Group-C Trisomy in Myeloid Metaplasia with Possible Leukemia

By Avery A. Sandberg, Takaaki Ishihara and Lois H. Crosswhite

Examination of the chromosome constitution in human leukemic cells has yielded fruitful, interesting, yet at times perplexing and provocative results. This is particularly true of the karyotypic findings in the various acute leukemias. Progress in cytogenetics of acute leukemia has been hampered by the inability to grow acute leukemic blood cells in vitro with consistency. In a previous study we reported that the examination of metaphases of bone marrow cells by a direct technic offered a more suitable and reliable means of establishing chromosomal anomalies in the acute leukemias than afforded by the blood culture method. From these observations, it appears that the only valid and representative karyotypic findings in acute leukemia are those based on results with bone marrow material without resort to culture. This inability to grow the cells of acute leukemia in vitro either from blood or marrow, contrasts with the ready ability of cells from chronic myelocytic leukemia (CML) to grow in blood cultures, with or without phytohemagglutinin, and the high incidence of the Ph autosome in the material examined.

Studies on the chromosome constitution in myeloid metaplasia and related myeloproliferative disorders have been few and generally negative, unless the disorders were complicated by a leukemic phase. One case of myeloid metaplasia in a patient with polycythemia vera, who had received radiation, with possible deletion of one of the group C chromosomes has been described. Nowell and Hungerford studied the chromosome constitution of marrow and blood metaphases in a group of 13 patients with a variety of myeloproliferative diseases and found karyotypic abnormalities in 4. Even though the cells of these 4 patients contained a variety of chromosomal abnormalities, it is noteworthy that in some of the metaphases of 2 of the patients possible trisomy of group C was present; and in another subject a group C chromosome was missing in some of the cells. In addition, it should be noted that 3 of 4 of the subjects with abnormal chromosome findings had received radiation (x-ray, P32); and 3 had “a subacute granulocytic leukemia” complicating the myeloproliferative phase of polycythemia vera.

In 12 previously studied cases of myeloid metaplasia due to various causes (myelofibrosis, polycythemia vera, etc.), we were unable to find any karyotypic abnormalities either in the marrow or blood cells. In this paper we wish to present cytogenetic studies in an unusual case of myeloid metaplasia with a possible acute leukemic-like terminal phase. The karyotypic abnormality was

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confined to trisomy of group C9, which was present in the preponderant number of cells in the marrow, and on one occasion in the blood cells, of this sole, cytogenetically abnormal case in our group of 20 patients with myeloproliferative disorders other than leukemia.

MATERIALS AND METHODS

The examination of chromosomes was performed on blood cells grown in short-term culture (66-72 hours) and by the direct bone-marrow technic described previously.1,3 In the latter method the cells are neither exposed to culture conditions nor to colchicine and, thus, the results represent the in vivo status of the marrow cells. The chromosomes were assigned to various groups, combining the numerical and alphabetical labels,8,9 as done in previous publications.3,5 Leukocyte alkaline phosphatase was determined according to the technic described by Kaplow,1 and the results were based on the "scoring" method suggested by him.

Case Description

The patient was a 69-year-old white woman who had had diabetes mellitus for over 10 years and had been treated by diet and Orinase. She complained of being easily bruised and of having nose-bleeds during the last 2 years. She received 5,400 rads (surface dose) to the bridge of the nose for basal cell carcinoma from March 10, 1958 to April 2, 1958 and 4,050 rads to a recurrence of a similar cancer on the right lower eyelid from December 14, 1959 to December 24, 1959. In August, 1962 the patient was admitted to a hospital because of a large furuncle of the labia majora. Splenomegaly (15 cm. below costal margin) was discovered, accompanied by leukocytosis, thrombocytopenia and anemia. The serum uric acid level was 6.2 mg. per cent. During the next 3 months the patient's leukocytosis increased, there was no improvement in the anemia and thrombocytopenia and in November, 1962 she was again hospitalized for abdominal pains and diarrhea. A benign gastric ulcer and diverticula of the colon were found. She died on November 22, 1962. Autopsy revealed unequivocal evidence of myeloid metaplasia and possible leukemia in lymph nodes, spleen and liver.

The hematologic findings on the specimens on which chromosome analyses were performed were as follows: Blood on August 9, 1962: hemoglobin 10.6 Gm. per cent, WBC 36,000 (5 per cent myeloblasts, 2 per cent promyelocytes, 10 per cent myelocytes, 25 per cent metamyelocytes, 33 per cent neutrophils, 3 per cent eosinophils, 12 per cent basophils, 10 per cent lymphocytes, 3 nucleated red blood cells/100 WBC), 54,000 platelets with some abnormal forms, no megakaryocytes.

Bone marrow on August 9, 1962: Moderately hypocellular with a greatly increased M:E (90:1); 3 per cent myeloblasts, 6 per cent promyelocytes, 19 per cent myelocytes, 22 per cent metamyelocytes, 37 per cent neutrophils, 2 per cent basophils, 9 per cent lymphocytes, 1 per cent plasma cells, 1 per cent normoblasts, no megakaryocytes.

Blood on November 21, 1962: Hemoglobin 10.2 Gm. per cent, WBC 74,000 (3 per cent myeloblasts, 8 per cent promyelocytes, 13 per cent myelocytes, 30 per cent metamyelocytes, 36 per cent neutrophils, 4 per cent eosinophils, 6 per cent lymphocytes, 29 nucleated red blood cells/100 WBC), 60,000 platelets, no megakaryocytes, reticulocytes 16 per cent.

Bone marrow on November 21, 1962: Slightly hypocellular with a greatly increased M:E (94:1); 13 per cent myeloblasts, 8 per cent promyelocytes, 21 per cent myelocytes, 21 per cent metamyelocytes, 21 per cent neutrophils, 4 per cent eosinophils, 6 per cent basophils, 5 per cent lymphocytes, 1 pcr cent normoblasts, no megakaryocytes.

Leukocyte alkaline phosphatase was determined on the blood and marrow cells and found to be of higher activity (65-80 units) than that observed in normal leukocytes (15-20 units). On all occasions blood smears were characterized by pronounced polychromatophilia of the erythrocytes, and toxic granulation in the neutrophils.
Table 1.—Chromosome Constitution of Marrow and Blood Cells in a Case of Myeloid Metaplasia with Possible Acute Leukemia

<table>
<thead>
<tr>
<th>Date</th>
<th>Source</th>
<th>Chromosome Number</th>
<th>Total Cells Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-7-62</td>
<td>Bone marrow</td>
<td>45 46 47</td>
<td>100</td>
</tr>
<tr>
<td>8-7-62</td>
<td>Blood culture</td>
<td>48 49 50 92 93 94</td>
<td>100</td>
</tr>
<tr>
<td>11-21-62</td>
<td>Bone marrow</td>
<td>97 188</td>
<td>50</td>
</tr>
<tr>
<td>11-21-62</td>
<td>Blood culture</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Blood culture*</td>
<td></td>
<td>1 14 0</td>
<td>15</td>
</tr>
</tbody>
</table>

*Grown for 138 hours.

RESULTS

The original clinical impression, based primarily on the size of the spleen, the leukocytosis and differential, was that the patient had CML. With this view in mind, the marrow and blood cells were examined for the presence of the Ph1 autosome. No Ph1 chromosomes were found, but the preponderant number of metaphases in the marrow and blood were hyperdiploid with a very sharp mode of 47 (table 1). Karyotypes of bone marrow and blood metaphases revealed an extra autosome in group C, probably C9 (figs. 1-3). Under certain conditions, this autosome can be identified with relative certainty, due to the presence of a secondary constriction near the centromere in cultured blood cells. Unfortunately, such a constriction was not very evident in most metaphases of bone marrow. The chromosome constitution was re-examined in marrow and blood cells about 3 months after the original examination. The metaphases in the marrow contained primarily 47 chromosomes and their karyotype was identical to that seen originally (table 1). On the other hand, the metaphases in the blood were characterized by a mode of 46 with a normal female karyotype. On both occasions the conditions of blood culture were essentially identical, including the addition of phytohemagglutinin, and the differential counts were not remarkably different, even though the WBC was higher on the second occasion. During the second examination, blood was cultured for a longer period of time in order to ascertain whether the abnormal metaphases would appear in larger number. The results did not differ at 138 hours as compared to those obtained at 66 hours. No blood culture was set up without phytohemagglutinin, since previous results in our laboratory have indicated that cells with gross aneuploidy did not divide in vitro at a rate or number sufficient for karyotypic analysis. Attempts to culture the skin of the patient failed, but the appearance of normal female diploid cells in the second blood culture would mitigate against the patient having had an abnormal genotypic picture in tissues other than hematopoietic.

Especially noteworthy was the presence of a substantial number (15 percent or more) of hypertetraploid metaphases in the marrows examined (table 1). In the metaphases with 94 chromosomes (fig. 4), it was shown that 6 autosomes were present in group C9 (normal 4) and double that number in the hyperoctoploid cells (188 chromosomes). In addition, an impressive number of metaphases (not included in table 1) with very high ploidy of chromosomes was observed (figs. 5 and 6). In the cultured blood no hypertetraploid metaphases were found.
Fig. 1.—Modal karyotype with 47 chromosomes from marrow of patient S. R. with myeloid metaplasia and possible acute leukemia. Note trisomy of group C9 and normal number of chromosomes in all other groups. No morphologically abnormal chromosomes could be seen.

Fig. 2.—Modal karyotype with 47 chromosomes from blood culture of patient S. R. Note trisomy of group C9.
Fig. 3.—Marrow metaphase with 47 chromosomes from patient S. R. with myeloid metaplasia and possible acute leukemia. Arrows point to the three group C9 chromosomes, the extra autosome accounting for the hyperdiploidy of the material examined.

Examination of buccal mucosa cells for sex-chromatin revealed the presence of only one sex-chromatin (Barr) body.

Discussion

Most cases of leukemia, either acute or chronic, present little difficulty in diagnosis or classification. Unfortunately, this statement does not apply to a substantial number of cases with myeloproliferative disorders other than leukemia. A peculiar complexity exists regarding the exact nature and definition of the terminal phase of the disease, which may resemble in some aspects that of acute myeloblastic leukemia or the acute phase of CML and, yet, differ from either in other respects. The presence of the Ph¹ autosome in blood or marrow metaphases and the alkaline phosphatase reaction of the leukocytes are of great help to the clinician in the diagnosis of complicated cases. Chromosomal abnormalities have been rarely observed in patients with myelo-
proliferative disorders other than leukemia. Nowell and Hungerford found chromosomal abnormalities in only 4 of 13 patients studied and the case presented is the only one cytogenetically abnormal in our group of 20 patients. It is interesting to note that chromosomal abnormalities were seen predom-
Fig. 6.—Metaphase with high polyploidy from marrow of patient S. R. with myeloid metaplasia and possible acute leukemia. Metaphases with high ploidy (94 chromosomes and higher), as shown in figs. 4–6, made up over 15 per cent of the metaphases examined.

...inantly in patients with a terminal leukemic stage of the myeloproliferative disorder. Our case has many points of resemblance to the clinical aspects of some of the cases studied by Nowell and Hungerford, but differs from any of their cases in the persistence, uniqueness and consistency of the karyotypic abnormalities found in the marrow samples and in blood culture. The karyotypic findings of the case described demonstrated: (a) the presence of group C9 trisomy; this indicated a relation of the final phase of myeloid metaplasia to acute leukemia, (b) the use of karyotype analysis as a diagnostic tool, (c) the relation of myeloid metaplasia to other neoplastic diseases and (d) the superiority of the examination of the karyotypes of marrow cells as contrasted to that of blood cells. These points will be discussed in some detail below.

The classification of the case presented was difficult since the clinical features did not appear to meet the usual criteria designated for acute leukemia or CML. Both states are most incompatible with the lack of hypercellularity of the marrows observed on two occasions and the positive alkaline phosphatase reaction of the leukocytes. The most likely diagnosis is myeloid metaplasia with an acute-leukemia-like terminal phase. It is possible that previous x-ray therapy to the face (approximately 10,000 rads surface dose, over 6,000 rads tumor dose) played a causal effect in the patient's ultimate hematologic picture.

The hyperdiploidy of the case presented was due to the presence of an
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extra autosome in group C9. Even though it is possible that this extra chromosome may possibly belong to one or another of group C, we have assigned it to group C9 on the basis of its size, location of the centromere, ratio of the arms and other morphologic characteristics. It is interesting to note that such trisomy has been shown to be one of the most frequent abnormalities in aneuploid cells from marrows of patients with acute leukemia, especially subjects with AML. The finding of C9 trisomy leads us to believe that the patient's terminal phase was an acute leukemic one (myeloblastic?), since we have not seen this karyotypic abnormality in any other state. The trisomy of C9 in our patient was related to her leukemic (?) granulocytic cells and not to a congenital or generalized genotypic defect, is pointed to by the presence of normal diploid cells in the blood cultures. It is unfortunate that the skin culture failed to grow, since the findings would have been of interest. The exact role played by the x-ray therapy in the causation of the cytogenetic abnormalities is difficult to evaluate, but should not be lost sight of.

The presence of a substantial number of cells with hypertetraploid or hyperoctoploid number of chromosomes is interesting. The frequency of metaphases with double the number (4n=tetraploidy) of chromosomes in normal marrow is in the order of 1-4 per cent. On the other hand, in the marrow of the case reported, such cells made up 16-18 per cent of the metaphases observed. The aneuploidy in these metaphases could be accounted for by the presence of an extra chromosome in group C9. In our experience, the occurrence of a remarkable number of tetraploid and higher ploidy metaphases in leukemic marrow is not uncommon, but its exact significance remains unknown. Since these abnormal metaphases in the marrow of the case presented are probably of leukemic origin, especially in view of the absence of the multi-nucleated megakaryocytes and very small number of normoblasts in the marrows sampled, the finding of the metaphases with a large number of chromosomes may point to another unusual karyotypic milieu in leukemia. A similar occurrence of cells with very high ploidy has been observed in metastatic human cancers. Whether this high incidence of high ploidy is indigenous or related to the neoplastic process is an area requiring more intensive investigation and its significance is, at the moment, obscure.

In previous studies we have demonstrated that when acute leukemic blood is cultured in the presence of phytohemagglutinin, the cells with abnormal metaphases fail to grow and normal metaphases predominate. On the other hand, examination of marrow material revealed aneuploid modes. In the present case, the aneuploid metaphases predominated in the blood; whereas on the last examination the mode was a sharp 46 in two cultures incubated for 66 and 138 hours, respectively. Thus, the results indicate that marrow examination remains paramount in establishing the karyotypic aspects of acute leukemia and related disorders. Unfortunately, the ease of securing blood and the refinements in the blood culture method, in contrast to the discomfort in obtaining a marrow sample, have resulted in the publication of a number of papers with inconclusive results regarding the genotypic picture in acute leukemia. It is to be regretted that more investigations, utilizing bone marrow...
material, have not been performed on large groups of patients in order to clarify and classify the genotypic findings in acute leukemia and related disorders.

**Summary**

A chromosomal abnormality in marrow and blood cells has been found in only one patient out of a group of 20 subjects with myeloproliferative disorders other than leukemia. The abnormal karyotypic finding consisted of group C9 trisomy in a patient with myeloid metaplasia and an acute leukemia-like picture and indicates a definite relationship to acute leukemia. The latter has been shown to be not infrequently accompanied by C9 trisomy. The trisomy was accompanied by the presence of a substantial number of hypertetraploid cells in the marrow but not in the cultured blood cells. As a matter of fact, the blood culture yielded predominantly metaphases with 47 chromosomes (C9 trisomy) on the first examination and metaphases with 46 chromosomes and a normal female karyotype on the second occasion. The superiority of bone marrow karyotype analysis over that of blood cells in leukemic states is thus indicated.

**Summario in Interlingua**

Un anormalitate chromosomal in cellulas de medulla e sanguine esseva trovate in un sol patiente ex un gruppo de 20 subjectos con disordines myeloproliferative altere que leucemia. Le anormal constatation caryotypic consisteva in trisomia de gruppo C9 in un patiente con metaplasia myeloide e un acute tableau leucemiioide. Isto indica un definite relation con leucemia acute. Quanto a leucemia, su association con trisomia de gruppo C9 ha non infrequentemente esiste demonstrate. Le trisomia in le presente caso esseva accompaniata de un numero substantial de cellulas hypertetraploide in le medulla sed non inter le cellulas de sanguine culture. De facto, le cultura sanguinee produceva, in le prime examine, metaphases predominantemente con 47 chromosomas (trisomia C9) e, in le secunde occasion, metaphases con 46 chromosomas e un normal caryotypo feminin. Assi le superioritate del analyse del caryotypo de medulla ossee super illo de cellulas sanguinee in statos leucemic es demonstrate.

**Acknowledgments**

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