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Chromosome Studies in a Case of Benzene-Induced Erythroleukaemia*

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INTRODUCTION

CHROMOSOME studies in some cases of acute erythroid leukaemia (Di Guglielmo's syndrome) have been reported in the last few years [1-9]. As in acute leukaemias the chromosome patterns are quite variable, but in most cases one or more cell lines with abnormal karyotypes were found [2-9]. In the present paper a case of benzene-induced erythroleukaemia is reported in which chromosome studies were carried out.

CASE REPORT

S.M., female, age 37. Nothing important in the family history. One normal pregnancy at the age of 29. One spontaneous abortion at the age of 31. The patient had been working from 1959 to 1966 in a small factory of beauty cases; she usually worked at packing, but she was also often engaged in the mounting of the cases with a glue containing latex and para rubber, which was diluted with benzene and applied directly on the lining with a finger. The whole processing of the beauty cases was carried out by 4-5 persons in a small room. The patient reported that, especially during winter, there was a strong smell of benzene in the room.

Since 1965 the patient had suffered from frequent metrorrhagias. In July 1966 the patient started complaining of weakness, anorexia, pallor, irregular fever (up to 39°C). In August 1966, following a severe metrorrhagia, the patient was admitted to a gynaecologic department to have a curettage and, due to the finding of anaemia, she was transferred to the Clinica del Lavoro on 1 September 1966.

At admission the blood findings were: Hb 8 g/100 ml, RBC 3,000,000/mm³, WBC 4500/mm³ with neutrophils 38%, eosinophils 2%, lymphocytes 14%, monocytes 6%, metamyelocytes 14%, myelocytes 26%, basophilic

erythroblasts 10/100 WBC; platelets 125,000/mm³ with some giant, bizarre elements.

The bone marrow obtained by sternal puncture (9 September 1966) was very rich in elements of both series, especially of the more immature forms. Both erythroid and myeloid elements showed atypical features: like nucleo-cytoplasmic maturation asynchronism, bizarre nuclei; some giant mono- and binucleated elements were present. The myeloid-erythroid ratio was 1:1.

The patient was treated with transfusions and low doses of corticosteroids and was discharged improved on 24 September 1966.

A few days after (8 October 1966) the patient was readmitted in emergency due to severe metrorrhagia, thrombophlebitis of the left leg, high fever. The blood findings were: Hb 6.2 g/100 ml, RBC 2,000,000/mm³, WBC 30,000/mm³ with neutrophils 69%, lymphocytes 4%, metamyelocytes 19%, myelocytes 2%, myeloblasts 1%, haemocytoblasts 5%; acidophilic erythroblasts 15, polychromatophilic erythroblasts 7, basophilic erythroblasts 1, proerythroblasts 1,100 WBC. Some of the immature elements of the erythroid series were PAS-positive. The leucocyte alkaline phosphatase score was high (262). At this time a direct chromosome preparation of peripheral blood was made.

From 22 October-1 November 1966 the patient was given a short course of 6-mercaptopurine (2.5 mg/kg/day), together with corticosteroids and transfusions. The patient had a partial clinical and haematological remission and was kept under check for a while.

On 23 December 1966 a new bone marrow sample was obtained, on which cytogenetic studies were performed. The pattern was similar to that previously described, but the bizarre and giant elements were more numerous. The myeloid-erythroid ratio was 0.46. Out of 1000 immature cells of both series, 2 mitoses of the myeloid and 6 of the erythroid precursors were found.

In the next months the patient became increasingly ill, had high irregular fever, complained of joint pains, and presented subcutaneous and submucous haemorrhages and liver and spleen enlargement.

The patient died on 16 February 1967. The autopsy findings were: bronchopneumonia, diffuse subcutaneous and submucous haemorrhages, liver and spleen enlarge-

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Table 1. Chromosome counts in direct preparations of peripheral blood and bone marrow

Date	Source	Chromosome counts						Near-tetraploid	Total cells counted
		≤ 43	44	45	46*	57	73		
14 Oct. 1966	Blood		3	3	5			2	13
23 Dec. 1966	Bone marrow	6		2	1	4	1	5	29

*Pseudodiploid cells with 2 markers.

ment due to massive infiltration with erythroid and myeloid elements.

MATERIAL AND METHODS

The chromosome studies were performed on direct preparations of peripheral blood and bone marrow. The peripheral blood was prepared by centrifugation of 10 ml of blood in 10 ml of 3% formalin for 45 min at 37°C. Cells were washed twice with Hanks solution, resuspended in diluted Hanks solution (Hanks 1 part, distilled water 4 parts), fixed in methanol:acetic acid (3:1), and chromosome preparations were made by the air-drying method, with slight flaming. No lymphocyte cultures were performed.

The leucocyte alkaline phosphatase was determined according to Kaplow's cytochemical score [10].

RESULTS

The direct preparation of peripheral blood was performed at a time when the patient had been treated only with corticosteroids and transfusions. The chromosome study of the bone marrow was made 11 months after a short course of 6-mercaptopurine; unfortunately no chromosome studies had been performed at the time of the first bone marrow biopsy in September 1966.

The results of the chromosome counts are summarized in Table 1. Complete chromosome analysis was performed on 11 metaphases

from the peripheral blood (Table 2) and on 12 metaphases from the bone marrow (Table 3). Both samples were characterized by a stem-line with a chromosome number of 46, but with a pseudodiploid pattern. In all pseudodiploid metaphases 2 chromosomes of the E group [17-18] were missing and 2 marker chromosomes were present (Figs. 1 and 2). The karyotype was designated as $46, XX, 2E-, mar 1-, mar 2-$ according to the nomenclature of the Chicago conference [11].

Marker 1 was a small ring chromosome, appearing most of the times divided into the chromatids (Figs. 1 and 2). Marker 2 was a minute fragment, whose centromere was evident only in some metaphases. Both markers were quite recognizable and were observed also in some other metaphases which were not suitable for counting. The markers, and especially marker 1, occupied preferentially a peripheral position in the metaphase spreads (Figs. 1 and 2).

As shown in Table 1 quite a few metaphases were near-tetraploid; in these cells each marker was generally represented twice or more (Tables 2 and 3).

In some of the best metaphases a Bp-chromosome was also somewhat evident; this chromosome was intermediate between chromosomes of pair 3 and chromosome 4 (Figs. 1 and 2). However, due to the quality of many metaphase spreads, it is difficult to state whether this chromosome abnormality was consistent.

Table 2. Chromosome analysis of 11 metaphases from direct preparation of peripheral blood

No. of cells	No. of chromosomes	A	B	C	D	E (17-18)	F	G	mar 1	mar 2
1	44			-1		-2			1	
1	44	-1	-2			-1			1	1
1	45			-1	-1	-1			1	1
1	45				-1	-2			1	1
1	45					-2				1
5	46					-2			1	1
1	86*		-1	-9	-1	-2			4	3

*In the near-tetraploid metaphases missing and extra-chromosomes are referred to the tetraploid karyotype.

Table 3. Chromosome analysis of 12 metaphases from direct preparation of bone marrow

No. of cells	so. of chromosomes	A	B	C	D	E 17-18	F	G	mar 1	mar 2
1	45			-1		-2			1	1
1	45					-2			1	
1	46				{1q-	-1				1
1	46			-1		-1 (1Eq-)			1	1
5	46					-2			1	1
1	57	+1		+4	+1		+1	+1	2	1
1	87*		-2			-5	-2		2	2
1	93*					-3			1	3

*See note below Table 2

DISCUSSION

The capacity of benzene to induce leukaemias is well known [12, 13]. Also cases of acute erythroleukemia due to benzene are reported [14, 15]. In our case the illness started after 7 years of exposure to benzene in concentrations supposedly well above the threshold limit values. Although the patient had not had blood examinations, the frequent metrorrhagias in the year preceding the onset of leukaemia seem to indicate that disorders of the blood-forming organs were already present. For this reason we can assume that this case of erythroleukaemia was induced by benzene.

As far as the chromosome findings are concerned, from the comparison of our data with those existing in the literature some considerations can be made. The presence of one or more stem-lines with an abnormal karyotype seems to be a very frequent occurrence in acute erythroleukaemia [2-9]. As for the other acute leukaemias, the abnormal chromosome patterns are different from case to case. In the present case, consistent structural chromosome abnormalities were demonstrated in all metaphases, both in the sample of peripheral blood taken before antileukemic treatment and in the sample of bone marrow obtained at a time of relapse after treatment. The structural abnormalities seem therefore to belong to the neoplastic stem-line.

In two cases of acute erythroleukaemia reported in the literature a ring chromosome was observed [2, 6]. In the case of Di Grado *et al.* [2] the ring chromosome was the size of chromosome 2; in the case of Krogh Jensen [6] with 3 stem-lines, 2 stem-lines had a ring chromosome, one the size of a C chromosome, one the size of an F chromosome. The ring chromosome observed in our case was somewhat smaller than that of the second stem-line of the case of Krogh Jensen [6]. This author reports

also in the same case the almost constant presence of a small acentric fragment in the metaphases with the marker ring chromosome. In our case the minute chromosome seemed actually to be centric.

A feature common to some reported cases of acute erythroleukaemia is a hypodiploid mode [1-3, 6, 7, 16]. In the case described the mode was pseudodiploid. The smaller size of the two marker chromosomes in comparison with the size of the two missing chromosomes of the E group suggests however a loss of genetic material, even though a translocation of part of this material on one B chromosome (1Bp+) cannot be ruled out. Loss of genetic material was also observed by Ricci [5] in his case of erythroleukaemia with a pseudodiploid pattern.

The involvement of chromosomes of the E group, particularly 17-18, in acute leukaemias and allied diseases, and in the acute transformation of chronic myeloid leukaemia, does not seem to be a rare occurrence [6, 7, 17-19]. Loss of 2 chromosomes [17-18] was a constant feature of the stem-line of the case described, even though in occasional hypodiploid cells only one E chromosome was missing together with chromosomes of other groups.

At variance with Castoldi *et al.* [9] no involvement of the G chromosomes was observed.

The tetraploid or near-tetraploid metaphases found in the direct preparation both of peripheral blood and of bone marrow represent probably mitoses of the abnormal giant elements, often binucleated, which were present in the smears.

The presence of the marker chromosomes in all the metaphases observed and analysed seems to indicate that all the dividing cells possessed an abnormal chromosome complement. As in the Giemsa-stained bone marrow smears made at the time of the chromosome prepara-

tion mitoses both of the erythroid and of the myeloid series were present, the abnormal metaphases observed seem to belong to both series. This fact, observed in acute leukaemias also by Krogh Jensen [6, 20, 21], seems to demonstrate that the abnormal cells derive from a common stem-cell with anomalous chromosome pattern.

Regarding the formation of the abnormal complement, it is noteworthy that some of the reponed patients with acute leukaemia or erythroleukaemia had a history of exposure to X-rays [1, 6]. The patient with erythroleukaemia observed by us had been for some years exposed to benzene during her work.

Benzene exposure is known to be able to produce chromosome changes similar to those induced by X-rays, both in lymphocytes in bone marrow cells [22-26.] The hypothesis can therefore be suggested that in our case benzene exposure might have induced stem-cell chromosome changes (probably breakage of chromosomes of the E group), and that the consequent rearrangement might have resulted in a cell with a pseudodiploid complement, which gave rise to the abnormal clone. The leukaemic process might have been initiated by the proliferation of such clone and by its differentiation into cells both of the erythroid and of the myeloid series.

RESUME

On a étudié les chromosomes d'un cas d'érythroleucémie aiguë chez une malade qui avait été exposée au benzène. A l'examen direct tant du sang que de la moelle osseuse, il y avait une lignée cellulaire avec 46 chromosomes, avec un caryotype pseudodiploïde par absence de deux chromosomes du groupe E et présence de deux chromosomes marqueurs (un chromosome en anneau et un petit fragment centrique). Le caryotype anormal paraissait être commun aux deux séries, puisque les chromosomes marqueurs étaient présents dans toutes les mitoses étudiées et appartenaient probablement tant à la série érythroblastique qu'à la série myéloïde. Les auteurs émettent l'hypothèse que l'exposition au benzène peut avoir induit des fragmentations et des réarrangements chromosomiques dans une cellule souche qui, en prolifération, produisait le clone érythroleucémique anormal.

SUMMARY

Cytogenetic studies were carried out in a case of acute erythroleukaemia, which occurred in a patient who had been exposed to benzene. In direct preparations both of peripheral blood and of bone marrow, a stem-line of 46 chromosomes was present, with a pseudodiploid karyotype, due to the absence of 2 chromosomes of group E and to the presence of 2 small markers (a ring chromosome and a minute centric fragment). As the markers were present in all the mitoses observed, most probably belonging both to the erythroid and to the myeloid series, the abnormal chromosome complement seemed to characterize a stem-cell common to both series. The hypothesis is suggested that the exposure to benzene might have induced chromosome breakages and rearrangements in a stem-cell, and that proliferation of such abnormal element might have resulted in the erythroleukaemic clone.

ZUSAMMENFASSUNG

Bei einem Patienten mit akuter Erythroleukämie, welcher der Einwirkung von Benzol ausgesetzt war, wurden die Chromosomen untersucht. Sowohl in der direkten Präparation des strömenden Blutes als auch in der des Knochenmarks war ein Zellstamm mit 46 Chromosomen vorhanden. Durch die Abwesenheit von 2 Chromosomen der Gruppe E und das Vorhandensein von 2 kleinen Markerchromosomen (ein Ringchromosom und ein kleines zentrisches Fragment) entstand eine pseudodiploides Bild. Da die Markerchromosomen bei allen beobachteten Mitosen vorhanden waren, welche vermutlich sowohl der myeloischen als auch der erythroblastischen Serie angehören, scheint der pathologische Chromosomenbestand beiden Serien anzugehören.

Die Autoren kommen zu der hypothetischen Folgerung, daß es durch die Wirkung des Benzols zu einem Bruch und einer Wiederanordnung der Chromosomen in einer Stammzelle gekommen war, wobei die Proliferation dieser Zelle zur Bildung eines anomalen Klons führte.

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