Absence of the 9q+ chromosome in Ph¹ negative chronic myelogenous leukaemia

JANET D. ROWLEY

Department of Medicine of the University of Chicago and the Franklin McLean Memorial Research Institute,*
Chicago, Illinois, USA

Summary. Bone marrow chromosomes from four patients with Ph¹ negative chronic myelogenous leukaemia have been examined with quinacrine fluorescence. The marrow cells of all four patients showed an apparently normal karyotype. This karyotype is different from that of the Ph¹ positive variant in which a possible translocation (9q+;22q-) has been observed.

A second consistent chromosomal abnormality has recently been reported in marrow cells of patients with Philadelphia (Ph¹) positive chronic myelogenous leukaemia (CML) (Rowley, 1973). The abnormality consists of an additional band of dully fluorescing material at the end of the long arm of one chromosome No. 9(9q+) (see Figs. 1a and 2a). This additional band has been observed in all 23 patients with Ph¹ positive CML whose cells have been examined with quinacrine fluorescence (J. D. Rowley, 1973 unpublished observations). It has been suggested that this material on the 9q+ chromosome may represent a translocation of the material missing from the Ph¹ chromosome, which is a No. 22 with a deletion of most of the long arm (22q-) (Caspersson *et al*, 1970; O'Riordan *et al*, 1971). The karyotype of these cells can be expressed as t(9;22)(q34;q11) (Paris Conference, 1971), if it is assumed that the translocation is reciprocal.

In several large series of patients whose clinical features suggest a diagnosis of CML, it was found that approximately 70–90% of these patients have a Ph¹ chromosome whereas 10–30% do not (Whang-Peng *et al*, 1968; Ezdinli *et al*, 1970). In several smaller series, no Ph¹ negative patients were observed (Pedersen, 1966; Woodliff, 1971). Clinically, Ph¹ negative patients are predominantly males, are older, have lower white blood cells and platelet counts, and have elevated serum and urine muramidase levels (Perillie and Finch, 1970). They do not

respond well to busulphan. They have a much poorer mean survival, namely, 15 months as compared to 40 months for Ph¹ positive patients (Whang-Peng *et al*, 1968).

It is of interest to determine whether cells from Ph¹ negative patients show any abnormalities involving chromosome 9 or 22 when the cells are examined with quinacrine fluorescence. The correctness of the diagnosis of CML must be clearly established, if the results of the analysis are to be germane to a comparison of the chromosomal patterns in Ph¹ positive and Ph¹ negative CML. Four patients with Ph¹ negative CML are presented here.

Case Reports

Case 1. This 58-year-old white male, first seen here on 26 August 1972, had lost weight and had had easy bruising, tiredness, and recurrent urinary tract infections for 6 months. On physical examination, the liver was enlarged and a few scattered lymph nodes were palpated. Results of the peripheral blood examination on admission were: red cell count, 2.84 million; haemoglobin, 8.2 g%; haematocrit, 25.2%; platelet count, 41,000/mm³; white cell count, 6300/mm³; neutrophils 33%, juveniles 2%, metamyelocytes 5%, neutrophilic myelocytes 12%, promyelocytes 2%, myeloblasts 2%, eosinophils 1%, small lymphocytes 36%, and monocytes 7%. There were three nucleated red cells per 100 white cells.

Bone marrow examinations on 28 August, 7 September, and 20 October 1972 showed an essentially similar pattern. Cellularity approached 100%; the myeloid: erythroid ratio was 10:1. The myeloid series showed occasional pseudo-Pelger cells with an increased number of promyelocytes and myeloblasts and no maturation arrest. The number of megakaryocytes was decreased.

Received 15 October 1973.

* Operated by the University of Chicago for the United States Atomic Energy Commission; formerly the Argonne Cancer Research Hospital.

Maturation in the erythroid series was abnormal due to an increase in megaloblasts. The leucocyte alkaline phosphatase score was zero on several occasions. Serum muramidase levels were 42 and 52 $\mu\text{g/ml}$. (normal range is 8–30 $\mu\text{g/ml}$.), and urine muramidase activities were 15 and 42 $\mu\text{g/ml}$. (normal is 0). The patient appeared to have an atypical myeloproliferate disorder, probably CML. He was given transfusions and was discharged on folic acid, 1 mg daily.

A subtotal colectomy with ileostomy was performed on 20 September 1972, because of segmental necrosis of the colon; the spleen was removed because of a subcapsular hematoma. The observation that the red pulp of the spleen was infiltrated with immature and mature granulocytic elements substantiated the diagnosis of CML. The patient died in March 1973 of bilateral pneumonia;

a necropsy revealed extensive leukaemic infiltration of all organs.

Cytogenetic Analysis. Bone marrow samples were processed as previously described (Rowley, Blaisdell, and Jacobson, 1966). Some slides, previously stained with Giemsa, were destained and examined with quinacrine fluorescence; others were stained initially with quinacrine and then with Giemsa (Rowley, 1973). The results of the chromosomal analyses of the first two bone marrow samples have been combined since they are similar, and are summarized in Table I.

The modal chromosomal number was 46. The routine karyotype of Giemsa-stained chromosomes appeared to be normal (46,XY); quinacrine-stained cells also showed a normal karyotype (Figs. 1b and 2b).

FIG. 1. Chromosomes 9 and 22 from four cells that have been examined with quinacrine fluorescence.

Line a: From a patient with *Ph*¹ positive CML. The 9q+ and 22q- (*Ph*¹) chromosomes are indicated by arrows.

Line b: Case 1. Note the normal size and banding pattern in the homologous chromosomes.

Line c: Case 2.

Line d: Case 3.

FIG. 2. The same chromosomes 9 and 22 as seen in Fig. 1, from three Giemsa-stained cells.

Line a: From patient with Ph^1 positive CML. The cells have been treated with NaOH to produce centromeric staining. The abnormal 9 and 22 are indicated by arrows.

Line b: Case 1. These cells were stained with Giemsa before quinacrine fluorescence.

Line c: Case 2.

Case 2. This 40-year-old Negro female, admitted on 11 August 1968 with a diagnosis of pancytopenia, secondary to busulphan therapy for CML, had been seen elsewhere in November 1967 for fatigue and low back pain. A white blood count at that time was 300,000/ mm^3 , and the bone marrow specimen showed infiltration of immature and mature myeloid elements with an increase in eosinophils and basophils. The physical examination on admission to Franklin McLean Memorial Research Institute was unremarkable. Results of the peripheral blood examination were: haemoglobin, 5.0 g%; haematocrit, 14%; platelet count, 12,000/ mm^3 ; white cell count, 2350/ mm^3 ; neutrophils 70%, small lymphocytes 18%, and monocytes 12%. Examination of the bone marrow specimen (13 August 1968) revealed slight hypercellularity with a myeloid:erythroid ratio of 1:2 and a decrease in megakaryocytes. The leucocyte alkaline phosphatase was 238/100 neutrophils (normal range is 43–123). The patient was transfused with red cells and discharged on oral folic acid.

In October 1968, her white cell count was 46,000/ mm^3 , with 60% neutrophils, 6% small lymphocytes, 14%

TABLE I
SUMMARY OF CHROMOSOMAL ANALYSES

Stain	Number of Cells Counted			Total No. of Cells
	Chromosome No. 44*	Chromosome No. 45*	Chromosome No. 46*	
<i>Case 1</i>				
Giemsa only	4(2)†	13(9)	71(40)	88(51)
Fluorescence and Giemsa	2(2)	7(2)	22(5)	31(9)
Fluorescence only		1(1)	2(2)	3(3)
Total	6(4)	21(12)	95(47)	122(63)
<i>Case 2</i>				
Giemsa only	10(4)	13(11)	117(30)	140(45)
Fluorescence and Giemsa	3(3)	2(2)	12(12)	17(17)
<i>Case 3</i>				
Giemsa only		1	46(14)	47(14)
Fluorescence and Giemsa		2(2)	6(6)	8(8)
<i>Case 4</i>				
Fluorescence and Giemsa	1(1)	5(5)	10(10)	16(16)

* Random loss of one or two chromosomes due to cell breakage.

† The number of cells analysed in detail are given in parentheses.

monocytes, 7% myelocytes, and 13% metamyelocytes. A bone marrow aspirate showed marked granulocytic hyperplasia and a myeloid:erythroid ratio that approached 100:1 in some areas; erythroid maturation was normal and megakaryocytes were reduced. The leucocyte alkaline phosphatase score was 76/100 neutrophils. The patient was maintained on busulphan until November 1969, when the white count increased to 39,500/ mm^3 with 10% blasts in the peripheral smear. Despite increased busulphan, and later cytosine arabinoside, the patient died on 9 February 1970, of infection and tracheal obstruction secondary to CML in blastic crisis. No necropsy was performed.

Cytogenetic Analysis. Chromosomal studies were performed on marrow samples obtained on 13 August, 25 October, and 30 December 1969. All diploid cells showed an apparently normal karyotype, and no cells with the Ph^1 chromosome were seen (see Table I). In 1973, slides prepared from the last sample, which had been stored in acetic acid-alcohol fixative in the freezer, were stained with quinacrine mustard and examined with fluorescence. Analysis of the fluorescence banding pattern revealed a normal karyotype; particular attention was paid to chromosomes 9 and 22 (Figs. 1c and 2c).

Case 3. This 72-year-old white female was seen on 6 May 1970 for evaluation of her response to treatment for CML, which had been detected in February 1970 when she had noted persistent bleeding following a dental extraction. Peripheral blood examination in February showed haemoglobin, 8.4 g%; platelet count, 44,000/ mm^3 ; and white cell count, 74,000/ mm^3 . The bone marrow sample was hypercellular with myeloid hyperplasia, especially myeloblasts and promyelocytes. The erythroid series was severely depressed; megakaryocytes

were sparse. The patient had received 6-mercaptopurine, busulphan, and later irregular doses of cyclophosphamide.

At the time of admission she was receiving prednisone, 5 mgm qid; haematocrit was 28%, platelets were 5,000/mm³; and the white cell count was 15,000/mm³; neutrophils 53%, small lymphocytes 12%, monocytes 10%, promyelocytes 4%, and myeloblasts 21%. The leucocyte alkaline phosphatase score was 341/100 neutrophils. Bone marrow examination showed a very hypercellular marrow with a large number of myeloblasts and promyelocytes and rare myelocytes and metamyelocytes. The number of erythroid elements was increased compared with the previous sample; megakaryocytes were practically absent. A diagnosis of CML in acute blast transformation was made; treatment with cytosine arabinoside and 6-thioguanine did not induce a remission and the patient died on 26 June 1970; no necropsy was performed.

Cytogenetic Analysis. The diploid cells obtained from the admission bone marrow sample appeared to have a normal karyotype (Fig. 1d). In 1973, Giemsa-stained cells were photographed, the slides destained, stained with quinacrine mustard, and examined with fluorescence. The slides were then treated with NaOH to produce centromeric staining. These cells also showed a normal fluorescence karyotype.

Case 4. This 73-year-old white female who entered the Michael Reese Hospital and Medical Center on 18 August 1973, had noted easy bruising for three months. On physical examination, the liver was enlarged and there were petechiae over the chest, abdomen, and extremities. The peripheral blood examination showed haemoglobin,

8 g%; haematocrit, 26%; platelet count, 225,000/mm³; white cell count, 78,000/mm³; neutrophils 36%, bands 12%, metamyelocytes 7%, myelocytes 9%, promyelocytes 6%, myeloblasts 8%, small lymphocytes 3%, and monocytes 19%. Some of these mononuclear cells were thought to represent neutrophilic precursors without significant granule formation.

The bone marrow was hypercellular with numerous megakaryocytes and an increased myeloid:erythroid ratio. There was a definite shift to the left in the myeloid series, with approximately 10% blasts. The leucocyte alkaline phosphatase score was 1/100 neutrophils. Serum muramidase was 92 µg/ml and urine muramidase, 244 µg/ml. Busulphan therapy was begun on 26 August 1973 and the patient was discharged 2 September 1973.

Cytogenetic Analysis. Chromosomal analysis was performed on the bone marrow specimen (20 August 1973) and on two samples of peripheral blood (20 and 24 August) cultured without phytohaemagglutinin and harvested at 24 and 48 hours. The results of the three analyses were similar and have been combined in Table I. The modal chromosomal number was 46; all analysed diploid metaphases showed a normal karyotype. Some slides previously stained with Giemsa were destained and restained with quinacrine mustard; other slides were stained with quinacrine mustard first and then with Giemsa. The karyotype of quinacrine-banded chromosomes appeared to be normal (Fig. 3).

Discussion

These cases appear to have been typical of Ph¹ negative CML in that the disease had a relatively rapid course, namely, 7, 28, and 6 months for cases

FIG. 3. Karyotype of a cell from case 4, obtained from peripheral blood which had been cultured for 24 hours without PHA. The cell was first stained with quinacrine mustard, and then restained with Giemsa.

1, 2, and 3, respectively. The suggestion has been made that the Ph¹ negative variant might have a different aetiology than Ph¹ positive CML since, on clinical grounds alone, these two groups of patients appear to be distinct (Whang-Peng *et al*, 1968; Ezdinli *et al*, 1970). It has been thought that this clinical difference might be related to the presumed loss of the long arm of No. 22. The evidence now suggests that the long arm of No. 22 (Ph¹) may have been translocated to the long arm of No. 9. If this is the case, cells from Ph¹ positive patients with a translocation between No. 9 and No. 22 may have the same, or nearly the same, amount of genetic material as the karyotypically normal cells from the Ph¹ negative patients presented here. Therefore, the clinical differences between these groups of patients do not seem to be accounted for by a loss of genetic material.

The mechanism leading to these clinical differences is unclear at present; however, there are at least three possible explanations. Firstly, Ph¹ positive and Ph¹ negative CML may be produced by different aetiological agents. Second, in the process of translocation in Ph¹ positive patients, there may be a loss of a critical portion of either chromosome No. 9 or No. 22 which is too small to be detected with current techniques. Third, as a result of the translocation, some or all of the chromosomal material from No. 22 might undergo a change which could produce a decrease in its genetic activity. This would lead to a functional deletion even though the amount of chromosomal material remained intact.

I thank the following for referring the four cases: Case 1—Drs Joseph Baron and R. A. Hovanessian; Case 2—Drs Joseph Baron and Daniel Roth; Case 3—Dr Edgar Moran; and Case 4—Drs Richard Desser and Arthur Billings. Expert technical assistance was provided by Miss Margaret Ikeda and Mrs Judith Mikuta and by Mr David Potter.

REFERENCES

- Caspersson, T., Gahrton, G., Lindsten, J., and Zech, L. (1970). Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. *Experimental Cell Research*, **63**, 238–239.
- Ezdinli, E. Z., Sokal, J. E., Crosswhite, B. S., and Sandberg, A. A. (1970). Philadelphia-chromosome-positive and negative chronic myelocytic leukemia. *Annals of Internal Medicine*, **72**, 175–182.
- O'Riordan, M. L., Robinson, J. A., Buckton, K. E., and Evans, H. J. (1971). Distinguishing between the chromosomes involved in Down's syndrome (trisomy 21) and chronic myeloid leukaemia Ph¹ by fluorescence. *Nature*, **230**, 167–168.
- Paris Conference (1971). Standardization in human cytogenetics. *Birth Defects: Original Article Series*, **8**, Pt. 7, 1972. National Foundation—March of Dimes, New York.
- Pedersen, B. (1966). The aneuploid, Ph¹-positive cell population during progression and treatment of chronic myelogenous leukaemia. *Acta Pathologica et Microbiologica Scandinavica*, **67**, 451–462.
- Perillie, P. E. and Finch, S. C. (1970). Muramidase studies in Philadelphia-chromosome-positive and chromosome-negative chronic granulocytic leukemia. *New England Journal of Medicine*, **283**, 456–458.
- Rowley, J. D. (1973). A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. *Nature*, **243**, 290–293.
- Rowley, J. D., Blaisdell, R. K., and Jacobson, L. O. (1966). Chromosome studies in preleukemia. I. Aneuploidy of group C chromosomes in three patients. *Blood*, **27**, 782–799.
- Whang-Peng, J., Canellous, G. P., Carbone, P. P., and Tjio, J. H. (1968). Clinical implications of cytogenetic variants in chronic myelocytic leukemia (CML). *Blood*, **32**, 755–766.
- Woodliff, H. J. (1971). *Leukaemia Cytogenetics*, p. 51. Year Book Medical Publishers, Chicago, Illinois.