**38. Clonal Origin of Ph¹-positive Cells in a Case of
46,XX/47,XXX/48,XXXX Mosaicism
Associated with Chronic
Myelocytic Leukemia**

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The clonal origin of the Philadelphia chromosome (Ph¹)-positive cells in chronic myelogenous leukemia (CML) has been suggested on the basis of the fact that in CML patients heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase (G6PD), the Ph¹-positive hematopoietic cells show a single enzyme type, A or B, whereas both A- and B-type G6PD are expressed in the Ph¹-negative skin fibroblasts or T-lymphocytes of the same patients (Fialkow *et al.* 1967, 1977). Further evidence for the clonal origin of CML has been obtained by making use of heteromorphic banding patterns of chromosome 9 (Hayata *et al.* 1974) or 22 (Gahrton *et al.* 1974), as well as a chromosome 9-linked marker enzyme adenylate kinase-1 in somatic cell hybrids between CML and Chinese hamster cells (Kessel *et al.* 1982). Similar data indicating the clonal development of CML have been presented in some sex-chromosome mosaic patients such as 46,XX/47,XXY (Fitzgerald *et al.* 1971), 46,XY/47,YYY (Moore *et al.* 1974) and 45,X/46,XX (Chaganti *et al.* 1981).

We report here a case of 46,XX/47,XXX/48,XXXX mosaicism associated with CML; in this case the Ph¹ chromosome was found to occur exclusively in the XXX cells, providing additional evidence for the clonal origin of CML.

Case report. The patient (HK 946), a 44-year-old phenotypically normal but infertile Japanese female, was admitted to the hospital on October 27, 1981 due to general fatigue with persistent low grade fever and discomfort in the upper left quadrant of the abdomen. Physical examination revealed marked splenomegaly and hepatomegaly. Hematologic data in peripheral blood were as follows: WBC, $363.9 \times 10^3/\text{mm}^3$; RBC, $361 \times 10^4/\text{mm}^3$; Hgb, 11.3 g/dl; Hct, 33.5%; and platelets, $16.5 \times 10^4/\text{mm}^3$, with a differential count of 45%

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blasts. The bone marrow aspirate showed $962 \times 10^3/\text{mm}^3$ nucleated cells (myeloblasts, 82.8%) and $15/\text{mm}^3$ megakaryocytes, with an M/E ratio of 80.6. Myeloperoxidase reaction was only partially positive (3%), and neither Auer rods nor Azur granules were detected. The neutrophil alkaline phosphatase appeared to be low (rate, 32; score, 57), as compared to the control value (rate, 90; score, 215). The patient was diagnosed as in the acute phase of CML. Although the regular combination therapy with vincristine (2 mg/week I.V.) and prednisolone (60 mg/day P.O.) was unsuccessful, a complete remission was induced when prednisolone was combined with Acracinomyacin (20 to 40 mg/day I.V.) and given to the patient for 17 days. In two weeks after discontinuation of the second therapy, the patient's general condition was greatly improved, showing a complete disappearance of hepatosplenomegaly, with no trace of blast cells in peripheral blood, and less than 2% of leukemic cells in the bone marrow. After four months duration of the chronic phase, she developed relapse and died on June 25, 1982.

Materials and methods. Cytogenetic studies were performed on bone marrow and peripheral blood cells, in the initial acute phase before therapy, as well as in remission and relapse. Bone marrow preparations were made by a direct air-drying method, after treatment with a hypotonic KCl solution (0.075 M) containing Colcemid ($0.0015 \mu\text{g}/\text{ml}$) for 20 mins at 37°C , and fixation in 3:1 methanol-acetic acid. Peripheral blood lymphocytes were cultured in Eagle's minimum essential medium containing 10% fetal bovine serum and a drop of mitogen, either phytohemagglutinin (PHA) or pokeweed mitogen (PWM). The PHA-added culture was incubated for 72 hrs, while the PWM-added one 144 hrs. Fibroblastic cells were also obtained from skin cultures of the patient. The cultured cells were treated with $0.003 \mu\text{g}/\text{ml}$ Colcemid for 1 hr before harvest, placed in 0.075 M KCl for 15 mins, fixed in 3:1 methanol-acetic acid, and air-dried on slides. Chromosome banding patterns were demonstrated by the Hoechst 33258-quinacrine mustard double staining method (Yoshida *et al.* 1975). The late replicating X chromosome was identified by the BrdU-acridine orange (AO) method (Dutrillaux *et al.* 1973) in short-term cultures of bone marrow cells without PHA, as well as in PHA-added blood cultures. Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN 1978).

Results. The first cytogenetic examination made in the blastic phase revealed that all of the 18 bone marrow cells analyzed by the Q-banding method had a Ph^1 chromosome and three X chromosomes. Seven of the 18 cells had a $47, \text{XXX}, \text{t}(9;22)$ karyotype, whereas the

remaining 11 showed various types of numerical and/or structural changes in addition to the X-trisomy and Ph¹. These additional changes appeared to be random, with or without markers of unknown origin. Two of the three X-chromosomes in the Ph¹-positive marrow cells were late replicating as ascertained by the BrdU-AO method. By contrast, all of the marrow cells examined in remission (26 metaphases) and relapse (19 metaphases) had a 47,XXX,t(9;22) karyotype. On the other hand, PHA-stimulated lymphocytes of this patient were found to comprise three cell lines, 46,XX (35%), 47,XXX (50%) and 48,XXXX (15%), based on a total of 500 metaphases examined, indicating a constitutional mosaicism. The number of late replicating X chromosomes was one for the 46,XX, two for the 47,XXX, and three for the 48,XXXX cells, respectively. While the XX/XXX/XXXX somatic constitution without Ph¹ was confirmed in the PWM-added blood culture of the same patient, her skin fibroblasts showed exclusively a 47,XXX karyotype among a total of some 50 metaphases so far analyzed.

Discussion. Four cases of sex-chromosome mosaicism associated with Ph¹-positive CML have been reported to date. The first case (Tough *et al.* 1961) was an XY/XXY mosaic patient whose leukemic marrow cells had a Ph¹ chromosome in both the XY and XXY cells, although the frequency of Ph¹-positive cells was much higher in the XY line than in the XXY line. The second case (Fitzgerald *et al.* 1971) showing also an XY/XXY somatic constitution was examined in the acute phase of leukemia; the Ph¹ chromosome was present in the XY line, but not in the XXY line. In this case, two other cell lines which were considered to be clonal derivatives of the Ph¹-positive XY cell line were observed. The third case (Moore *et al.* 1974) was an XY/XYY mosaic, with the XYY line predominating in his cultured lymphocytes and skin fibroblasts, whereas the direct marrow preparations demonstrated an additional XY line including a Ph¹, which predominated in both the chronic and acute phase. The last case (Chaganti *et al.* 1982) was a Turner syndrome patient with an X/XX mosaic constitution whose Ph¹-positive leukemic marrow cells always had a single X-chromosome, in spite of the fact that the proportion of 45,X and 46,XX cells in both cultured lymphocytes and skin fibroblasts of this patient was 1:9. These findings have been thought to favor the clonal origin of the Ph¹-positive cells in CML, being in line with the results obtained through the enzymatic or chromosomal marker studies as mentioned before.

Thus the present XX/XXX/XXXX mosaic case may provide additional evidence for the clonal development of CML in that all

the marrow metaphases examined showed an XXX+Ph¹ constitution throughout the clinical course including the initial acute phase, as well as the subsequent remission and relapse. However, since the data at hand are still meager and insufficient, whether all of the CML cases are actually of unicellular origin seems to be a matter of debate. In fact, the first reported case of XY/XXY mosaic (Tough *et al.* 1961) showed a Ph¹ in both cell lines, and hence could be biclonal in origin, even though an alternative possibility that the Ph¹-positive XY line might have derived from the Ph¹-positive XXY line, or vice versa, can not be excluded. Our patient who showed a single cell line in the skin culture was apparently a somatic mosaic comprising three cell lines in cultured lymphocytes stimulated by PHA and PWM. Nevertheless, her bone marrow cells showed exclusively an XXX+Ph¹ constitution throughout the clinical course even in remission. This suggests that the Ph¹-positive marrow cells of this patient have originated from a single stem cell of the myeloid series, if we exclude the possibility that the XXX cells are more prone to acquire Ph¹ or to undergo neoplastic transformation as compared to the XX and XXXX cells. In order to test the latter possibility, we have attempted to find out any heteromorphic marker band, as revealed by C- or Q-banding, between homologues of chromosomes 9 and 22, although the results obtained were not informative in this case, unlike the cases reported by Hayata *et al.* (1974) and Gahrton *et al.* (1974).

It has been reported that individuals with certain constitutional chromosome abnormalities, such as Down syndrome, are associated with a high risk of malignant diseases including leukemia (Sandberg 1980), and that somatic cells of such patients are hypersensitive in terms of the yield of chromosome aberrations or sister chromatid exchanges induced by radiation (Sasaki *et al.* 1970; Higurashi and Conen 1972; Countryman *et al.* 1977; Crossen and Morgan 1980; Preston 1981), viruses (Higurashi *et al.* 1973), and chemicals (O'Brien *et al.* 1971; Kaina *et al.* 1977; Sugimoto *et al.* 1982). Although the unique occurrence of Ph¹ in the XXX cells of our patient might be related to a certain genetic predisposition of such aneuploid cells, Ph¹-positive leukemic cells in other cases of sex-chromosome mosaic have been reported to occur in chromosomally normal as well as abnormal cells, irrespective of the proportion of cell lines involved in a given mosaicism as mentioned before. Further information and some experimental studies on Ph¹-positive CMLs developed in constitutional mosaic patients may shed light on the etiology and pathogenesis of CML and related disorders.

Summary. A 44-year-old female diagnosed as having chronic myelogenous leukemia (CML) in the acute phase is found to possess

three cell lines, 46,XX (35%), 47,XXX (50%), and 48,XXXX (15%), in cultured lymphocytes, although her skin culture shows exclusively a 47,XXX karyotype. By contrast, all of the bone marrow cells examined in the acute phase as well as in remission and relapse has a 47,XXX,t(9;22) constitution, with some random karyotypic variations in the initial acute phase before therapy. These findings seem to provide additional evidence for the monoclonal origin of Ph¹-positive cells in CML.

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