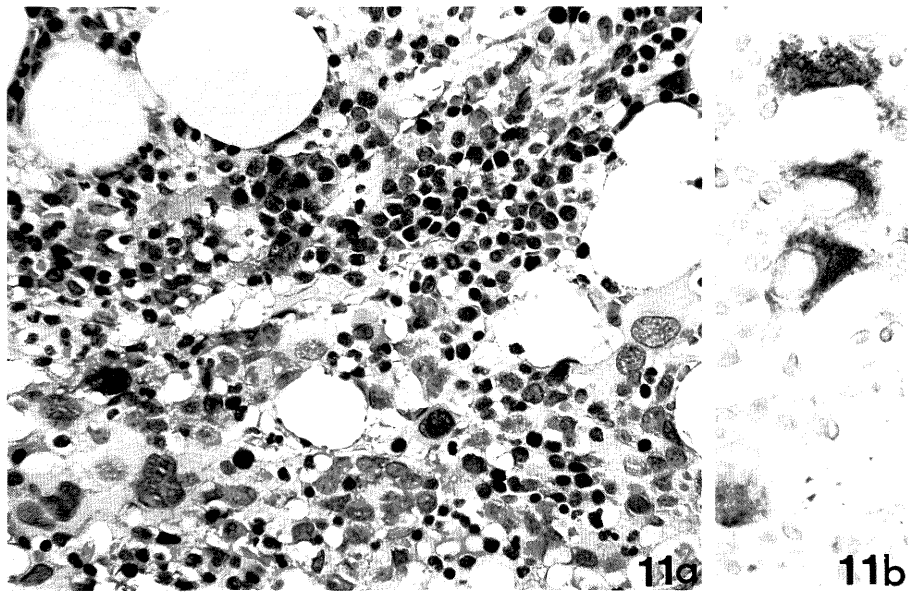

Fig. 11. The bone marrow section from the initial needle biopsy in case 3, showing panmyelosis including prominent immature megakaryocytes (11a ×300), which have strong activity for factor VIII (11b ×600).

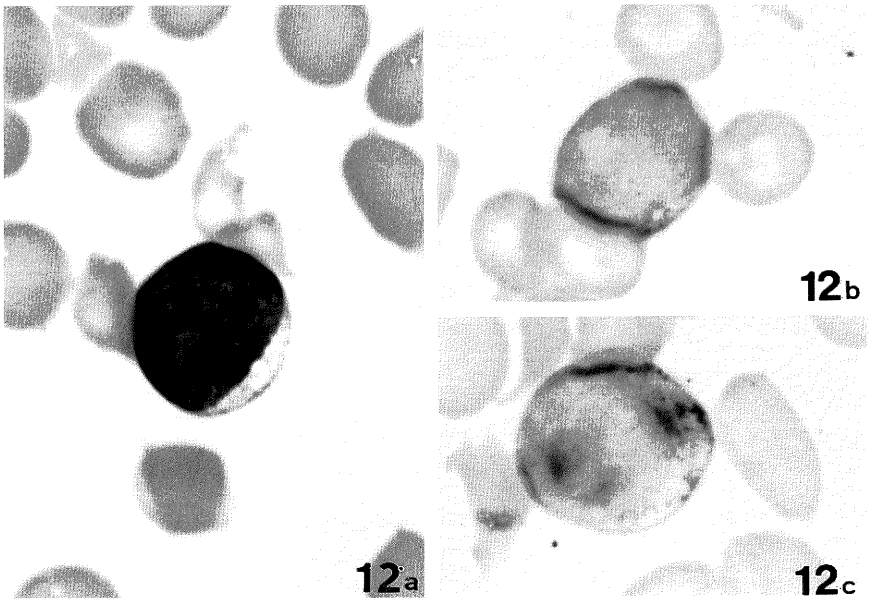

Fig. 12. The blast cell from the patient in case 3, showing small lymphoblastoid morphology (12a May-Giemsa, ×1,500). The cell is weakly reactive with acid phosphatase (12b ×1,500) and moderately reactive with aminopeptidase (12c ×1,500).

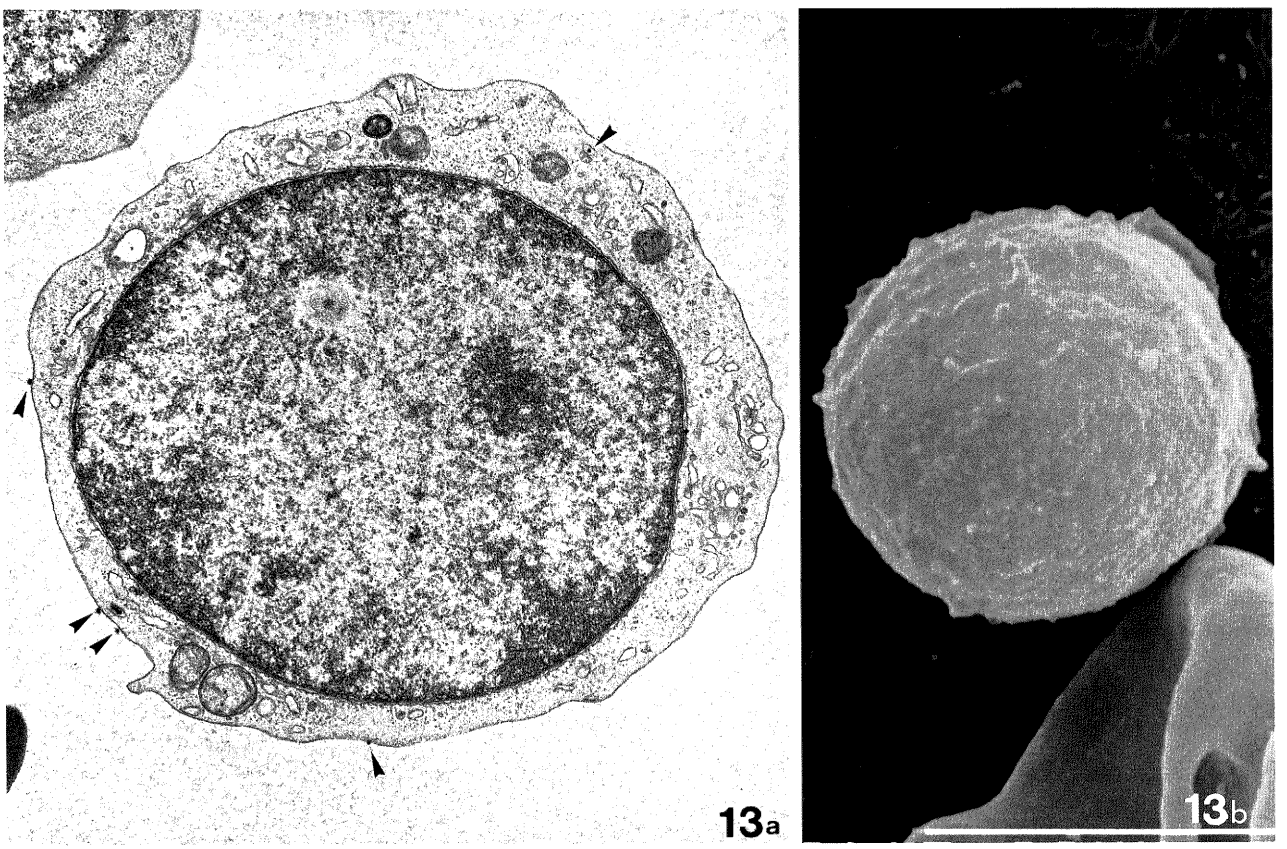

Fig. 13. The lymphoblastoid cell in case 3 reacts with HPCA-I (arrow) by immunoelectron microscopic study (13a ×11,550). Scanning electron microscopy of the cell shows a very smooth surface (13b ×16,000).

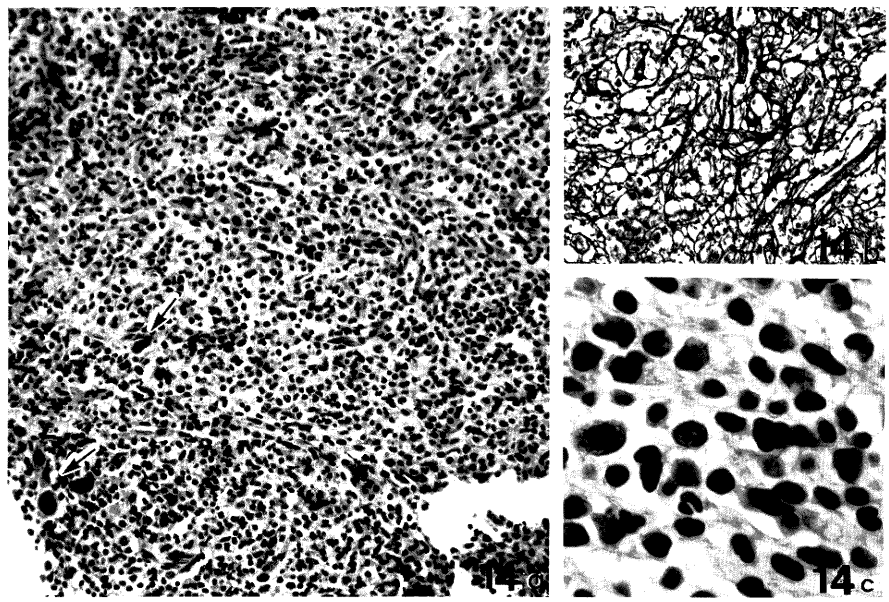

Fig. 14. Case 3. The section of the second bone marrow biopsy. Note the almost complete filling of the intertrabecular space with predominantly lymphoblastoid leukemic cells (14a ×150, 14c ×600, H & E), a few of immature megakaryocytes (arrow) and reticulin fibrosis (14b Reticulin, ×150).

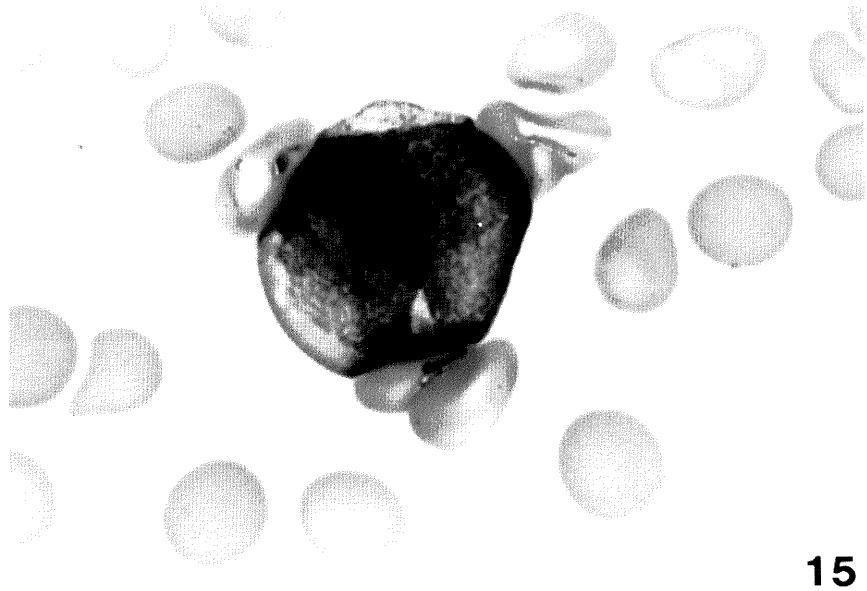

Fig. 15. Case 4. Peripheral blood blast showing nuclear indentation with strongly basophilic cytoplasm (May-Giemsa, ×1,500).

proliferation of blast cells was not found in the biopsy specimen, myeloid crisis of CML was considered through hematological data. Therefore, chemotherapy with DVP (daunomycin, vincristin and prednisolon) was chosen rather than bone marrow transplantation.

MATERIALS AND METHODS

The subjects in this study were 4 patients with MPD and the related diseases accompanied by myelofibrosis (MDS, ET, CML and the other chronic myelo-

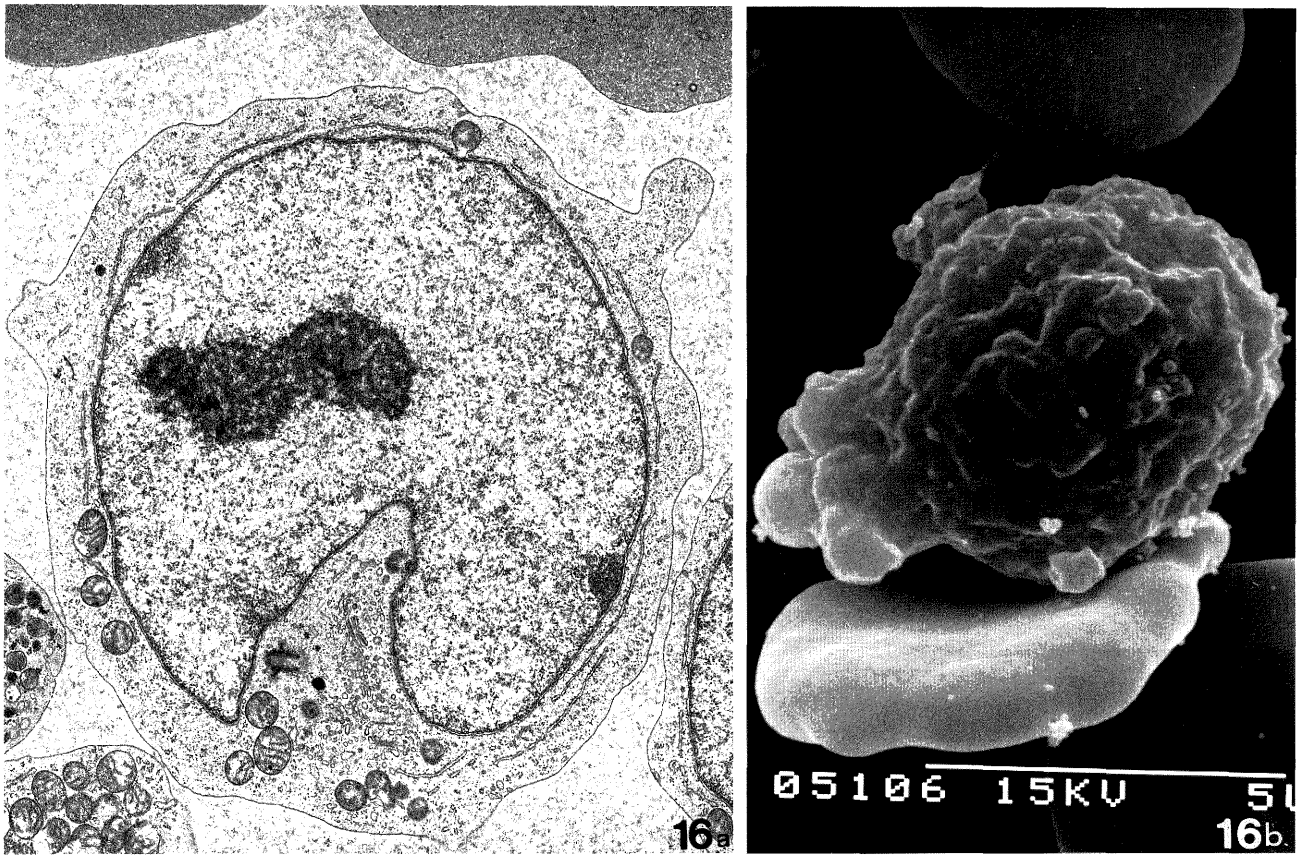

Fig. 16. Transmission electron microscopy of circulating large blast in case 4 showing nuclear indentation, distinct nucleolus, small Golgi complex, a small amount of mitochondria, long strands of rough endoplasmic reticulum, centriole and irregular cytoplasmic borders (16a ×8,085). Scanning electron microscopy showing characteristic cytoplasmic projections (×13,500).

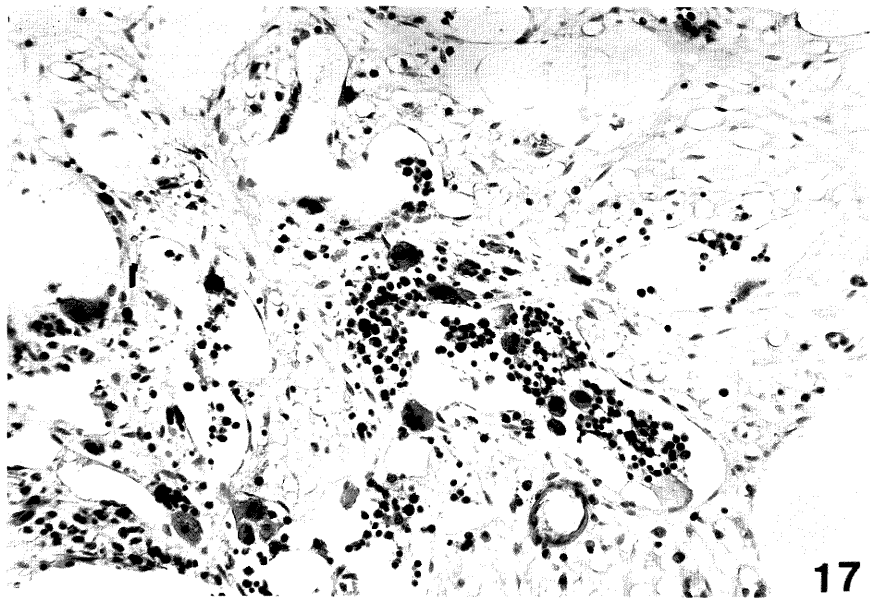

Fig. 17. Bone marrow section from case 4. Increase of loose connective tissue with dilated irregular sinuses showing megakaryocytes and erythroblasts (H & E, ×150).

proliferative disorder).

Needle biopsies: The bone marrow biopsies were obtained from the iliac crest. The specimens were fixed in 10% neutral buffered formalin and decalcified by EDTA (ethylenediaminetetraacetic acid) fluid. Sections were stained with not only hematoxylin and eosin but also periodic acid-Schiff (PAS), chloroacetate esterase, muramidase, Azan-Mallory and reticulin. Immunohistochemical localization of anti-Factor VIII antigen, anti-HbA and anti-HbF were performed using the indirect peroxidase antiperoxidase (PAP) method described by Sternberger.[9]

Blood smears and cytochemical studies: Blood films were stained with May-Giemsa stain. Cytochemical reactions for myeloperoxidase (MPO), Sudal black B, Chloroacetate esterase, α-naphthyl butyrate esterase (ANBE), double staining both of chloroacetate and ANBE, aminopeptidase, PAS, acid phosphatase and β-glucuronidase were performed on all cases using standard techniques.

Immunoelectron microscopic study: Immunoelectron microscopic study was performed by the immunoglobulin gold colloid staining method, using goat anti-mouse IgG-gold coupled to 20 nm or 30 nm gold particles (E-Y Lab. Inc. USA). As a control of immunogold labelling, the primary antibody was replaced with non-immune mouse immunoglobulin.

Scanning electron microscopic study: Scanning electron microscopic study was also performed on the buffy coats obtained by centrifugation of heparinized blood.

Monoclonal antibodies: The following were used: human progenitor cell associated antigen (HPCA)-1, Leu 4, Leu 12, Ia (Becton Dickinson), My 4, My 7, My 8, My 9, B4, Glycoprotein IIbIIIa complex (GpIIbIIIa) (Coulter Immunology), Glycophorin A (GPA) (Immunotech) and human pan-myeloid-monocyte antigen (MCS)-2 (Nichirei).

RESULTS

Summary of cytochemical reaction and immunoelectron microscopic study of blast cells in the peripheral blood are shown in Tables 3 and 4.

In case 1, the blast cells had a high nuclear cytoplasmic ratio and oval or irregular nuclei with distinct single nucleolus. Cytochemical staining of the cells revealed that they were variably positive for ANBE, PAS and acid phosphatase. On the other hand, both MPO and chloroacetate esterase were negative. Immunoelectron microscopic study demonstrated that the blasts reacted with MCS-2 (Fig. 3). and responded negatively to both GpIIbIIIa and GPA.

The morphology of the blasts in case 2 were similar to the cell in case 1 on the May-Giemsa stain. However, the blasts showed little activity for acid phosphatase and no activity for ANBE, chloroacetate esterase, MPO, PAS and aminopeptidase. On the other hand, immunoelectron microscopic study showed activity for MCS-2 and Ia (Fig. 8). Scanning electron microscopic study demonstrated that the blast cells had numerous microvilli that were diffusely distributed over the hemisphere surface (Fig. 6b).

The blasts in case 3 were composed of populations of both small and medium-sized lymphblastoid cells. They contained no MPO, chloroacetate esterase and ANBE activity, whereas acid phosphatase and

Table 3. Cytochemical Reactions in the Blast Cells

Patient	Esterase			MPO	Acid Phosphatase	PAS	Amino-peptidase
	ANBE	Chloroacetate	Double staining				
1	+	−	− ⟆ (+)	−	+*	− ⟆ +	−
2	−	−	−	−	+*	−	−
3	−	−	−	−	+*	−	− ⟆ +
4	−	−	−	− ⟆ (+)	+*	−	−

ANBE: α naphthyl butyrate esterase
MPO: Myeloperoxidase
*NaF sensitive, diffuse and finely granular
( ) Small population

Table 4. Immunoelectron Microscopic Studies on Blast Cells

Patient	HPCA-I	MCS-2	GpIIbIIIa	GPA	Others
1	nd	+	−	−	
2	nd	+	−	−	Ia (+)
3	+	+	−	−	My 4 (−) My 9 (+) Leu 4 (−) Leu 12 (−)
4	nd	nd	nd	nd	Leu 4 (−) B 4 (−)

nd: not done

aminopeptidase were weakly positive (Figs. 12b, c). Immunoelectron microscopic study supported that these cells had a nature of both very primitive cells and granulo-monocyte series because they showed activity for not only HPCA-1 (Fig. 13a) and My 9 but also MCS-2. On the other hand, the cells reacted negatively to Leu 4, Leu 12, GpIIbIIIa and GPA. Scanning electron micrograph of the cells showed a smooth surface with a very small amount of microvilli (Fig. 13b).

The blasts in case 4 composed of two cell populations: large cells and medium-sized cells. A majority of the cells were large, more than 20 μ in length, contained irregular folding nuclei, large nucleoli, basophilic cytoplasm with a few azurophilic granules and irregular cytoplasmic projection. A small amount of the medium-sized blasts reacted for MPO, whereas the large cells were positive only for acid phosphatase. Ultrastructural study revealed the folded irregular nucleus without chromatin condensation, distinct nucleoli, small mitochondria, long strands of rough endoplasmic reticulum, small azurophilic granules, small Golgi complex, centriole and irregular cytoplasmic borders (Fig. 16a). The characteristic cytoplasmic projections were observed clearly by the scanninge electron microscope (Fig. 16b).

Summary of the histopathology of the bone marrow section is shown in Table 5. The cellularity increased in moderate to marked degree except in case 4. The proliferated cells were composed of the three hemopoietic lineages in all cases. Immature megakaryocytes, which had a high nucleo-cytoplasmic ratio and no lobulated nucleus with fine chromatin, reacted with PAS and anti-factor VIII and they were prominent in case 1, and in the initial specimen of case 3. Erythroblasts were present in variable number. Small clusters of orthochromic erythroblasts were always observed except in the second biopsy specimen of case 3. The granulocytic series was observed through all stages of granulopoietic development. Although the reticulin network was slightly or moderately increased, the collagen fibriosis was present only to a very slight degree except in case 4, which showed proliferation of megakaryocytes and erythroblasts within the dilated sinus lumina. In case 3, bone marrow biopsy samples were obtained twice in the clinical course. The initial bone marrow biopsy section revealed panmyelosis with increase of immature megakaryocytes and reticulin fibrosis. The second biopsy was performed 4 years later. Myelofibrosis was still present then , however, monotonous proliferation of small lymphoblastoid cells was also seen.

Table 5. Histopathologic Findings of Bone Marrow Biopsy Specimen

Patient	Cellularity	Proliferated cells					Fibrosis	
		Blasts	Megakaryocyte		Granulocytes	Erythroblasts	Reticulin fibers	Collagen fibers
			Immature cells	Mature cells				
1	++	±	++	+	+	+*	++	±
2	++	−	+	++	++	±*	++	+
3 1)	+++	−	++	+	+	+*	++	±
2)	+++	+++	±	−	±	−	++	±
4	+	±	±	+	±	±*	++	+

+: slight degree ++: moderate degree +++: marked degree *: small cluster formation
1) The initial bone marrow biopsy. 2) The second bone marrow biopsy.

DISCUSSION

The present cases are MPD and the allied diseases accompanied by bone marrow fibrosis. The myelofibrosis was recognized at the time of the onset of symptoms or during their clinical course. Case 1 was made a diagnosis of MDS because pancytopenia, a small amount of blast cells and several dysplastic changes in three hemopoietic cells were observed. In case 2, it was difficult to decide between ET or IMF. However, the case was considered ET on the following findings: continuous thrombocytosis more than 100×10^4/cmm, no evidence of typical leucoerythroblastosis and tear drop anomaly of erythrocytes in the peripheral blood and absence of huge splenomegaly. Case 3 had a unique clinical course. At first, she was diagnosed as thrombocytopenia and suspected ITP. After splenectomy, her platelet count gradually increased to greater than 100×10^4/cmm. 42 months after the splenectomy, bone marrow fibrosis was discovered. In addition, 4 years after the initial biopsy, acute leukemia had developed. Case 4 was a Ph^1 positive CML complicating bone marrow fibrosis and blast crisis 28 months after the onset of symptoms.

In general, myelofibrosis has been classified into two groups: IMF and secondary myelofibrosis which is accompanied by metastatic malignancy, leukemias, malignant lymphomas, myeloma, tuberculosis and so on. IMF is a rare disease in Japan and is included in MPD.[10] This disease has the following clinical and histological characterstics: leucoerythroblastosis with abnormality of circulating erythrocytes, fibrosis of the bone marrows showing proliferation of all lineages (panmyelosis), and striking hepatosplenomegaly with extramedullary hematopoiesis and chronic course of disease. However, unusual myelofibrosis with a rapidly fatal course was reported as a malignant myelosclerosis by Lewis and Azur.[11] They considered it a variant of myelofibrosis that was different from the terminal ("acute") phase of IMF. Several other cases with similar clinical and histological findings have been reported under the title of acute myelofibrosis (AMF) or acute myelosclerosis.[12-14] Bearman et al.[13] reported these clinicopathologic features of AMF: 1. pancytopenia, 2. minimal or no anisocytosis and poikilocytosis, 3. a fibrotic bone marrow showing hyperplasia and immaturity of all three cell lines, with particular prominence of megakaryocytes and their precursors, 4. almost always, an absence of splenomegaly, 5. a rapidly fatal course. In addition, there were some cases in our study which did not fall into any type stated above and occupied an intermediate position between IMF and AMF.[15] Therefore, the authors consider that myelofibrosis, except so-called secondary myelofibrosis, can be classified into three groups: acute, intermediate and chronic.

Development of bone marrow fibrosis is common in CML, especially in the terminal stage.[16] In general, fibrosis occurring in CML is localized to a slight degree. However, our co-worker Homma[17] reported a rare case representing diffuse severe myelofibrosis and osteosclerosis following CML and mentioned that CML had a close relationship morphologically with IMF and that caused high frequency of secondary myelofibrosis after busulfan therapy. On the other hand, although ET is one of the MPD, bone marrow fibrosis is an uncommon complication.[2] Homma made a report of a very rare case of ET showing blastic transformation and myelofibrosis in the terminal stage. He pointed out that ET had the possibility to develop blast crisis as the other MPD did and bone marrow fibrosis in MPD was one of the important prognostic factors.[18]

The authors have experienced MDS complicated myelofibrosis from the onset of the illness.[15] It was very difficult to distinguish MDS with bone marrow fibrosis from AMF. We considered the presence and the degree of morphological dysplasia of three hemopoietic cells were very important to make a diagnosis of MDS. However, as a result of analysis of 15 patients with AMF, we concluded that AMF was a heterogenous disorder including MDS (6 cases), acute megakaryoblastic leukemia (3 cases), erythroleukemia (1 case), intermediate form (2 cases) and strict case of AMF (3 cases).[15] Shibata has already pointed out the relationship between MDS and AMF and presented a new classification of myelofibrosis syndrome.[19] Our opinion on the mutual relation of disorders representing myelofibrosis is shown in Fig. 18.

It is generally accepted that MPD is a stem cell disorder.[20-23] In all present cases, proliferated cells in the bone marrows were composed of not only a single cell line but also three hemopoietic series. Moreover, in case 3 the clinical and pathological pictures changed many times during her long clinical course and terminated in overt leukemia. In addition, it is very interesting that the leukemic cells showing small lymphoblastoid morphology reacted with HPCA-1, which detects more primitive cells. These findings may support that this case is a stem cell disorder.

There are many theories on the pathogenesis of bone marrow fibrosis. Recently glucose-6-phosphate

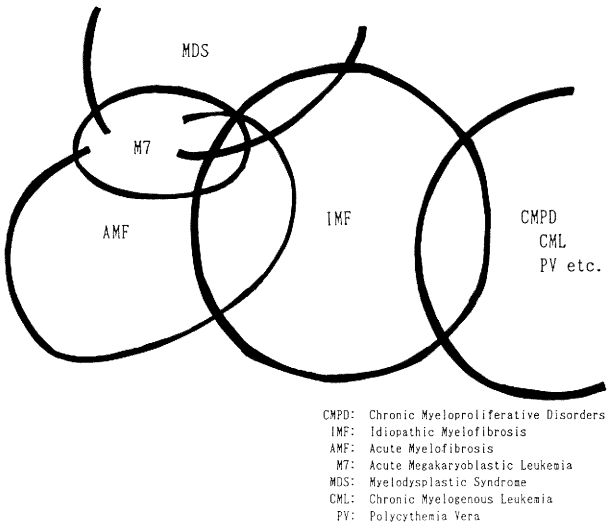

Fig. 18. Relationship between chronic myeloproliferative disorders and allied diseases.

dehydrogenese isoenzyme analysis has proved that the fibrosis in IMF is a reactive phenomenon.[21] It has been considered that panmyelosis, especially predominant proliferation of megakaryocytes, might play an important role as a cause of bone marrow fibrosis. On the other hand, Bain et al.[5] mentioned that the small numbers of blasts in the peripheral blood in the AMF were found to be megakaryoblasts by a platelet-peroxidase reaction at the ultrastructural level. And they considered that AMF might be synonymous with acute megakaryoblastic leukemia. Moreover, Castro-Malaspina et al.[4] reported the relationship between the growth factor derived from platelets and stimulation of fibroblasts proliferation and collagen secretion. In addition, Breton-Gorius et al.[24] reported a case of acute megakaryoblastic leukemia with myelofibrosis which showed a lack of α-granules in the cytoplasm of megakaryocytes while the plasma thromboglobulin level was normal. They considered that the α-granular proteins were synthesized but not retained in α-granules, and the increased marrow level of platelet-derived growth factor would favor the proliferation of fibroblasts and the synthesis of collagen. In our cases also, it was possible to consider the relation between megakaryocytic proliferation and bone marrow fibrosis. However, the blasts in the peripheral blood had a nature of granulo-monocytic series or more primitive cells. These results may indicate that the morphological picture in the bone marrow which showed myelofibrosis does not always agree with the feature in the peripheral blood. The authors would like to conclude that blast cells which increased in the peripheral blood of disorders complicating myelofibrosis is not always megakaryocytic series.

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