86. On the Monoclonal Origin of Chronic Myelocytic Leukemia

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Whether cellular transformation is of unicellular or multicellular origin is an important problem for the understanding of oncogenesis. Fialkow et al. (1967) have supported the uniclonal origin of leukemia with enzyme genetic studies in chronic myelocytic leukemia (CML). Evidence for the unicellular origin of CML is presented in this study in relation to the Ph1 chromosome translocation, recently described in CML by Rowley (1973).

Case report. The initial diagnosis in this patient was acute myelocytic leukemia (AML), when he was 44 years old. Following admission to Roswell Park Memorial Institute, cytosine arabinoside and thioguanine were used for therapy and he responded well. In December, 1970, a marrow specimen showed a slightly increased M:E ratio, a decreased number of megakaryocytes, and a slight increase in myeloblasts. One and a half years later the bone marrow showed a greatly increased cellularity and M:E ratio, no megakaryocytes, and a definite increase of promyelocytes and mature basophils. The marrow at this time appeared to be more compatible with CML than AML. In all probability we are dealing with a case of CML, which first presented itself in a very blastic phase. The first chromosomal study was made in December, 1970. Only one out of forty-six cells from the marrow had a Ph1 chromosome and all other cells were Ph1 negative. Chromosomal studies in January, May, and July, 1972, revealed that all of the cells (61 metaphases) from the marrow had a Ph1 chromosome. Subsequently, all cells from either the marrow or cultured peripheral blood (without PHA) were 100% Ph1 positive.

Materials and methods. The marrow aspirate was brought to the laboratory, being kept in RPMI 1640 culture medium with 17% fetal calf serum. The cells were treated with colcemide (0.02–0.03 μg/ml) for 20 min. at 37°C in a 0.075 M KCl hypotonic solution and fixed with acetic alcohol (1:3). Blood cultures were made with leukocyte rich plasma in RPMI 1640 medium containing 30% fetal calf serum for 24 hours without PHA, in order to obtain metaphases of leukemic origin, and for 72 hours with PHA for obtaining karyotypes from normal lymphocytes. The metaphases of leukemic origin
were treated with 0.010–0.015 μg/ml of colcemide for 70 min. and the controls with 0.05 μg/ml of colcemide for 120 min. Hypotonic treatment of cultured cells was done with a 0.075 M KCl solution without colcemide for 25 min. at 37°C and the cells were fixed with acetic alcohol. The slides were made by means of an air-drying method with special modifications. Buffered (McIlvain's phosphate buffer, pH 6.8) quinacrine mustard was used for fluorescent staining, according to the method of Caspersson et al. (1970). Trypsin-Giemsa banding slides were made with a slightly modified technique of Seabright (1971). C banding followed the process of Arrighi and Hsu (1971), with slight modifications.

Results and remarks. The patient's leukemic cells had a non-homologous No. 9 chromosome pair showing a different banding pattern on the long arm of one member, in addition to the translocation at the terminal end (Fig. 1, a & b). Characteristics of the banding pattern of the No. 9 chromosome with the translocation consisted of two parts: 1) a large negative staining Q or G band at the proximal part (region lq or 9qh), and 2) the loss of an intermediate pale Q band or a negative G band between evenly spaced medium fluorescent

Fig. 1a. Q banding karyotype of Ph1 positive cell from the CML patient presented.
bands or dark stained G bands at the middle part (region 2q) on the long arm (Fig. 2, a & b). C banding studies indicated a larger C band on the No. 9 chromosome with the translocation, of which the long arm beyond the C band was longer than that of the homolog (Fig. 3a). This heteromorphism of the C band was also present in normal cells (Fig. 3b). Q and G banding analysis of the chromosomes from normal lymphocytes of this patient revealed a non-homologous band in the 9qh region, but with a homologous banding pattern in the other regions (Fig. 4, a & b). Thus, the Ph\(^1\) translocation occurred onto either a maternal or paternal No. 9 chromosome with a bigger C band or the translocation possibly induced an additional chromosomal change in the middle part of the long arm. To-date, none of the 15 cases of CML with Ph\(^1\) translocation studied in our laboratory (unpublished data) has shown a change of the banding pattern in the original part of the No. 9 chromosome with the translocation.

Fitzgerald et al. (1971) and Motomura et al. (1973) discussed clonal evolution of CML, each describing a case of sex chromosome mosaicism with CML. A monoclonal evolution of the karyotype in the blastic transformation of CML was indicated by their reports. However, it was not clear as to whether the appearance of the Ph\(^1\) chromosome was of unicellular or multicellular origin. Tough et al.
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(1961) observed a Ph1 chromosome both in 46, XY and 47, XXY cell lines in a case of Klinefelter's mosaicism with CML, whereas Moore et al. (1974) found a Ph1 chromosome only in a 46, XY line in a case of 46, XY/47, XYY mosaicism with CML. Moore et al. (1974) suggested that the presence of the 46, XY, Ph1 line in the former mosaicism might be the result of transformation occurring after the appearance of Ph1 chromosome, since the skin cells were carrying only 47, XXY. With fluorescent banding techniques Gahrton et al. (1973) reported a case of CML with a Ph1 derived from a maternal No. 22 chromosome which had a characteristic Q band at the satellite region.

The possible multiclonal origin of the CML in our case, though extremly unlikely, cannot be absolutely ruled out. Thus, it is possible to envision that the modified No. 9 chromosome preferentially attracted the translocation from No. 22 and, hence, the karyotypic changes consistently involved the modified No. 9 chromosome. The additional modification of the substructure of the No. 9 chromosome, however, may be due to the leukemic process or induced by the translocation.

In the present case, the Ph1 positive cells always had two unusual marker bands, as described above. Even if there is a predilection for

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Fig. 2, a and b. No. 9 pairs of chromosomes from Ph1 positive cells showing non-homologous Q (Fig. 2a) and G (Fig. 2b) banding patterns.

Fig. 3, a and b. Heteromorphic C banding of No. 9 pairs of chromosomes from leukemic cells (Fig. 3a) and normal lymphocytes (Fig. 3b).

Fig. 4, a and b. Q (Fig. 4a) and G (Fig. 4b) banding patterns of chromosome No. 9 from normal lymphocytes.
the chromosomal change to exist in one of the chromosomes of pair No. 9, it is difficult to explain with the multicellular origin concept of CML the fact that in the present study the No. 9 chromosome with the translocation always had a missing band. Hence, the present findings strongly favor the monoclonal origin of CML.

Summary. A male patient with CML had an unusual No. 9 chromosome with a typical Ph1 chromosome translocation. This unusual No. 9 chromosome showed heteromorphism at the 9qh region and a missing band in the middle of its long arm. These characteristic features of the leukemic cells strongly support the monoclonal origin of CML.

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