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Chromosome Studies in Children and Adults with Leukemia*

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A. Introduction

The study of the chromosome pattern (karyotype) of human leukemic cells is one of the most rapidly progressing areas of clinical research. As more patients are examined, we are now able to correlate the karyotype with the type of leukemia and with the patient's response to therapy. Chromosome analysis of leukemic cells thus can provide information of both diagnostic and prognostic significance. The clinical usefulness is largely the result of new technical advances in staining procedures that allow us to identify each chromosome precisely with banding techniques.

Many fewer patients with lymphoid leukemias have been studied than those with myeloid leukemia; only two unselected series of patients with acute lymphocytic leukemia (ALL) whose cells have been examined with the new banding techniques have been reported, and thus correlations between the karyotype and the type of leukemia and response to therapy are generally inconclusive. Chromosome abnormalities will be described in various myeloid leukemias including chronic myeloid leukemia, Ph[1]-positive acute leukemia, and acute nonlymphocytic leukemia. Within each category, the data that have been collected will be correlated with clinical information.

B. Methods

An analysis of chromosomal patterns, to be relevant to a malignant disease, must be based on a study of the karyotype of the tumor cells themselves. In the case of leukemia the specimen is usually a bone marrow aspirate that is processed immediately or cultured for a short time (Testa and Rowley, to be published). In patients with a white blood cell count higher than 14,500 and with about 10% immature myeloid cells, it is possible to culture a sample of peripheral blood for 24 or 48 h without adding phytohemagglutinin (PHA). In most instances cells from unaffected tissues will have a normal karyotype. The chromosome abnormalities observed in the leukemic cells thus represent somatic mutations in an otherwise normal individual.

Chromosomes obtained from bone marrow cells, particularly from patients with leukemia, frequently are very fuzzy, and the bands may be indistinct. The observation of at least two "pseudodiploid" or hyperdiploid cells or three hypodiploid cells, each showing the same abnormality, is considered evidence for the presence of an abnormal clone; a clone is defined as a group of cells all of which have arisen from a single cell. Patients with such clones are classified as abnormal; those whose cells show no alterations or in whom the alterations involve different chromosomes in different cells are considered to be normal. Isolated changes may be due to technical artifacts or to random mitotic errors.

In the following discussion the chromosomes are identified according to the Paris Nomenclature (Paris Conference 1972), and the karyotypes are expressed as recommended under this system. The total chromosome number is indicated first, followed by the sex chromosomes, and then by the gains, losses, or rearrangements of the autosomes. A "+" sign or "−" sign before a number indicates a gain or loss, respectively, of a whole chromosome; a "+" or "−" after a number indicates a gain or loss of part of a chromosome. The letters "p" and "q" refer to the short and long arms of the chromosome, respectively; "i" and "r" stand for "isochromosome" and

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"ring chromosome". "Mar" is marker, "del" is deletion, "ins" is insertion, and "inv" is inversion. Translocations are identified by "t" followed by the chromosomes involved in the first set of brackets; the chromosome bands in which the breaks occurred are indicated in the second brackets. Uncertainty about the chromosome or band involved is signified by "?".

C. Ph¹-Positive Myelogenous Leukemia

Cytogenetic studies on patients with chronic myelogenous leukemia (CML) have been the keystone for karyotype analysis of other human malignancies. New discoveries that have resulted from examination of CML have subsequently been confirmed in other hematologic malignancies and in many solid tumors as well.

I. Chromosome Studies of Chronic Myelogenous Leukemia

Nowell and Hungerford in 1960 reported the first consistent chromosome abnormality in human cancer; they observed an unusually small G-group chromosome which appeared to have lost about one-half of its long arm in leukemic cells from patients with CML (Nowell and Hungerford 1960). The question whether the deleted portion of the long arm of the Ph¹ chromosome was missing from the cell or whether it was translocated to another chromosome could not be answered at that time, because it was impossible to identify each human chromosome precisely with the techniques then available. Furthermore, the identity of this chromosome as either a No. 21 or No. 22 could not be established. Despite this uncertainty, the Ph¹ chromosome was a very useful marker in the study of patients with CML.

Bone marrow cells from approximately 85% of patients who have clinically typical CML contain the Ph¹ chromosome (Ph¹+) (Sandberg 1979; Whang-Peng et al. 1968); the other 15% of the patients usually have a normal karyotype (Ph¹−). A perplexing observation, still not explained, was that patients with Ph¹+ CML had a much better prognosis than those with Ph¹− CML (42− vs 15-month survival). However, as was shown by Whang-Peng et al. (1968) a change in the karyotype was a grave prognostic sign; the median survival after such a change was about 2 months.

Chromosome banding techniques were first used in the cytogenetic study of leukemia for identification of the Ph¹ chromosome as a deletion of No. 22 (22q−) (Caspersson et al. 1970; O'Riordan et al. 1971). Since quinacrine fluorescence revealed that the chromosome present in triplicate in Down's syndrome was No. 21, the abnormalities in Down's syndrome and CML were shown to affect different pairs of chromosomes.

The question of the origin of the Ph¹ (22q−) chromosome was answered in 1973, when Rowley reported that the Ph¹ chromosome results from a translocation rather than a deletion as many investigators had previously assumed (Rowley 1973a). Additional chromosome material was observed at the end of the long arm of one. No. 9 (9q+) and was approximately equal in length to that missing from the Ph¹ chromosome; it had staining characteristics similar to those of the distal portion of the long arm of No. 22. It was proposed, therefore, that the abnormality of CML was an apparently balanced reciprocal translocation t(9;22)(q34;q11). Karyotypes of 802 Ph¹+ patients with CML have been examined with banding techniques by a number of investigators, and the 9;22 translocation has been identified in 739 (92%). It is now recognized that variant translocations may occur (Rowley 1980a; Sandberg 1979); one is a simple translocation involving No. 22 and some chromosome other than No. 9, which has been seen in 29 patients. The other is a complex translocation involving three or more different chromosomes; except in two cases, two of the chromosomes involved were found to be No. 9 and No. 22. This type of translocation has been observed in 31 patients. The great specificity of the translocation involving Nos. 9 and 22 remains an enigma. The survival curves for patients with variant translocations appeared to be the same as those for patients with the standard t(9;22).

When patients with CML enter the terminal acute phase, about 20% appear to retain the 46,Ph¹+ cell line; additional abnormalities are superimposed on the Ph¹+ line in 80% of patients (Rowley 1980a; Sandberg 1979). In a number of cases the change in the karyotype preceded the clinical signs of blast crisis by 2 to 4 months. Bone marrow chromosomes from 242 patients with Ph¹+ CML who were in

acute phase have been analyzed with banding techniques (Rowley 1980a). Forty showed no change in their karyotype, whereas 202 patients had additional chromosome abnormalities. The most common changes frequently occur in combination to produce modal numbers of 47 to 52. The chromosome aberrations observed most often are a gain of No. 8, a duplication of the Ph[1], an isochromosome for the long arm of No. 17, and a gain of No. 19.

Only two reports have been published relating specific chromosome changes in the acute phase to survival of patients, and the results are conflicting. Thus, Prigogina et al. (1978) found that patients who did not shown additional abnormalities in the acute phase had a longer survival than those whose karyotypes showed such changes. Sonta and Sandberg (1978), however, found no difference in survival between these two groups.

II. Ph¹-Positive Acute Leukemia

Our interpretation of the biologic significance of the Philadelphia chromosome has been modified over the course of the last nine years as our clinical experience with this marker has widened. Thus, earlier it was proposed that cases of acute myeloblastic leukemia (AML) in which the Ph[1] chromosome was present should be reclassified as cases of chronic myeloid leukemia in blast transformation. This notion, which was broadened to include the cases that appeared to be ALL at diagnosis, was generally accepted until about 1977. More recently, however the tendency has been to refer to patients who have no prior history suggestive of CML as having Ph[1]-positive leukemia (Rowley 1980a). It is becoming increasingly evident that the observed interrelations of Ph[1]+ leukemias are complex indeed. Thus, some patients have a high percentage of lymphoblasts, others have a high percentage of myeloblasts, and still others have a mixture of myeloblasts and lymphoblasts. In the future the use of cell surface markers will help to define these groups of patients further.

D. Acute Nonlymphocytic Leukemia

The use of chromosome banding techniques has markedly increased our understanding of the types and frequency of chromosome abnormalities in acute nonlymphocytic leukemia (ANLL), including AML, erythroleukemia, and acute monocytic leukemia. Extra, missing, or rearranged chromosomes previously described on the basis of morphology alone can now be identified precisely in terms of the particular chromosomes or chromosome bands involved. We and others have also shown that the karyotype pattern of the leukemic cells is correlated with survival. Patients with a normal karyotype have a significantly longer median survival (10 months) than do patients with an abnormal karyotype (4 months) (Golomb et al. 1978). As illustrated in Fig. 1, this difference in survival is more pronounced for AML than for acute myelomonocytic leukemia (AMMol). Leukemic patients who are alive at 1 year are much more likely to have only normal metaphases.

Approximately 50% of the patients studied with banding have detectable karyotypic changes; these abnormalities are present prior to therapy and usually disappear when the patient enters remission. The same aberrations reappear in relapse, sometimes showing evidence of further karyotypic change superimposed on the original abnormal clone. Although the karyotypes of patients with ANLL may be variable, the chromosome changes in

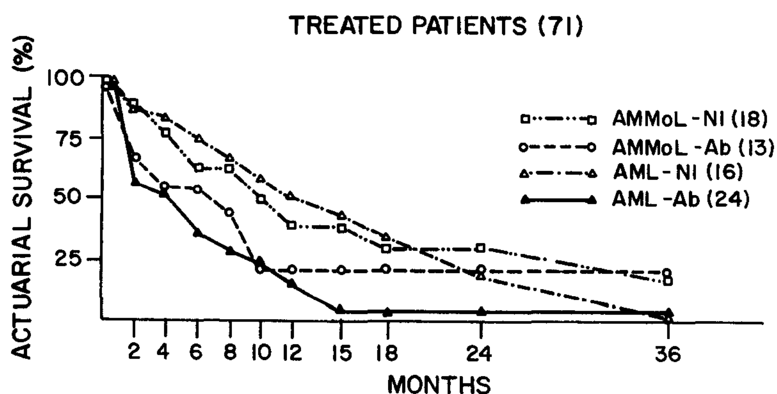

Fig. 1. Actuarial survival of treated patients versus time in months according to cytologic diagnosis (AML, AMMoL) and normal (Nl) or abnormal (Ab) karyotype. Reproduced from Golomb et al. (1978), with permission

191 chromosomally abnormal patients are available for analysis (Testa and Rowley 1980). Roughly one-third of the patients have 46 chromosomes but with an abnormal pattern, one-fourth have fewer than 46 chromosomes, and the remainder have 47 or more chromosomes. The nonrandom distribution of chromosome losses and gains is particularly evident in patients with 45–47 chromosomes. A gain of No. 8 is the most common abnormality in ANLL and loss of one No. 7 is the next most frequent change (Fig. 2). Gains or losses of some chromosomes occurred only in patients with more complex karyotypes; they are likely to represent secondary changes occurring in clonal evolution rather than primary events.

Two structural rearrangements seen in ANLL appear to have special significance. The more common of these was described as the complex pattern, −C, +D, +E, −G. The precise nature of the abnormality was resolved by Rowley (Rowley 1973b) who used the Q-banding technique to determine that it is a balanced translocation between Nos. 8 and 21 [t(8;21)(q22;q22)]. This translocation is frequently associated with loss of a sex chromosome, about one-third of males with the 8; 21 translocation are −Y, and one-third of the females are missing an X (Rowley 1973b; Second International Workshop on Chromosomes in Leukemia, 1980). This association is especially noteworthy, since sex chromosome abnormalities are otherwise rarely seen in ANLL. The translocation appears to be restricted to patients with acute myeloblastic leukemia (M_2 in the FAB classification). According to data collected at the Second International Workshop (Second International Workshop on Chromosomes in

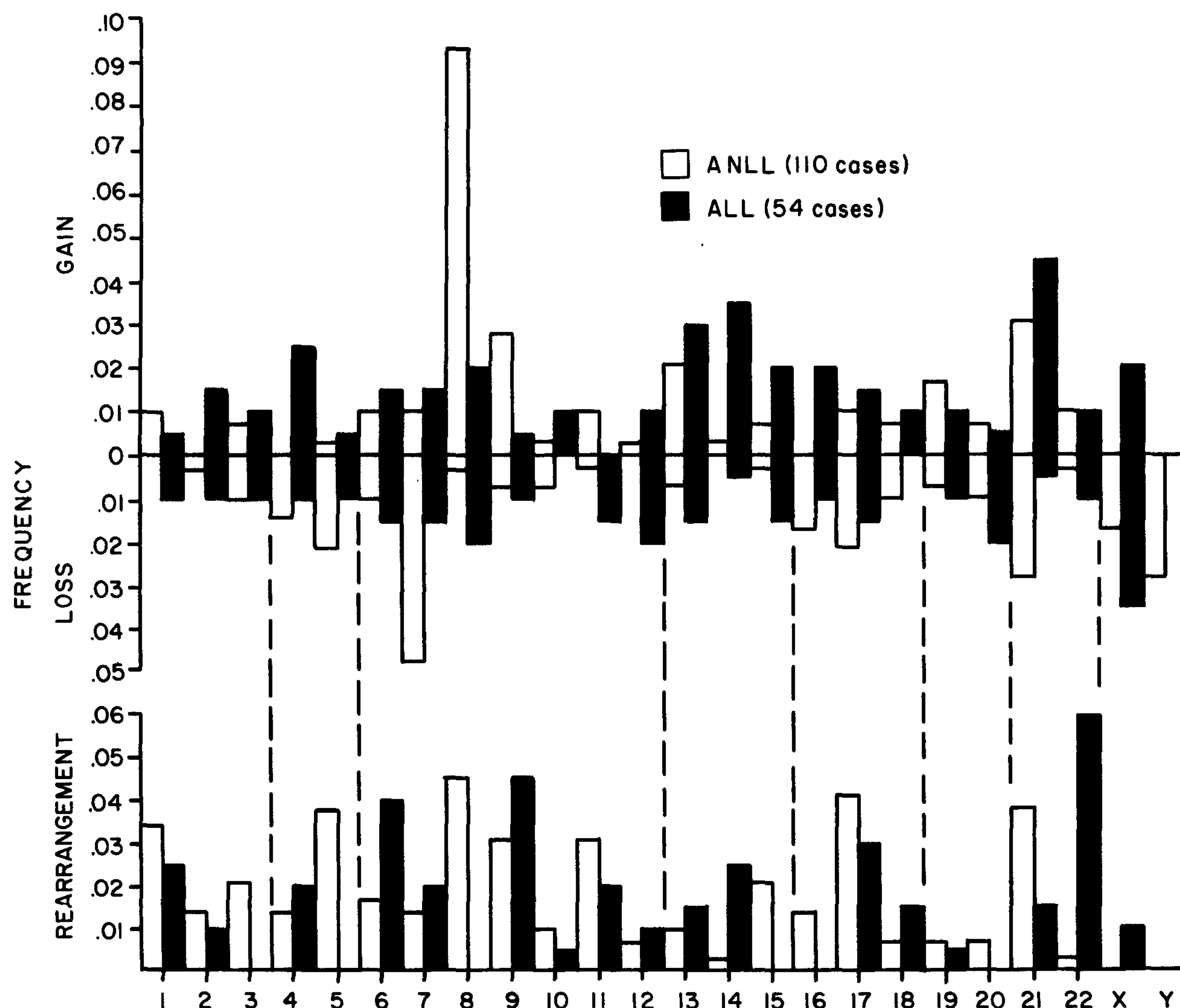

Fig. 2. Histogram of chromosome abnormalities (gains, losses, and rearrangements) in 110 cases of ANLL and in 54 cases of ALL, excluding documented cases of secondary karyotypic evolution. The frequency of each abnormality is calculated as a proportion of all abnormalities. Reproduced from Cimino et al. (1979), with permission

Leukemia, 1980), the median survival of patients with the t(8;21) was 11.5 months, with some subgroups having a median survival as long as 15 months.

Another significant structural rearrangement is that observed in acute promyelocytic leukemia (APL), which is a unique form of acute leukemia characterized by hemorrhagic episodes, disseminated intravascular coagulation (DIC), and infiltration of the marrow with atypical "hypergranular" promyelocytes. The FAB cooperative study group recently recognized that not all patients have coarse granules, and therefore it added a category called the M_3 variant. The variant category was identified largely on the basis of the clinical features and a specific chromosome abnormality, namely, a translocation involving the long arms of Nos. 15 and 17 [t(15;17)(q25;q22)] (Rowley et al. 1977; Second International Workshop on Chromosomes in Leukemia, 1980). It is important to make a correct early diagnosis because the initial therapy, which includes the use of heparin for control of bleeding, is associated with a significant improvement in survival. Whereas the correct diagnosis should not be difficult in typical cases, the M_3 variant, in which granules may be lacking or reduced in number, may cause confusion. Every one of our M_3, variant patients also had the typical translocation.

E. Acute Lymphoblastic Leukemia

Chromosome abnormalities have been observed in about one-half of the patients with ALL (Sandberg 1979). It has long been recognized that aneuploid patients with ALL have higher modal chromosome numbers than do patients with ANLL. Many fewer data are available on the types and frequency of chromosome changes in ALL than in ANLL.

Only two unselected series of patients with ALL, each studied with banding, have been reported; Oshimura et al. (1977) described results in 31 patients, and Cimino et al. (1979) described the results in 16 patients. There have been a number of other reports on one or a few patients, all selected for some unusual cytogenetic abnormalities, most frequently the presence of a Philadelphia (Ph^1) chromosome (Rowley 1980a). The small number of patients and the complexity of the karyotypes make the identification of nonrandom patterns in ALL very difficult at present. Despite these handicaps, at least one karyotypic abnormality seems to be relatively specifically associated with one type of ALL classified with the use of immunologic markers. It seems reasonable to assume that other associations will become apparent in the future as we gain additional information about both the karyotypic pattern and the cell surface markers of subpopulations of lymphoid cells that will be defined with more sophisticated immunologic techniques.

The chromosome pattern in 53 patients with ALL has recently been reviewed (Rowley 1980b). The most frequent single change in this group is a gain of one No. 21; the second most frequent change is a gain of one No. 14, and the next most frequent is a gain of one No. 13 (Fig. 2). The only chromosome lost with any frequency is one X chromosome. Abnormalities of the Y chromosome have not been described. The most common deletion is that involving the long arm of No. 6; the break point in 6q appears to be somewhat variable, involving the region from 6q11 to 6q25.

Patients with B-cell ALL constitute a small percentage (about 4%) of those with ALL, and they are identified because their cells express surface immunoglobulin. With rare exceptions, every ALL patient whose leukemic cells have been identified as B-cells had an abnormality of No. 14 which was the result of a translocation of material from another chromosome to the end of the long arm (14q+) (Rowley 1980b). The 8;14 translocation was regularly seen in patients who also had a solid tumor phase of Burkitt's lymphoma (Berger et al. 1979; Slater et al. 1979). Other abnormalities in addition to the 14q+ chromosome have been trisomy for part or all of the long arm of No. 1, structural rearrangements involving both the long and short arms of No. 6, and an additional No. 7. Cells from some patients were examined for Epstein-Barr virus (EBV) and were found to be negative (Slater et al. 1979). In one patient the cells had a characteristically low level of adenosine deaminase (Cimino et al. 1979).

A 14q+ chromosome is a frequent occurence in other malignant lymphoproliferative diseases, particularly, though not exclusively, in those of B-cell origin. The translocation was first discovered in Burkitt lymphoma. A translocation involving the long arms of No. 8 and No. 14 has been detected in almost all Burkitt

tumors of both African and non-African origin, independent of whether they are EBV-positive or -negative (Kaiser-McCaw et al. 1977; Zech et al. 1976). A 14q+ abnormality is rarely observed in myeloid disorders, and therefore the proposal that No. 14 may carry genes that are important in lymphocyte proliferation has been supported by all recent data (Kaiser-McCaw et al. 1977).

The data on chromosome patterns in ALL are only preliminary; however, some differences between the types of abnormalities seen in ANLL and ALL are apparent (Fig. 2). In ANLL the most common single change is a gain of one No. 8, followed by loss of No. 7 and rearrangements of No. 8 and No. 21 (Testa and Rowley 1980). Gain or loss of No. 21 is frequent, and loss of a sex chromosome, X or Y, usually occurs in association with structural aberrations of No. 8 or No. 21, or both. In ALL, the most common change is gain of one or two No. 21s, followed by gains of Nos. 13 or 14 and then gain of one No. 15 or an X, usually in males (Cimino et al. 1979). Two ALL patients showed the gain of one No. 8 initially, and evolution of the karyotype in three others involved the gain of one No. 8. The most frequent structural change in ALL is a deletion of No. 6, usually affecting the long arm. Rearrangements of No. 17 are often observed in both leukemias. In ANLL, gains of Nos. 6, 14, and 15 are rare and are seen only in patients with complex patterns; gain of the X has not been seen.

As described earlier, the presence of an abnormal clone of cells in patients with ANLL has a significant association with response to therapy and, therefore, with patient survival (Golomb et al. 1978). The lack of a sufficient number of ALL patients studied with banding makes such a correlation in patients with ALL difficult at the present time. Whang-Peng et al. (1976) related the unbanded karyotypic patterns to survival in 331 patients with ALL and concluded that "the appearance of aneuploid cells in the bone marrow at the onset or later in the disease is of no prognostic significance but persistence of these lines and the development of total aneuploidy signals a poor prognosis".

There are very few data relating the survival time of patients with ALL to the presence at diagnosis of clonal chromosome abnormalities identified with banding. Thus, in the series reported by Oshimura et al. (1977), length of survival was listed only for patients with an abnormal karyotype, and one-half of these were treated prior to the chromosome analysis. The median survival of the eight abnormal patients whom they studied prior to treatment was 13 months. In our limited series at The University of Chicago (Cimino et al. 1979) the median survival of eight patients with an abnormal karyotype was 10+ months (range, 2 to 33 months), as compared with 23+ months (11 to 56+ months) for eight patients with an initially normal karyotype. The duration of the initial remission differed considerably, being only 1 month (0 to 18+ months) in chromosomally abnormal patients, whereas it was 12+ months (1.5 to 46+ months) in chromosomally normal patients.

However, in a recent report, Secker Walker et al. (1978) concluded that the presence of an abnormal clone was not an adverse factor. Of six patients who had only abnormal cells at diagnosis, one relapsed after 4 years and the others remained in first remission for a longer period than this. These data are relevant to the observations of Bloomfield et al. (1978) and Chessells et al. (1979) that the survival of patients with Ph^1-positive ALL is very short. The range is 2 to 24 months, with a median for both children and adults of about 12 months. This contrasts with a median survival of more than 2 years in adult Ph^1-negative ALL (Bloomfield et al. 1978). However, if one compares the median survival of patients with Ph^1+ ALL (12 months) with that of other ALL patients who have aneuploidy (10–13 months), the survival times are the same.

F. Conclusions

The primary focus in this review has been on the identification of nonrandom chromosome changes in various myeloproliferative and lymphoproliferative disorders. Although the data available for these disorders are quite variable both with regard to the number of patients studied and the quality of banding, patterns of chromosome changes can be discerned that differ among the various groups. Wherever possible these patterns have been related to structural and functional characteristics of these cells as determined by others as well as to the clinical correlations of particular chromosome changes. In the future these correlations must be extended to relate specific

chromosome aberrations, particularly translocations and deletions, to alterations of the function of genes located at these sites.

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