Chromosomal DNA Cytophotometry in 20q— Nonspecific Myeloid Disorders

A. V. Carrano, B. H. Mayall, J. R. Testa, L. K. Ashworth, and J. D. Rowley

ABSTRACT

DNA cytophotometry was used to quantify the chromosomal alterations in the bone marrow and blood of three patients with nonspecific myeloid disorders. All patients possessed a population of cells with a morphologically abnormal chromosome 20, del(20q11). In two of the patients, the abnormal chromosome 20 showed nearly identical DNA measurements with a net loss of 0.37% of the total autosomal DNA in one patient and 0.38% in the second. The third patient had a net loss of only 0.25% of the autosomal DNA. Analysis of the DNA content of the long arm and short arm of the abnormal No. 20 indicated that all three cases had chromosomal material deleted to the short arm (0.10 to 0.14% of the autosomal DNA). About 0.50% of the autosomal DNA was deleted from the long arm in two of the patients; only 0.35% of the autosomal DNA was deleted from the long arm in the third case. Within the limit of resolution, there is no evidence that the material lost has been translocated intact to another chromosome. The origin of the 20q— chromosome as the result of an incomplete pericentric inversion is suggested.

INTRODUCTION

Partial deletion in an F-group chromosome is associated with two specific hematological disorders, namely, PV* (2, 6, 7, 22) and IARSA (5). With the use of banding techniques, the deletion in both of these disorders was established as occurring in the long arm of chromosome 20 (3, 4, 17, 18, 23—25). Although in 2 cases a translocation involving chromosome 20 was reported (1, 25), there is no evidence to indicate that the missing material is consistently translocated to another chromosome. In 3 cases of IARSA reported by de Grouchy et al. (5) before the use of chromosomal banding, it seemed, however, that the affected F-group chromosome had undergone a pericentric inversion, but the possibility of loss of material could not be ruled out. Recently, Testa et al. (21) reported the presence of a 20q— chromosome [del(20q11)] in 5 patients with nonspecific myeloid disorders; i.e., the clinical findings were inconsistent with the diagnosis of PV or IARSA.

Because of increasing evidence that nonrandom chromosomal changes are associated with specific hematological disorders (18), it is important to characterize precisely the cytogenetic lesions in such disorders to determine whether they are consistent among patients and whether similarities exist among diseases. Previously, we applied DNA cytophotometry to study the 9q+ /22q— translocation associated with chronic myelogenous leukemia and showed that the translocation is reciprocal, that it involves an exchange of 0.325% of the autosomal DNA, and that no other cytogenetic lesion was present in the individuals studied (10, 11). In the present investigation, we examine the alterations in chromosomal DNA in 3 of the patients with nonspecific myeloid disorders and a 20q— chromosome reported by Testa et al. (21). Our results extend beyond the resolution of conventional cytogentic banding techniques and suggest the occurrence of an incomplete pericentric inversion of a chromosome 20 in all 3 individuals, i.e., breakage on opposite sides of the centromere, inversion of the centric piece, and subsequent reattachment at only one breakpoint.

MATERIALS AND METHODS

Patient Summary. Details of the clinical and cytogenetic evaluations of these patients are reported elsewhere (21). The patient number in parentheses identifies the individual in this previous study. The bone marrow culture procedure used for the cytogenetic analyses has been described (19). Peripheral blood was cultured essentially according to the method of Moorhead et al. (16).

Case 298 (Patient 2) is a 76-year-old white female who was diagnosed in 1969 as thrombocytopenic and who continues in that condition. Cytogenetic evaluation of the bone marrow in 1976 and 1977 indicated the presence of a del(20q11) in about one-half of the cells (Fig. 1). Cultured peripheral blood lymphocytes had a normal karyotype. Six normal cells and 6 bone marrow cells containing the 20q— chromosome were evaluated by cytophotometry.

Case 287 (Patient 4) is a 57-year-old white female initially evaluated for anemia in September 1976. A bone marrow sample at this time showed 46% abnormal metaphases. Approximately one-half of the cells possessed only a del(20q11) (Fig. 1), and the remainder had extra chromosomes 9 and 21 in addition to the 20q—. A second bone marrow biopsy in October 1976 indicated that this latter clone was present in 66% of the metaphases. Eleven bone marrow cells containing the 20q— chromosome were evaluated by cytophotometry. At the present time, this patient has pancytopenia.

Case 294 (Patient 5) is a 69-year-old black male initially evaluated in December 1975 for leukocytosis. Following a bone marrow biopsy in November 1976, this patient was diagnosed as having a myeloproliferative disorder of uncertain type. Cytogenetic evaluation in December 1976 on metaphases from unstimulated peripheral blood indicated the presence of 47 chromosomes and an XXY chromosome complement in all cells. Approximately one-half of the metaphases also had a...
from the NOD value. This does not introduce significant error.

Del(20q)(q11) (Fig. 2). An earlier stimulated peripheral blood culture indicated that this patient was a mosaic XY/XXY. The patient died in December 1976. Twelve cells from the unstimulated peripheral blood culture were evaluated by cytophotometry. All cells possessed the 20q— chromosome.

DNA-based Cytophotometry. Chromosomes from well-spread metaphase cells were identified initially with quinacrine banding to ensure accurate chromosome classification. The quinacrine-stained metaphases were destained in absolute methanol:glacial acetic acid (3:1), treated with RNase, and stained with gallocyanin chrome alum according to our standard protocol (13) under conditions that are specific and stoichiometric for DNA (8). The stained slides were mounted in oil (refractive index, 1.556).

CYDAC, a flying-spot scanning cytophotometer (14), was used to scan the gallocyanin chrome alum stained metaphases and digitize their absorbance. Digitized images were analyzed interactively by computer on a color television monitor (10). Initially, the chromosomes were analyzed for total absorbance, and these values were normalized on a cell-by-cell basis so that 100 units is the sum of all 44 autosomes (15). Thus, 1 unit corresponds to about 120 fg of DNA. The NOD represents the total relative amount of DNA-stain content of a chromosome. Subsequent to the analysis of NOD, the digitized images were further processed to determine the location of the centromere of each chromosome. The algorithm that identifies the centromere position was reported (12). The procedure is now improved to allow more human interaction. When the centromere is determined, the operator can orient the chromosome, based on the quinacrine karyotype, so that the short and long arms of the metacentric chromosomes are labeled appropriately. The partitioning of the chromosome at the centromere permits the division of the total absorbance into short arm (PNOD) and long arm (QNOD) normalized absorbance. These values are also expressed relative to total autosomal DNA (100 units). It is not always possible to identify the centromere in every chromosome for which NOD has been determined, primarily because the chromosome is severely bent, folded, or otherwise distorted. Thus, for any chromosome type, more chromosomes may be contributing to average NOD than to average PNOD and QNOD, and the sum of PNOD and QNOD may differ slightly from the NOD value. This does not introduce significant error.

RESULTS

The normalized DNA-stain content measurements of NOD, PNOD, and QNOD for chromosomes 20 and 20q— in the 3 patients are given in Table 1. All values are given as a mean ± S.D. of the normalized values. Table 1, Column 1, lists the standard values of each of the 3 parameters obtained by analyzing the chromosomes of 10 normal individuals (9). The number of chromosomes measured for each patient is shown in parentheses.

Changes in the DNA-stain content are determined by subtracting the value of the individual mean from the standard mean; a positive value indicates a gain and a negative value a loss in DNA-stain content. These comparisons were made directly to the standard value for 3 reasons: (a) insufficient normal cells were available from 2 of these patients to provide the desired internal standard (10); (b) artifacts may be introduced by any normal homolog DNA heteromorphisms within the patient; and (c) changes may have occurred in the morphologically normal chromosome 20 during transformation of the normal cells.

Case 298 illustrates this last point dramatically. Because a sufficient number of cells lacking the No. 20q— chromosome were present in the bone marrow of Patient 298, the measurements of normal and abnormal cells were processed separately, i.e., Cases 298N and 298, respectively. This permits a direct comparison between the abnormal clone and normal constitutive cells of the marrow. No difference was detected between the homologous No. 20 chromosomes in the normal cells of 298N. The values given in the table for NOD, PNOD, and QNOD, therefore, are the combined mean ± SD, of both homologs. Further, there is no significant difference between the chromosome 20 standard values and those of 298N.

In abnormal cells of the same individual (Case 298), however, the DNA content of the normal No. 20 chromosomes was markedly altered. This is evident from the decrease in the amount of short-arm DNA in the normal chromosome 20 from the abnormal clone (0.43 unit) compared to the No. 20 chromosomes in normal cells (0.52 unit). The amount of DNA in the long arm of the normal chromosome 20 increased in the abnormal clone (0.82 unit) over that in normal cells (0.68 unit). Despite changes in PNOD and QNOD, the total chromosomal DNA in the normal No. 20 chromosomes is the same (1.21 versus 1.20 units). Because of the small number of abnormal cells measured, the loss of 0.09 unit in the short arm of chromosome 20 in the abnormal cells compared to the normal...
cells is not statistically significant ($p = 0.07$). The gain of 0.14 unit in the long arm is statistically significant ($p < 0.01$).

In addition to chromosome 20, the combined mean (i.e., mean of both homologs) of the long arms (QNOD) of the 2 No. 8 chromosomes and the total DNA (NOD) of the No. 2 chromosomes increased significantly in the abnormal clone compared to normal cells, $p < 0.001$ and $p < 0.01$, respectively. No differences were detected between the homologs of these chromosomes. These minor variations of the DNA might have occurred either at the initiation or during the proliferation of the abnormal clone. The existence of this variation suggested that it would be more reasonable to compare individual chromosomal means in the abnormal cells to the standard mean rather than to compare absolute differences between homologs in the abnormal cells of our patients.

Although the QNOD value for the normal chromosome 20 in Case 298 was increased, the normal chromosomes 20 in Cases 287 and 294 did not deviate significantly from the standard mean of either NOD, PNOD, or QNOD. Compared to the standard mean, the 20q—chromosome is missing 0.25, 0.37, and 0.38 units, respectively. If the DNA were missing entirely from the long arm, this should be reflected in similar values in QNOD. As occurred either at the initiation or during the proliferation of the abnormal clone, we evaluated the im-

### Table 1

**Normalized DNA stain content measurements of chromosomes 20 and 20q— in 3 individuals with nonspecific myeloid disorders**

<table>
<thead>
<tr>
<th>Sum of autosomal chromosomes is 100 units.</th>
<th>Normal chromosome 20</th>
<th>20q—</th>
<th></th>
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<tbody>
<tr>
<td>Standard mean ± S.D.*</td>
<td>Case Mean ± S.D. Δ</td>
<td></td>
<td></td>
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<tr>
<td><strong>Total chromosomal DNA (NOD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.16 ± 0.06</td>
<td>287 1.18 ± 0.05 (9)</td>
<td>0.02</td>
<td>-0.25</td>
</tr>
<tr>
<td></td>
<td>294 1.19 ± 0.08 (12)</td>
<td>0.00</td>
<td>-0.37</td>
</tr>
<tr>
<td></td>
<td>298 1.21 ± 0.07 (6)</td>
<td>0.05</td>
<td>-0.38</td>
</tr>
<tr>
<td></td>
<td>298N* 1.20 ± 0.07 (12)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Short-arm DNA (PNOD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.49 ± 0.06</td>
<td>287 0.50 ± 0.06 (7)</td>
<td>0.01</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td>294 0.43 ± 0.08 (11)</td>
<td>-0.06</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>298 0.43 ± 0.10 (5)</td>
<td>-0.06</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>298N* 0.52 ± 0.06 (8)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td><strong>Long-arm DNA (QNOD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.67 ± 0.05</td>
<td>287 0.68 ± 0.10 (7)</td>
<td>0.01</td>
<td>-0.35</td>
</tr>
<tr>
<td></td>
<td>294 0.74 ± 0.12 (11)</td>
<td>0.06</td>
<td>-0.49</td>
</tr>
<tr>
<td></td>
<td>298 0.82 ± 0.10 (5)</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>298N* 0.68 ± 0.08 (8)</td>
<td>0.01</td>
<td></td>
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</tbody>
</table>

* Pooled mean ± S.D. from the measurements of approximately 10 cells from each of 10 normal individuals.

** DISCUSSION **

We have used DNA cytophotometry to confirm the loss of material from one chromosome 20 in 3 patients with nonspecific myeloid disorders. In addition, we have surpassed the resolution of conventional cytogenetic techniques and demonstrated the addition of material to the short arm of this chromosome.

From DNA cytophotometry of the other chromosomes in these patients, it is not likely that the missing material was translocated as an intact segment. The sensitivity of the method is such that the intact segment would have been detected if present. We cannot rule out the possibility that the deleted segment may have been further fragmented and translocated to more than one chromosome. Further, other consistent chromosomal DNA alterations in these patients are not indicated. All variations detected in these individuals can be attributed to normal DNA heteromorphisms that are also found in our set of normal individuals (9). Recently, Shabtai et al. (20) reported a high incidence of C-band polymorphism in chromosome 19 from individuals with PV. If these polymorphisms exist in our 3 patients, they do not significantly alter the DNA of chromosome 19 and would therefore exist within the limits of normal variation.

The DNA alteration in chromosome 20 might be explained by an incomplete pericentric inversion, i.e., breakage on op-
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