THE CHROMOSOMES IN THE MYELODYSPLASIAS

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The discovery in 1960 (1) that chromosomal abnormalities occurred in leukemia raised the question of the relationship of these abnormalities to the etiology of leukemia. Approximately 90% of patients with chronic myelogenous leukemia have a specific abnormality in dividing leukemic marrow cells. (2, 3) This abnormality consists of the deletion of about 1/2 of the long arm of a G group chromosome, resulting in an abnormally small chromosome that has been called the Philadelphia chromosome or Ph^1 (Fig. 1). Cells with this abnormality contain the normal number of chromosomes (46) but they lack about 0.6 μ of chromatin, which has been estimated to represent 10^7 nucleotide pairs. (4) The Ph^1 is found only in the leukemic cells of these patients, not in their lymphocytes or skin. (5) It usually persists in the transformation to acute leukemia, when additional abnormalities may occur.

In contrast to this consistent finding in chronic granulocytic leukemia, chromosomal abnormalities are present in about 50% of patients with acute leukemia. (6, 7) Cells may have too few chromosomes or too many (Fig. 2). Leukemic cells may have the normal number of 46, but show structural rearrangements that result in genetic imbalance. Although chromosomal alterations differ from one patient to another, the pattern tends to remain relatively stable within a single patient. Occasionally, there is evidence of clonal evolution with a gradual gain or loss of chromosomes. (8) Again, the aberration is confined to the leukemic cells and is not found in lymphocytes or skin.

These chromosomal abnormalities have been found in patients who already mani-

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fest leukemia, and who can give no information as to the etiologic significance, if any, of the genetic imbalance.

Leukemia is a relatively rare disease, and it is not feasible to do cytogenetic studies on a population large enough to provide data on the very few who may subsequently develop it. In addition, as I have already indicated, chromosomal abnormalities are confined to leukemic marrow cells. Thus, for an investigation to have any relevance to the problem, we should have to use marrow cells, the extraction of which is not done without valid indication.

To try to study the relationship of chromosomal aberrations to leukemia, we selected a population that appeared to have an increased incidence of leukemia. In 1953, Bloch, et al reported on the pre-leukemic state. (9) They observed twelve patients, including ten women, who had been followed in the University of Chicago Clinics for 3 to 17 months for unexplained refractory anemia, neutropenia, and thrombocytopenia. Bleeding or easy bruising was a prominent symptom. All of these patients died of acute leukemia. Numerous bone marrow specimens seemed to exhibit one general pattern which included the presence of aplasia and maturation arrest in the granulocytic series, sudden or gradual shift to hyperplasia with maturation arrest of the granulocytic lineage, and finally an overwhelming of the marrow by stem cells and immature granulocytic precursors. This group of patients, with these diverse myelodysplastic abnormalities, and with a higher than normal incidence of leukemia, appeared to be a population that might provide answers to the following questions:

- 1) Do chromosomal abnormalities appear before the patient develops leukemia, or is the abnormality a secondary phenomenon?
- 2) Are chromosomal abnormalities useful in predicting which patients will develop leukemia?
- 3) Is the appearance of a chromosomally abnormal clone of cells indication

by itself, for starting anti-leukemic therapy?

We have studied 92 patients with some hematologic abnormality that justified a bone marrow aspiration. Every patient has had a chromosomal analysis using bone marrow cells within one to two hours of the time of aspiration. (10, 11) Thus, the errors due to cell selection in culture are avoided. It is a well-established fact that leukemic cells often do not tolerate in vitro culture, and the number of chromosomally abnormal cells may diminish rapidly within 8 to 48 hours after culture. (12)

In patients with myelofibrosis, from whose marrow no mitotic cells could be obtained, peripheral leucocytes were cultured for 24 hours without phytohemagglutinin. Mitotic cells presumably represented circulating immature myeloid cells. Peripheral blood cultures in other patients were established according to a modification of Moorhead et al. (13)

Of 92 patients originally examined, 16 have been excluded because their hematologic problems were transient, and were diagnosed, in retrospect, as iron-deficiency anemia, or mildly low white count, or their hematologic abnormality was the result of another disease. In 12 other patients we were unable to find any analyzable metaphases. This leaves 64 patients. Twenty-one of these have anemia, either due to toxic substances, inhibiting factors,, sideroachrestic anemia or unexplained. The group also includes 14 patients with pancytopenia, 7 with low white counts, 9 with low platelet counts, 4 with both low white blood cell and low platelet counts. Fourteen patients have high platelet counts, 5 have myelofibrosis and 2 have miscellaneous abnormalities. We have been able to examine 30 patients on two or more occasions. Twenty-four patients (more than 1/3 of the total) have died since 1964, all of them of infection, hemorrhage, or cardiovascular difficulties. None has developed leukemia, so the first question I have raised is unanswered.

It is pertinent to enquire at this point whether our prospective study is examining the same population as that included in the retrospective study of Bloch et al. The answer to this question is: I don't know. 10 of their 12 patients (or more than 80%) were women; 23 of 64 in this current series are women. All of Bloch's patients had abnormal marrows; about 75% of the patients in our study had abnormal marrows.

Ten of the 64 patients have had major chromosomal abnormalities in 25 to 95% of their mitotic marrow cells. Twenty-three more patients have basically a normal karyotype, but they have a variable, usually small, proportion of chromosomally abnormal cells. The significance of these cells is uncertain. The ten patients with abnormalities have such diverse myelodysplastic problems as pancytopenia, refractory anemia, thrombocytopenia, and thrombocytosis. I would like to describe three of these patients in more detail, and indicate what insight they provide in the understanding of our basic problem.

The first patient* (11) is a 23-year-old man, first referred to the Hematology Clinic in May 1964, with a diagnosis of aplastic anemia, possibly related to chloramphenicol. Chromosomal analysis in July 1964 revealed that less than 10 percent of his marrow cells had a normal pattern with 46 chromosomes, whereas the majority of cells had 45 chromosomes. One medium-sized chromosome, belonging to the group conventionally identified as group C, was missing (46,XY/45,XY,C-) (14) (Fig. 3). Chromosomal studies of peripheral blood lymphocytes revealed a normal chromosomal number and pattern. The marrow study has been repeated three different times over 3 years, and on each occasion 90 to 95 percent of the cells in metaphase have shown this same abnormality. This patient continued to have anemia, and low platelet count, despite

*(Case 1 in reference 11)

a variety of therapeutic agents. He died in a local hospital in August 1967 of an intracranial hemorrhage. The marrow obtained at autopsy revealed no evidence of leukemia.

Here is a patient, who for a minimum of 3 years, had a major chromosomal abnormality affecting over 90% of his dividing marrow cells. These cells continued to function, in a very inefficient way it is true, and to respond to the normal control mechanisms. The lack of a C group autosome has never been found in all somatic cells of a viable individual, and may be presumed to be lethal in embryogenesis. This abnormality has heretofore been reported only in patients with acute leukemia, but it apparently was not sufficient for the development of clinical leukemia in this patient. Whether the leukemic state might eventually have evolved must remain unanswered.

The second patient* (11)(15) is a 60-year-old woman with thrombocytosis, whose sternal marrow sections revealed normal overall cellularity, although megakaryocytes and platelets were unusually prominent. In her case the majority of marrow metaphases had a normal chromosomal pattern. In the first examination in January 1965, 25% of the dividing cells had 48 chromosomes, and each of these contained two extra C group chromosomes (46,XX/48,XX,2C+) (Fig. 4). Whether this represents two of one chromosome, or one each of two chromosomes cannot be determined with present techniques. Chromosomes from peripheral blood were normal. Because of her high platelet count, this patient was treated with Myleran and the 32P in May 1965. In July 1965, seven weeks later, although almost 100 metaphases were counted, no cells with an abnormal chromosomal pattern were observed. Marrow examinations have been repeated 4 times since July 1965, and the abnormal chromosomal pattern has gradually reappeared. In the most recent sample, in May, 1967, 6% of the cells had 47 chromosomes with one extra C group chromosome, and 12% of the cells had 48 chromosomes, including two extra* (Case 3 in reference 11)

extra C group chromosomes. It is interesting that although the chromosomally abnormal cells are increasing in number, the platelet count has remained within normal limits.

Before discussing this patient further, I should mention the work of Reisman et al (16, 17) and Smith and Pierce (18). Some children with acute leukemia may respond very well to anti-leukemic drugs with the result that they may have several periods of remission before the final, fatal outcome of their disease. During these periods of remission, the bone marrow appears microscopically normal, and the white count is also essentially normal. The marrow cell chromosomes of these children have been studied before treatment and during subsequent remissions and relapses. If a child has an abnormal chromosomal number before treatment, the abnormality disappears (often completely) during remission. The same abnormal chromosomal number, however, returns if there is a relapse. In the second or third relapse, the originally observed abnormal chromosomal number always re-emerges after remissions of varying periods up to 4 months. This pattern of normal chromosomal number in remission and abnormal number before treatment and in relapse, has led Reisman et al to conclude that a change in the chromosomal constitution of the malignant cell is one of the basic alterations in acute leukemia, being present in the earliest phase of the disease that can be detected. (17)

If our second patient is viewed in light of these experiences with the leukemia of childhood, she shows the same pattern of disappearance of the abnormal chromosomal number following treatment, the change being associated with clinical improvement. Whether the re-emergence of some cells with abnormal chromosomal patterns will be coupled with a rise in platelet count will be known only after continued observation. It is possible that we have detected the recurrence of a few genetically abnormal cells before their effects are clinically apparent. Thus it

appears that, as in acute leukemia of childhood, vigorous treatment can cause the disappearance, at least temporarily, of a genetically abnormal group of cells, even when these cells could not be classified as leukemic by accepted criteria. A similar pattern has been noted in polycythemia vera (19). This patient demonstrates an unsuspected pitfall. If we had obtained our first sample for karyotyping after the ^{32}P therapy, we would have found only chromosomally normal cells. We would have assumed that the subsequent marrow examinations revealed the development, de novo, of a chromosomally abnormal clone of cells.

The third patient is a 61-year-old woman with aplastic anemia like that affecting the first patient described. In both of these patients recurrent infections have been treated with chloramphenicol. The effect of chloramphenicol as related to chromosomal abnormalities or to leukemia is largely unknown. I have successfully studied chromosomes in seven patients who developed anemia or thrombocytopenia following chloramphenicol therapy. Five of the patients had normal karyotypes; the others are cases one and three in this report. Brauer and Dameshek (20) recently reported on 3 patients (all women) who developed anemia up to 8 years after chloramphenicol ingestion, and all of whom died of acute leukemia. No chromosomal studies were obtained on these patients. Previously, Fraumeni (21) investigated cases of chloramphenicol toxicity and found only one patient who developed leukemia, presumably as a result of the chloramphenicol.

Chromosomal studies on our third patient were first performed in June, 1964 at which time all dividing cells had normal chromosomes. The patient's aplastic anemia was treated with iron and blood transfusions and she made a good recovery. The bone marrow was examined again in March 1966 when the chromosomal pattern showed a remarkable change. Only 25% of the cells had normal chromosomes. Seventy percent of the metaphases lacked a C group chromosome, which appeared to be replaced by a small

G group chromosome. The simplest explanation of this change is the loss of material from the long arm of one C chromosome, leaving it with only the short arm (46, XX/46, XX, ?Cq-) (Fig. 5). The marrow was processed immediately so that this observation is not an in vitro artifact. This patient has had 3 subsequent marrow examinations, the most recent of which was in December, 1967, and the chromosomes have been normal.

I know of no other example of a chromosomal abnormality that has arisen in an individual with previously normal metaphases, and where the abnormality has disappeared spontaneously with no cytotoxic treatment. Microscopically, the bone marrow sections appear more normal in the recent sample than in the one examined in June 1964. Clinically, the patient is doing well.

One can only wonder how often this development of a chromosomally abnormal clone of cells occurs. What permits their appearance, and, of equal importance, what causes their apparently spontaneous disappearance? What will be the long-term effect of this apparently transient abnormality?

A pertinent question, which may have already occurred to you, is how often do you find chromosomal abnormalities in a normal population? Court Brown (22) published studies on peripheral blood chromosomes obtained from a random population of about 2000 males and females. He found very few consistent abnormalities (0.5%) in these people, and most of these were interpreted as representing minor, normal, chromosomal polymorphism. You will recall that all our patients had chromosomally normal peripheral blood cultures, so the control for our study requires bone marrow chromosomes. We have no problem in obtaining specimens from student volunteers, but we have had great difficulty in getting specimens from supposedly normal males and females, aged 40-70. Of the 14 individuals we have studied to date, all have had consistently normal karyotypes.

Since none of our patients has developed overt leukemia, at least in the gen-

erally accepted clinical use of the term, I cannot directly relate the information obtained from these patients to the development of leukemia. In 1965, Peter Nowell (23) reported on the predictive value of chromosomal abnormalities. He found that of 23 patients studied, 5 of 10 with chromosomal abnormalities died of leukemia within 3 months, whereas only 2 of 13 without chromosomal abnormalities died of leukemia within 7 months.

Our first patient demonstrates that a stable chromosomal abnormality, present in over 90% of marrow cells for at least 3 years, is not sufficient by itself to lead to leukemia. Our second patient illustrates the observation that chromosomal abnormalities in cells that are not leukemic in character can disappear with treatment, just as the leukemic cells disappear in children with acute leukemia during remission. The third patient raises the question of how frequent is the apparently transient occurrence of chromosomally abnormal cells, and what is its ultimate effect?

You may have noted that all three of these patients have abnormalities involving C group autosomes. Unfortunately, not only is this the largest group of chromosomes, consisting of 14 in all, but it is impossible to distinguish one pair from another. Therefore, I cannot say whether the chromosome that is missing in the first patient is the one that is duplicated in the second, and deleted in the third. It is always tempting, in such a situation, to postulate the presence of a genetic balance mechanism which is upset by a deficiency or excess of material. However, we are a long way from any concrete evidence to substantiate such a hypothesis. It is worthwhile to note here, that in the majority of patients reported to have chromosomal abnormalities in hematologic disorders other than leukemia, the chromosomal abnormality most frequently involved is in the C group (11). The frequent occurrence of C group abnormalities in leukemia has been noted many times. (6)

Individuals with constitutional chromosomal abnormalities such as mongolism,

have a higher than expected incidence of leukemia. (24) Similarly, patients with Bloom's Syndrome (25) and Fanconi's anemia (26), in which a high incidence of chromosomal breakage has been observed in in vitro culture, have high risk of leukemia and lymphoma. It is possible that chromosomal abnormalities render these cells more susceptible than are genetically normal cells, to the action of leukemogenic agents, which then transform abnormal, but partially controllable, cells to abnormal and autonomous cells.

Chromosomal analysis may be a method of diagnosing leukemia before it is clinically apparent. With this interpretation, at least two of these patients already has leukemia, although it is not manifested clinically. If this is so, leukemia could be diagnosed in some individuals, who have marrow chromosomal abnormalities, during the "latent" period, and appropriate chemotherapeutic agents might be used to suppress these genetically abnormal cells before they become uncontrollable. It is evident that a great deal of painstaking patient observation will be required before the questions raised in this study can be answered.

This work was begun with the close collaboration of Dr. Richard K. Blaisdell (now Professor and Chairman of the Department of Medicine, University of Hawaii). It has continued with the full cooperation of Drs. Stanley Yachnin, Sanford Krantz, Alvin Tarlov, and other members of the Section of Hematology, Department of Medicine, University of Chicago, whose support is gratefully acknowledged. The technical competence of Miss Judith Mikuta assisted by David Potter and Miss Julie Slott is also acknowledged. Dr. Margot Doyle and Mrs. Frances Skozen have given valuable editorial assistance.

Fig. 1 Metaphase plate and karyotype of a bone marrow cell obtained from a patient with chronic myelogenous leukemia. This cell contains 46 chromosomes. The chromosome labeled Ph^1 has a deletion of about one-half of its long arm (46, XY, G- Ph^1).

Fig. 2 Metaphase plate and karyotype of a bone marrow cell obtained from a patient with acute myelogenous leukemia. There are 59 chromosomes in this cell, additional chromosomes being present in groups A, C, D, F, and G-Y. A marker chromosome is also present. This patient had idiopathic thrombocythemia which transformed into acute leukemia (59, XY, A2+5C+2D+3F+1G+Mar+).

Fig. 3 Metaphase plate and karyotype of a bone marrow cell obtained from the first patient, with aplastic anemia. This cell has only 45 chromosomes and is lacking one C group autosome (45, XY, C-).

Fig. 4 Metaphase plate and karyotype of a bone marrow cell obtained from the second patient, with idiopathic thrombocythemia. This cell contains 48 chromosomes; the two extra chromosomes belong to the C group (48, XX, 2C+).

Fig. 5 Metaphase plate and karyotype of a bone marrow cell obtained from the third patient, who also had aplastic anemia. The cell contains 46 chromosomes but the C group has one less chromosome. The extra "G" chromosome (placed to the left of the remainder of the group) probably represents the short arm and centromere of a C group chromosome which has lost its long arm (46, XX, ?Cq-).

REFERENCES

1. NOWELL, P. C. and D. A. HUNGERFORD. 1960. Chromosome studies on normal and leukemic human leukocytes. J. Nat. Cancer Inst. 25:85.
2. TOUGH, I. M., P. A. JACOBS, W. M. COURT BROWN, A. G. BAIKIE and E. R. D. WILLIAMSON. 1963. Cytogenetic studies on bone marrow in chronic myeloid leukemia. Lancet I:844.
3. TJIO, J. H., P. CARBONE, J. WHANG and E. FREI. 1966. The Philadelphia chromosome and chronic myelogenous leukemia. J. Nat. Canc. Inst. 36:567.
4. RUDKIN, G. T., D. A. HUNGERFORD and P. C. NOWELL. 1964. DNA contents of chromosome Ph¹ and chromosome 21 in human chronic granulocytic leukemia. Science 144:1229.
5. TOUGH, I. M., W. M. COURT BROWN, A. G. BAIKIE, K. E. BUCKTON, D. G. HARNDEN, P. A. JACOBS and J. A. WILLIAMS. 1962. Chronic myeloid leukemia: Cytogenetic studies before and after splenic irradiation. Lancet II:115.
6. SANDBERG, A. A., T. ISHIHARA, Y. KIKUCHI and L. H. CROSSWHITE. 1964. Chromosomal differences among the acute leukemias. Ann. N. Y. Acad. Sci. 113:663.
7. KIOSSOGLOU, K., W. J. MITUS and W. DAMESHEK. 1965. Chromosomal aberrations in acute leukemia. Blood 26:610.
8. GROUCHY, J. de, C. de NAVA, J. M. CANTU, G. BILSKI-PASQUIER and J. BOUSSER. 1966. Models for clonal evolutions: A study of chronic myelogenous leukemia. Amer. J. Human Genet. 18:485.
9. BLOCK, M., L. O. JACOBSON and W. F. BETHARD. 1953. Preleukemic acute human leukemia. J. A. M. A. 152:1018.
10. TJIO, J. H. and J. WHANG. 1962. Chromosome preparations of bone marrow cells without prior in vitro culture or in vivo colchicine administration. Stain Technol. 37:17.
11. ROWLEY, J. D., R. K. BLAISDELL, and L. O. JACOBSON. 1966. Chromosome studies in preleukemia. Blood. 27:782.
12. SANDBURG, A. A., T. ISHIHARA, L. H. CROSSWHITE and T. S. HAUSCHKA. 1962. Chromosomal dichotomy in blood and marrow of acute leukemia. Cancer Res. 22:748.
13. MOORHEAD, P. S., P. C. NOWELL, W. J. MELLMAN, D. M. BATTIPS and D. A. HUNGERFORD. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell. Res. 20:613.
14. CHICAGO CONFERENCE - 1966. Standardization in human cytogenetics Birth Defects: Original article Series 2 (The National Foundation, New York).

15. ROWLEY, J. D. and R. K. BLAISDELL. 1966. Karyotype of treated thrombocythemia. *Lancet* II:104.
16. REISMAN, L. E., M. MITANI and W. W. ZUELZER. 1964. Chromosome studies in leukemia. I. Evidence for the origin of leukemic stem lines from aneuploid mutants. *New Eng. J. Med.* 270:591.
17. REISMAN, L. E., W. W. ZUELZER and R. I. THOMPSON. 1964. Further observation on the role of aneuploidy in acute leukemia. *Cancer Res.* 24:1448.
18. SMITH, S. and M. I. PIERCE - Personal communication.
19. KAY, H. E. M., S. D. LAWLER and R. E. MILIARD. 1966. The chromosomes in polycythemia vera. *Brit. J. Haemat.* 12:507.
20. BRAUER, M. J. and W. DAMESHEK. 1967. Hypoplastic anemia and myeloblastic leukemia following chloramphenicol therapy. *New Eng. J. Med.* 277:1003.
21. FRAUMERI, J. F. 1967. Bone marrow depression induced by chloramphenicol or phenylbutazone. *J. A. M. A.* 201:828.
22. COURT BROWN, W. M. 1967. Human population cytogenetics. John Wiley and Sons, Inc., New York.
23. NOWELL, P. C. 1965. Prognostic value of marrow chromosome studies in human "preleukemia." *Arch. Path.* 80:205.
24. STEWART, A., J. WEBB and D. HEWITT. 1958. Survey of childhood malignancies. *Brit. M. J.* I:1495.
25. GERMAN, J., R. ARCHIBALD and D. BLOOM. 1965. Chromosomal breakage in a rare, probably genetically determined syndrome of man? *Science* 148:506.
26. BLOOM, G. E., S. WARNER, P. S. GERALD and L. K. DIAMOND. 1966. Chromosome abnormalities in constitutional aplastic anemia. *New Eng. J. Med.* 274:8.

A

1

2

3

B

4-5

Marker

C

X-6-12

D

13-15

E

16-18

F

19-20

G

21-22

A

1 2 3

B

4-5

C

6-12

D

13-15

E

16-18

F

19-20

G

21-22 X Y