

**EDITORIAL REVIEW****The Role of Cytogenetics in Hematology**

By Janet D. Rowley

**I**N 1951, Dameshek<sup>1</sup> coined the term “myeloproliferative syndromes” to identify a group of hematologic disorders characterized by proliferation of one or more of the cell types that occur in the bone marrow. This concept provided a unifying hypothesis which has proved useful in the analysis of the similarities and differences between the various disorders. A particularly valuable part of Dameshek’s concept was the provision for the observed transitions and overlaps between the different myeloproliferative states. Besides undergoing such transitions, the subgroups polycythemia rubra vera (PV), essential thrombocythemia, chronic myelogenous leukemia (CML), and myelosclerosis with myeloid metaplasia (MMM) could terminate, with differing frequencies, in acute myelogenous leukemia (AML).

Chromosome analysis of bone marrow cells by use of the recent techniques of quinacrine fluorescence and Giemsa banding has provided new perspectives about the relationship of these diseases to each other and to a number of other hematologic disorders, such as the refractory anemias and acute leukemia. My purpose here is to inform hematologists regarding three important contributions that can be made by cytogenetic analysis of bone marrow cells. First, in some ill-defined disorders such as the refractory anemias, the presence of a particular abnormal chromosomal pattern may identify a specific subset of patients.<sup>2</sup> Second, the presence of an abnormal karyotype in a patient with other signs suggestive of acute leukemia allows the clinician to make the diagnosis earlier and therefore to start treatment sooner than if the karyotype had not been determined.<sup>3,4</sup> Finally, in chronic myelogenous leukemia,<sup>5</sup> and probably also in some types of acute leukemia,<sup>6</sup> the karyotype provides a valuable prognostic indicator of the patient’s response to treatment and of the clinical course.

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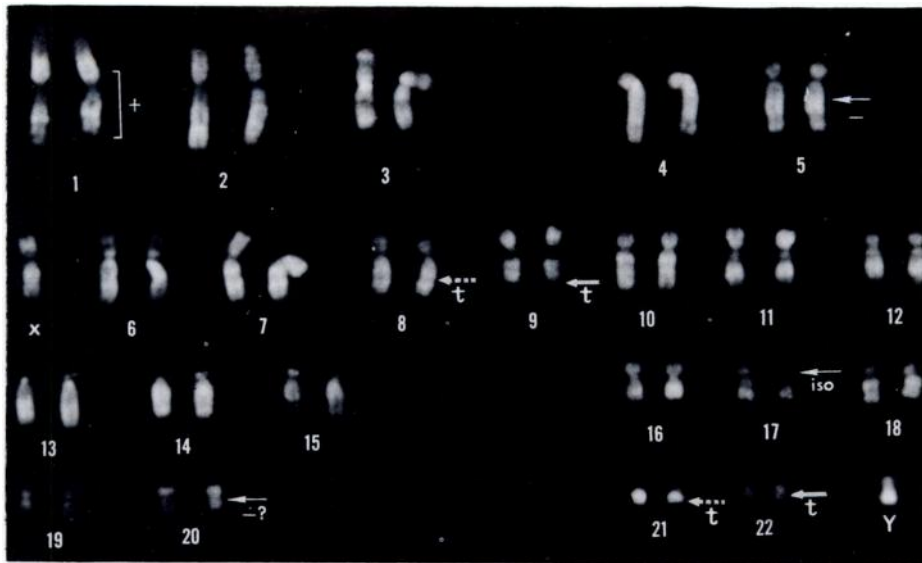
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## TECHNIQUES

Before specific chromosomal abnormalities are discussed, a brief description of the techniques used in the preparation and identification of human chromosomes may be helpful. In most laboratories, chromosomes are obtained from bone marrow samples that are processed within a few hours after aspiration. The marrow cells in mitosis have undergone DNA synthesis *in vivo*, and thus the possibility that cells with a particular karyotype may grow better than others in culture is avoided. In patients from whom it is not possible or convenient to obtain a marrow sample, and in whom circulating myeloid cells capable of division (myelocytes or younger) are present, peripheral blood can be cultured for 24 or 48 hr without the addition of phytohemagglutinin (PHA). Although the proportion of normal and abnormal cells may be different, the chromosomal pattern of these dividing myeloid cells is identical to that seen in marrow cells. A part of the peripheral blood sample can also be cultured for 72 hr with PHA to stimulate lymphocytes to divide; the karyotype of such cells is representative of the patient's constitutional chromosomal pattern.

The nomenclature of chromosome classification has been standardized at a series of conferences, the first of which was held in 1960 and the most recent in Paris in 1971.<sup>7</sup> The normal complement of 46 chromosomes includes 44 autosomes (22 pairs) which are numbered in order of decreasing size, from 1 to 22. There are seven morphologically similar groups of chromosomes, identified by the capital letters A-G; the two sex chromosomes, XX in females and XY in



**Fig. 1.** Normal male karyotype in a marrow cell stained with quinacrine mustard and examined with ultraviolet light. The arrows identify the sites of chromosome breakage. All or part of the long arm of chromosome 1 included in the bracket may be present in a trisomic state in patients with PV. The deletions of chromosomes 5 and 20 are indicated by minus signs. The translocation involving 8q,t(8;21)(q22;q22), frequently seen in patients with AML, is identified by  $\leftarrow$ , and that involving the Ph<sup>1</sup> chromosome t(9;22)(q34;q11) is identified by  $\leftarrow$ . The iso-chromosome for the long arm of 17 is labeled "iso."

males, are not numbered. The X chromosome is morphologically similar to group C chromosomes, and the Y may be similar to the G chromosomes.

A discovery made in 1970 has revolutionized cytogenetics. Each human chromosome can be precisely identified on the basis of its unique banding pattern when quinacrine fluorescence<sup>8</sup> (Fig. 1) or special Giemsa-staining<sup>9</sup> techniques are used. In the Paris Nomenclature,<sup>7</sup> the bands are numbered from the centromere to the end of each chromosome arm. Each individual chromosome is identified by number; the short arm and long arm are designated by "p" and "q," respectively. An abnormality of part of a chromosome is designated by the band numbers involved,<sup>7</sup> with an indication whether material is gained (+) or lost (-) by the chromosome. The chromosomal abnormalities can be summarized in each patient's karyotype; for example, 47,XY,+8 would describe a male with an extra chromosome 8; 46,XX,t(9,22)(q34,q11) would describe a female with a translocation (t) involving chromosomes 9 and 22, in which the break point is in band 34 of the long arm of chromosome 9 and in band 11 in the long arm of chromosome 22. This latter example identifies the chromosomal aberration commonly seen in CML, in which the 22q chromosome is the Philadelphia (Ph<sup>1</sup>) chromosome and the 9q+ chromosome is the recipient of the material "lost" by chromosome 22.<sup>10</sup>

#### CLINICAL IMPLICATIONS OF CHROMOSOMAL ABNORMALITIES

##### *Correlation of Chromosomal Patterns and Hematologic Disorders*

Reliable data on the frequency of abnormal karyotypes in cells from patients with hematologic disorders do not exist, except for the Ph<sup>1</sup> (22q-) chromosome, which is present in about 85% of patients with CML. Other aberrations are found in 30%-50% of patients with AML, in about 20%-25% of patients with PV, and in about 10% of patients with other hematologic disorders.<sup>11</sup>

Although the number of patients whose karyotype has been determined by banding techniques is small, some very important observations have already been made. For example, it has been discovered that the Ph<sup>1</sup> (22q-) chromosome represents a translocation and not a deletion of chromosomal material as had been thought earlier.<sup>10</sup> The C group abnormalities which are commonly observed in a variety of hematologic conditions are not random, but tend to involve specific chromosomes.<sup>12</sup> Rearrangements and deletions can now be detected in patients previously considered to have normal karyotypes.<sup>13</sup>

Some chromosomal aberrations are virtually diagnostic for a particular clinical entity, e.g., the Ph<sup>1</sup> (22q-) chromosome in CML. Others, such as the 20q- chromosome seen in PV<sup>14</sup> and refractory anemia,<sup>15,16</sup> seem to be associated with diseases of a particular cell type (red blood cell precursors in this example). Still other abnormalities are nonspecific; for example, an additional chromosome No. 8 is found in a variety of leukemic and nonleukemic conditions.<sup>12</sup> The sites of chromosomal breakage seen in the deletions or the common translocations are shown in Fig. 1. A diagram of the relationship of these chromosomal patterns to hematologic disorders is presented in Fig. 2 and Table 1. Patterns have been included if they were observed in the author's laboratory or reported in the literature in at least five patients.

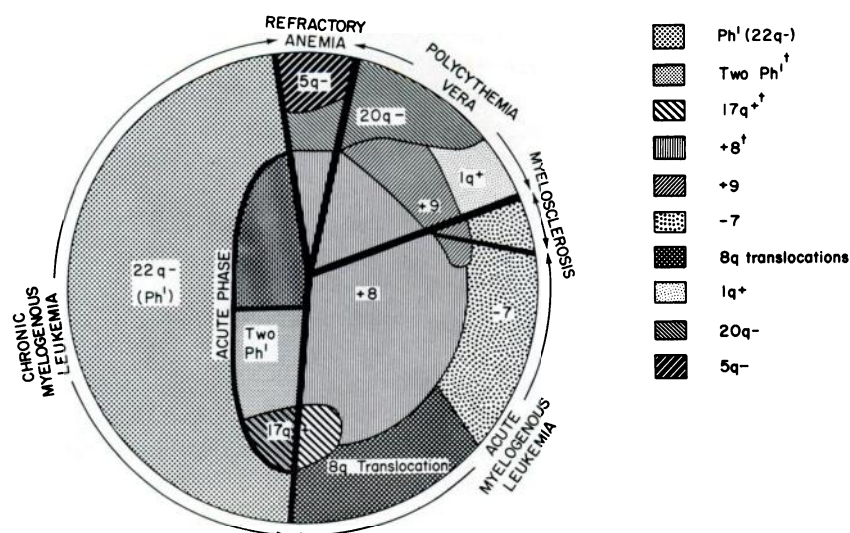


Fig. 2. Diagram showing the most frequent chromosomal abnormalities associated with several hematologic disorders. The space occupied by each disorder is roughly proportional to the percentage of chromosomally abnormal patients with that disorder. Thus, the largest segment is occupied by CML, with 85% of the patients showing the  $Ph^1$  chromosome, and the next is AML with about 50% of patients having abnormal patterns. The smallest segment is myelosclerosis, in which patients infrequently show abnormalities. In CML, the "acute phase" portion shows the additional abnormalities superimposed on the original  $Ph^1$ . Within each disorder, the space occupied by the various chromosomal patterns represents the frequency of that abnormality on the basis of present, rather imprecise, estimates.

### Refractory Anemia

The information presently available has made certain questions significant for investigators analyzing chromosomal patterns in relation to hematology. Within a disease category, what are the detectable clinical or biochemical correlates in patients with different karyotypes? Recent reviews have emphasized the heterogeneous nature of the idiopathic acquired refractory anemias.<sup>17,18</sup> Patients may have a microcytic anemia or, infrequently, a macrocytic anemia; a variable percentage of sideroblasts with or without ringed sideroblasts may be

Table 1. Chromosome Abnormalities Associated With Hematologic Disorders

Disorder	Trisomy for All or Long Arm of No. 1	5q-	-7	+8	8q Transl.	+9	17q+	20q-	$Ph^1$ (22q-)	Double $Ph^1$
Chronic myelogenous leukemia									+	
Acute phase				+			+		+	+
Acute myelogenous leukemia										
Polycythemia vera	+		+	+	+	+	+			
Refractory anemia	+			+				+		
Myelosclerosis with or without myeloid metaplasia			+			+				

present. Three different abnormal chromosomal patterns, namely, +8,<sup>12</sup> 5q-,<sup>2</sup> and 20q-<sup>15,16</sup> have been observed in patients with these disorders. It has recently been suggested that the 5q- karyotype is present in a distinct group of patients who have a macrocytic anemia without ringed sideroblasts.<sup>2</sup> If the genes in cells with an additional No. 8 are functionally expressed, one would anticipate biochemical differences in these patients in comparison to patients whose cells are lacking part of chromosome 5 (5q-), or of chromosome 20 (20q-). De la Chapelle<sup>19</sup> has observed increased levels of glutathione reductase in red cells from two patients with sideroblastic anemia who had an extra C chromosome, identified as a +8 in one of them. A normal level of glutathione reductase is found in patients who have a macrocytic anemia and the 5q- abnormality;<sup>2</sup> no information is available for patients with the 20q- abnormality. These different chromosomal patterns may thus be useful not only in distinguishing subgroups of patients who would otherwise have been classified together in one heterogeneous disorder, but also in the mapping of specific gene products on specific chromosomes.

#### *Chronic Myelogenous Leukemia*

Another question that can be asked concerns the influence of different chromosomal patterns on the response to treatment and prognosis. In CML, it is well established that patients with the Ph<sup>1</sup> chromosome have a much longer median survival (42 mo) than patients who are Ph<sup>1</sup> negative (15 mo).<sup>5</sup> Although the Ph<sup>1</sup> translocation involves chromosomes 9 and 22 in more than 90% of patients, 14 cases with a variant translocation or with a more complex rearrangement have been reported.<sup>11</sup> The clinical implications of these variants are as yet unknown.

Moreover, a change in the karyotype of a Ph<sup>1</sup>-positive patient strongly suggests that the patient is in the acute phase of CML; this change may precede the clinical signs of blast crisis by 3–4 mo.<sup>20</sup> The chromosomal evolution in the acute phase usually involves one or more of the following changes superimposed on the Ph<sup>1</sup> positive cell line, listed in the order of their frequency: (1) double Ph<sup>1</sup>, (2) addition of chromosome 8, and (3) abnormalities of 17q, such as the structural rearrangement of No. 17 to produce an isochromosome for the long arm.<sup>11</sup>

#### *Acute Myelogenous Leukemia*

Prior to chromosome banding techniques, investigators who attempted to correlate the chromosomal pattern seen in acute leukemia with patient survival arrived at contradictory conclusions. Preliminary data<sup>6</sup> from patients studied with banding techniques, however, strongly suggest that the careful analysis of the karyotype of patients with acute myelocytic leukemia provides valuable information on the prognosis and response to therapy. In a series of patients studied in our laboratory,<sup>6</sup> those with AML and a normal karyotype had an 87% rate of induction of complete remission and a median survival of 18 mo. Patients with an abnormal karyotype had a 20% remission rate and a 2-mo median survival. Patients with acute myelomonocytic leukemia demonstrated no difference in median survival irrespective of the presence or absence of an

abnormal karyotype. Although the karyotype of patients with acute leukemia shows considerable variability, nonrandom chromosomal patterns are observed, including an additional No. 8, loss of No. 7, and a translocation between No. 8 and No. 21.<sup>13</sup>

#### *Chromosomal Patterns in Other Hematologic Disorders*

Chromosomal abnormalities, which are confined to bone marrow cells, have been observed in other hematologic disorders often referred to as "preleukemia." The question of the significance of these abnormalities, which frequently involve an extra No. 8, is unresolved. Nowell has stated that, if patients with aneuploidy do not develop overt leukemia within 3 mo, they do not appear to have an increased risk of developing leukemia.<sup>3</sup> On the other hand, I have observed several patients whose marrows showed a chromosomal abnormality  $1\frac{1}{2}$ – $2\frac{1}{2}$  yr prior to the overt manifestation of leukemia (Rowley, unpublished observations). The significance of the frequent involvement of No. 8 in both malignant and nonmalignant hematologic disease is not clear.

Nonrandom karyotypic changes are not limited to disorders of bone marrow cells. When the banding techniques are applied to cells of lymphatic origin, they reveal that chromosome No. 14 is particularly vulnerable to rearrangements. Thus, cells from patients with Burkitt's lymphoma<sup>21</sup> and multiple myeloma<sup>22</sup> may have an additional band at the end of one No. 14; in African Burkitt's lymphoma, this band has recently been shown to represent a translocation from the end of chromosome No. 8.<sup>23</sup> The PHA-stimulated lymphocytes from patients with ataxia telangiectasia show a break in the proximal part of No. 14, with translocation of the long arm to several other chromosomes.<sup>24</sup> Rearrangements involving chromosome No. 14 have also been observed in a number of malignant lymphomas.<sup>25</sup>

On the basis of models formulated from data obtained in experimental animals, it has been proposed that nonrandom chromosomal changes in man may be specifically related to particular etiologic agents.<sup>26</sup> Although there is no evidence on this point at the present time, it would be possible by studying human tumors induced by known carcinogens to determine whether or not the hypothesis is correct.

#### CONCLUSION

Evidence is accumulating that various nonrandom chromosomal patterns are found in myeloproliferative disorders. Although none of these patterns shows the same degree of specificity as the Ph<sup>1</sup> chromosome in CML, their detection lends support to the hypothesis that chromosomal changes play an important role in these diseases. Chromosomal analysis of bone marrow cells by use of banding techniques has become an integral part of the careful investigation of virtually any group of patients with a particular hematologic disorder and may prove useful in guiding the clinician in the prognosis and therapy of these disorders. The correlation of the clinical findings with the cytogenetic, morphological, and biochemical analyses will provide a much more complete understanding of these diseases than can be achieved with any one of these techniques used alone.

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