Chromosomal Evidence for the Secondary Role of Fibroblastic Proliferation in Acute Myelofibrosis

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A 22-year-old white woman with acute myelofibrosis and agnogenic myeloid metaplasia is reported. She was found to have a consistent chromosomal aberration in her myeloblasts, interpreted as a 1-3 translocation. Bone marrow fibroblasts were successfully cultured, yielding a normal karyotype. The lymphocyte karyotype in this patient was also normal. It is proposed that these findings favor a secondary role of the fibroblastic proliferation in myelofibrosis and suggest that the primary cellular disturbance resides only in the hematopoietic cell lines.

Despite the fact that myelofibrosis with agnogenic myeloid metaplasia is universally considered one of the myeloproliferative diseases, its relationship to other members of the group remains controversial. One particular area of controversy relates to the nature of the fibroblastic proliferative activity in the marrow of patients with myelofibrosis. Does this process occur as a secondary reaction to marrow injury unrelated to the proliferative activity in the hematopoietic elements, or does it represent a primary response of the primitive mesenchymal reticulum cell toward uncontrolled proliferation of both fibroblastic and hematopoietic derivatives? Recently a patient with an acute variant of myelofibrosis with agnogenic myeloid metaplasia afforded us the opportunity to explore this problem by karyotyping multiple cell lines.

Case Report

C.S. (HFH 139 61 10-0) is a 22-year-old white housewife, who was admitted to the Henry Ford Hospital in mid-October 1969 for evaluation of severe anemia, easy fatigability and moderate exertional dyspnea. These symptoms were first noticed about 2 months after the delivery of a stillborn in late May. The patient’s local doctor had found her to be anemic and also described some bizarre nucleated cells in her peripheral blood. She had been unresponsive to iron and B12 medications.

The patient’s past history was unremarkable. She had had two uncomplicated pregnancies prior to the stillborn. There was no history of exposure to radiation or toxic substances. Her family history was significant only in that one younger sister had had idiopathic thrombocytopenic purpura.

The physical examination after admission to the hospital showed a well developed and well nourished young white woman with marked pallor of the skin and mucous membranes. There were no other abnormal physical signs. Specifically there was no enlargement of lymph nodes, spleen or liver, nor were there any petechiae or purpuric lesions.
Significant laboratory values were as follows: Hb, 6.7 Gm./100 ml., WBC 7400/cu. mm., PCV 19 per cent, platelet count 215,000/cu. mm. and reticulocyte count 0.1 per cent. The peripheral blood smear showed moderate hypochromasia and basophilic stippling in the red blood cells. Elliptical forms and target cells were also seen. Ten nucleated erythroblasts/100 WBC were seen. The white blood cell differential showed 14 per cent myeloblasts, 3 per cent early myelocytes, 2 per cent myelocytes, 7 per cent metamyelocytes, 8 per cent bands, 17 per cent segmented neutrophils, 35 per cent lymphocytes, 12 per cent monocytes, 2 per cent immature monocytes. The platelets showed markedly abnormal forms with gigantism; a rare megakaryocytic nucleus was also observed. The serum lactic dehydrogenase was 1640 units, and the serum iron was 190 μg./100 ml. with the TIBC 198 μg./100 ml. Hemoglobin electrophoresis yielded adult A hemoglobin only. The Coombs test was negative. Normal values were obtained for the urinalysis, blood urea nitrogen, serum creatinine, alkaline phosphatase, serum bilirubin, plasma fibrinogen and sedimentation rate. In addition, the serum protein electrophoresis was normal.

No marrow tissue was obtained by the routine aspiration technique. Accordingly, an open left iliac crest biopsy was done. This showed a classical picture of myelofibrosis with entrapment of megakaryocytes and, in addition, early changes of ossification in the trabeculae suggesting osteosclerosis (Fig. 1). Despite a normal sized liver, the liver biopsy showed an intense amount of myeloid metaplasia (Fig. 2).

X rays of the chest, skull, cervical spine and pelvic bones were all normal. A coagulation survey indicated only an impaired prothrombin consumption which was felt to reflect a decrease in platelet factor 3 activity. The leukocyte alkaline phosphatase reaction gave a score of 85 (normal range 13-130).

This patient has been followed for a period of 6 months since the diagnosis of acute myelofibrosis with agnogenic myeloid metaplasia was established. She requires periodic blood transfusions to maintain a satisfactory hemoglobin level.
FIBROBLASTIC PROLIFERATION IN ACUTE MYELOFIBROSIS

Fig. 2.—Section of liver obtained by needle biopsy. Immature hematopoietic elements of the erythroid, myeloid and megakaryocytic cell lines fill the sinusoids in this characteristic microscopic picture of myeloid metaplasia. Hematoxylin and eosin stain. x 780.

COMMENT

This patient's disease was not typical of classical myelofibrosis and myeloid metaplasia with respect to the early age of onset, the absence of a grossly enlarged spleen, the unusually high number of myeloblasts in the peripheral blood, the lack of tear drop erythrocytes and the rapid progression to an advanced myelofibrosis in 4 months' time. On the other hand, favoring such a diagnosis were: the leukoerythroblastic peripheral blood picture with morphologically bizarre platelets, the unequivocal histologic evidence in the marrow and liver, the high normal leukocyte alkaline phosphatase content, and the lack of known exposure to radiation or other marrow toxins. We, therefore, consider the best designation to be an acute variant of myelofibrosis with agnogenic myeloid metaplasia as previously described by Lewis and Szur.1 It is noteworthy that the pathologic changes demonstrated are identical with those of classical myelofibrosis and that the atypical features can be explained solely by the rapidity of the process in acute myelofibrosis. For these reasons, we believe that the pathogenesis is probably the same in both classic myelofibrosis and the acute variant.

CYTOGENETIC STUDIES

Chromosome studies were performed on myeloblasts derived from peripheral blood by a modification of the technique of Moorhead et al.2 These cultures were set up without phytohemagglutinin or other mitogenic agents and terminated in 24 hours. By using this technique the chromosomes of the immature cells can be studied without contamination by lymphocytes. A second peripheral blood specimen was obtained 6 weeks after the first and terminated after 72 hours in culture, using the standard technique for peripheral blood lymphocyte cultures. Pokeweed mitogen was used as a stimulating agent. Fibroblasts obtained by a McFarland needle biopsy from the marrow cavity of the posterior iliac crest were established in tissue culture using modifications of the technique of Harnden et al.3
RESULTS

There were 26 metaphases studied from the cultures of the first specimen which were unstimulated and terminated after 24 hours. The modal chromosome number was 46. Five cells with a hypomodal number exhibited random loss of chromosomes. A complete analysis of 24 cells was carried out. The quality of one cell was such that analysis could not be done. A consistent abnormality was present (Fig. 3). In the A group the number 2 chromosomes were normal, only one normal number 1 chromosome was seen, and there was a marker chromosome larger than the normal number 1 but with a submetacentric centromere. Only one number 3 chromosome was seen and there was an extra submetacentric chromosome the size of a C group chromosome. No other abnormality was present. These observations are interpreted as representing a 1-3 translocation. The amount of chromatin material missing from the num-

Fig. 3.—Karyotype from myeloblast in peripheral blood demonstrating the translocated chromosomes.

Fig. 4.—Group A chromosomes from four metaphases demonstrating the translocated chromosomes.
Fig. 5.—Normal female karyotype from fibroblast in bone marrow culture. Identical karyotype was present in the patient's lymphocytes.

Number 3 chromosome and that added to the number 1 is consistent with a balanced translocation. An alternate interpretation of the karyotype is that a 1-1 translocation is present. Figure 4 is the A group from four other metaphases. Since in some cells the number 1 chromosome could be distinguished from the number 3 by its larger size, we believe that a 1-3 translocation is more likely than a 1-1 translocation. The results of the chromosome analysis on the peripheral blood specimen, terminated after 72 hours and stimulated with pokeweed, were as follows: 27 cells were counted; the chromosome number was consistently 46. All were analyzed. Fifteen had the identical karyotype of the myeloblast culture, and 12 had a normal female karyotype. The results of this culture are interpreted as representing a mixture of (1) normal lymphocytes which had been stimulated by the pokeweed and (2) myeloblasts. The fibroblast culture obtained by bone marrow biopsy was terminated for chromosome analysis after one subculture. In the 19 cells counted, the chromosome number was 46 (Fig. 5). All were analyzed in detail and found to have a normal female karyotype. Table 1 summarizes these findings.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>With Marker Chromosome</th>
<th>Without Marker Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour culture unstimulated</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>72-hour culture with pokeweed</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Bone marrow fibroblasts</td>
<td>0</td>
<td>19</td>
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DISCUSSION

The proliferative character of myelofibrosis has convinced a few hematologist that it represents a true neoplasia of marrow elements, i.e., an atypical form of leukemia. Perhaps the fourfold increase in myelofibrosis found in the population exposed to radiation from the atomic bomb at Hiroshima\(^4\) is further evidence favoring its neoplastic character. Certainly at the present time, there seems to be a consensus that myelofibrosis represents an abnormal proliferative marrow disorder associated with myeloid proliferative activity in other organ sites, whether malignant or benign. One of the particular features of this disorder is a remarkable degree of fibroblastic activity, seen mostly in the marrow, but also in the sites of metaplasia. Vaughan and Harrison,\(^5\) with later support from Dameshek,\(^6\) Hutt et al.,\(^7\) and Hayhoe,\(^8\) considered that this fibroplasia was the result of a primary disorder of the mesenchymal reticulum cell, but others have compared the fibroblastic reaction to the process seen in cirrhosis of the liver, which may appear predominant, but in reality is a secondary reparative reaction to obscure injury.\(^9\)

A number of previous chromosomal studies supply evidence favoring the hypothesis that the primary defect in these patients resides in the hematopoietic elements rather than in the fibroblasts. Mitus et al.\(^10\) reported cytogenetic abnormalities in two patients with acute myelofibrosis. They studied chromosomes from peripheral blood cultures using the technique of Moorhead. Of 21 karyotyped metaphases, 16 showed structural or numerical changes; the other five were normal. They did not report culturing the myeloblasts only. Therefore, in all probability their cultures represented a mixture of myeloblasts and lymphocytes in mitosis. In their second patient, bone marrow was cultured and of 16 karyotyped metaphases, one was normal and 15 showed structural and numerical changes. They found large marker chromosomes in all but one of 24 metaphases examined. In this case the peripheral blood lymphocytes were not studied.

Engel et al.\(^11\) reported their findings in a patient with myelofibrosis. Using techniques similar to those used in the studies on our patient, they found a marker chromosome (Dq\(^-\)) in the hemopoietic cells but not in the lymphocytes or skin fibroblasts. They refer to a personal communication from Macias-Alvarez and Back in which a patient with myelofibrosis was also found to have a Dq\(^-\) marker chromosome. Kiossoglou et al.\(^12\) found abnormalities in all four of their patients with typical myelofibrosis, including a patient with a trisomy C cell line. Sandberg et al.\(^13\) found a group C trisomy in a patient with myeloid metaplasia. They interpreted this as representing possible leukemia in spite of a consistently hypocellular marrow.

In none of the above patients with myelofibrosis were bone marrow fibroblasts cultured in an attempt to resolve the question of whether the fibroblastic proliferation was part of the myeloproliferative disorder or a reaction to the loss of the hematopoietic elements. In a closely related disorder, chronic granulocytic leukemia, Maniatis et al.\(^14\) have demonstrated the lack of the Ph’ chromosome in fibroblast-like cells grown from the marrow of seven patients with Ph’ positive hematopoietic cells. We have demonstrated in our
patient a consistent chromosome abnormality in the myeloblasts, which is absent in the circulating lymphocytes and absent in the fibroblasts derived from a bone marrow biopsy. Although the chromosome abnormality may or may not be etiologically related to the pathologic process, it almost certainly marks the abnormal cells. Therefore, we find it difficult to accept the concept that the basic abnormality in this disease is one of fibroblastic proliferation but, rather, we contend that the chromosome abnormality in the myeloid cells marks these elements as being primary aberrations and that a similar abnormality should be identifiable in the marrow fibroblasts if these were derived from the same primitive abnormal stem cells.

REFERENCES

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