G-Banding Analysis of Complex Aneuploidy in Multiple Myeloma Bone Marrow Cells

By Preben Philip and Aage Drivsholm

Chromosome studies with the banding technique have been performed in a considerable number of cases of myeloproliferative diseases, but technical difficulties have so far prevented detailed studies of chromosomal abnormalities in multiple myeloma. The karyotypes of bone marrow cells from two patients with multiple myeloma have been analyzed by a trypan-Giemsa banding technique. Evidence is given for clonal evolution which in one patient has probably occurred by cell fusion and subsequent chromosome loss. Eight different marker chromosomes are characterized. Nonrandom chromosomal participation in the translocations and the existence of specific vulnerable points on chromosomes 1, 3, and 16 are suggested.

Chromosome abnormalities are found in bone marrow cells in about one-half of patients with multiple myeloma (MM). Abnormal clones have been reported with different patterns of chromosome composition, but so far no consistent abnormality characteristic of MM has been found. However, large marker chromosomes similar in size to the A-B group chromosomes or even larger, but with varying centromeric position, have repeatedly been described in malignant monoclonal gammopathies. Such markers were first observed in Waldenström's macroglobulinemia and accordingly termed "W chromosomes." Later this type of marker chromosomes was also found in other types of malignant monoclonal gammopathies, and consequently Houston et al. introduced the term "MG chromosomes."

In a survey of 54 reported cases of MM Siebner found large supernumerary chromosomes described in 35% of the cases, while it was not reported in any of eight patients with benign paraproteinemia. Large marker chromosomes are not often seen in marrow cells from patients with myeloproliferative diseases originating in the myeloid cell lines and thus appear to be particularly related to MM or other malignant monoclonal gammopathies.

The banding methods of chromosome preparation have provided several new interesting observations in myeloid myeloproliferative diseases. The technical difficulty in preparing myeloma bone marrow specimens for cytogenetic analysis is well known. Accordingly cytogenetic investigations of bone marrow cells from MM patients using the banding methods have been limited to two reports. Wurster-Hill et al. described two cases (one MM and one plasma cell leukemia). In both cases an additional segment on one chromosome, 14,
Philip and Drivsholm reported the detailed composition of the three large markers of patient 1 presented in this article. None of these cases was analyzed beyond the description of the marker chromosomes.

We describe here in detail the chromosomal abnormalities in rather complicated states of aneuploidy in the marrow cells from two patients with MM with a rapidly fatal course.

**MATERIALS AND METHODS**

Immediately after aspiration, 0.3–0.5 ml bone marrow was dispersed in 10 ml Fib 41 B (Fibiger Laboratory, Copenhagen, Denmark) with 20% fetal calf serum, 6 U/ml heparin, and 0.05 μg/ml colchicine. The cell suspension was then incubated at 4°–5°C. After 2 hr the cells were transferred to 0.075 M KCl for 20 min as a hypotonic treatment and subsequently fixed in methanol:acetic acid 3:1. Air-dried spreads were prepared according to the trypsin method introduced by Seabright but using 0.06% trypsin. Karyograms were made from microphotographs on Agfa-Ortho-25-Professional film; the chromosomes were classified according to the conventions given by the Paris Conference (1971).

**CASE HISTORIES**

**Patient 1**

The patient was a 76-yr-old male with chronic bronchitis. On July 4, 1973, he was admitted to Bispebjerg Hospital on suspicion of MM. On admission the hemoglobin concentration was 11.7 g/dl, reticulocytes were 0.8%, WBC was 8.5 × 10⁹/liter with 65% polymorphs, 2% stabs, 1% myelocytes, 1% eosinophils, 1% monocytes, and 30% lymphocytes. The marrow was hypercellular with about 50% plasmablasts. In the plasma an IgA-kappa M component, 43 g/liter, was found, and x-ray examination of the skull showed osteolytic lesions.

Due to the severe pain in the sternum and the ribs, x-ray treatment was started immediately (total dose, 6000 r). On August 18, treatment with cyclophosphamide, 2 g intravenously every 6th wk, was initiated for a total dose of 8 g. As a result of the treatment, the M component decreased to 18 g/liter. However, as the M component abruptly again increased to 38 g/liter, the treatment was changed on January 17, 1974, to melphalan, 40 mg intravenously every 6th wk. The last dose of melphalan was given April 18, and at the same time vincristine, 2 mg, was given intravenously.

On June 12, 1974, the patient died abruptly from cardiac failure. Autopsy showed multiple myeloma and coronary atheromatosis.

**Patient 2**

The patient was a 63-yr-old male. Since 1972 he had been treated for hypertension with Centyl (a thiazide diuretic), hydralazine and propranolol. Because of abdominal pains he was admitted to Rigshospitalet on July 10, 1974. On admission the hemoglobin concentration was 8.3 g/dl, reticulocytes were 1.6%, WBC was 2.8 × 10⁹/liter with 39% polymorphs, 7% stabs, 1% eosinophils, 3% monocytes, 42% lymphocytes, and 8% immature blast-like cells. The marrow was hypercellular; the dominating cell type was plasmacytoid ranging from mature looking plasma cells to undifferentiated blast-like types. In the plasma an IgG-lambda M component, 54 g/liter, was found. Serum urate was elevated (115 mg/liter), and so was serum calcium (146 mg/liter), plasma albumin was 18 g/liter, and serum creatinine was 1.7 mg/dl.

On July 26 treatment was initiated with cyclophosphamide, 2400 mg intravenously over 8 days under cover of allopurinol, continued by cyclophosphamide, 50 mg orally per day, until December 16. Prednisone was given for the hypercalcemia, initially 30 mg, later 10 mg/day.

Uric acid and calcium levels were normalized. The M-component, however, persisted in unaltered concentration, and the bone marrow remained largely unaltered except for the last sample of January 3; this was hypocellular but with a high proportion of immature plasma cells.

During July the course was complicated by left-sided thoracic *Herpes zoster*. On December 17 the patient was operated upon for bleeding gastric ulcer. Postoperatively, his condition deteriorated rapidly because of pulmonary infection and septicemia, and the patient succumbed on January 4, 1975. Autopsy revealed myeloma infiltrations in the spine, lungs, renal pelvic walls, and in the gastric wall.
### Table 1. Karyotypes From Bone Marrow Cells of Patient 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Chromosome Composition</th>
<th>Number of Mitoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>52,XY;−1,+3,+5,+7,+8,+9,+11,+18,−20,+t(1;16)</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>52,XY;−1,+3,+5,+7,+8,+9,+11,−15,+18,−20,+t(1;15),+t(1;16)</td>
<td>19</td>
</tr>
<tr>
<td>C</td>
<td>51,XY;−1,+3,+5,+8,+9,+11,−15,+18,−20,+t(1;15),+t(1;16)</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>52,XY;−1,+7,+8,+9,+11,−15,+18,−20,+t(1;15),+t(1;16),+rcp(3;5)</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>52,XY;−1,+3,+5,+7,+8,+9,+11,−13,−15,−16,+18,−20,+22,+t(1;15),+t(1;16),+t(13;16)</td>
<td>1</td>
</tr>
</tbody>
</table>

### RESULTS

**Patient 1**

Twenty-four mitoses were analyzed from one marrow sample obtained June 4, 1974. Five different cell types were identified. The chromosomal compositions are given in Table 1.

No normal mitoses were seen. Type B (Fig. 1) is the modal cell type. When the components of the marker chromosomes are also taken into consideration, this clone is characterized by loss of one of the following chromosomes or parts of chromosomes: 1pter → 1p36, 15pter → 15p1? (see legend to Table 3), 20— and by one extra of the following: 1q12 → 1pter, 3, 5, 7, 8, 9, 11, 16p13 → 16pter, 18. The rest of the chromosome complement appears to be present in normal amount. Type A has two normal chromosomes 15, but no t(1;15). Otherwise it is identical with type B. Type C differs from the modal type B only by having two normal chromosome 7 instead of trisomy 7. Type D differs from B only in exhibiting a reciprocal translocation between chromosomes 3 and 5. Type E is

![Fig. 1. Karyogram of type B mitosis. This mitosis is chosen for illustration because of the comparatively clear banding pattern and well-spread chromosomes without overcrossing. This single mitosis would hardly permit identification of the missing F group chromosome as a chromosome 20. The left-hand chromosome 18 may not be entirely normal.](image-url)
PHILIP AND DRIVSHOLM

Table 2. Karyotypes From Bone Marrow Cells of Patient 2

<table>
<thead>
<tr>
<th>Type</th>
<th>Chromosome Composition</th>
<th>Number of Mitoses</th>
<th>Sample Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6-11-74</td>
<td>3-1-75</td>
</tr>
<tr>
<td>K</td>
<td>46,XY</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>L</td>
<td>45,XO, -1, -3, +11, 14q+ , +t(1;3)</td>
<td>2</td>
<td>6*</td>
</tr>
<tr>
<td>M</td>
<td>44,XO, -1, -3, +t(1;1), +t(1;3)</td>
<td>2†</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>78, +3, +t(1;1), +t(1;1); 79</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Per cent metaphases with chromosome count about 75–80 (100 mitoses counted) 10 2

*Including one mitosis with an additional loss of chromosome 9.
†Including one mitosis in which three of the G-Y group chromosomes were too blurred to allow identification of the one missing.

The chromosomes of groups B–G were too blurred to allow identification.

identical with B except for containing an extra chromosome 22 and a translocation between a chromosome 13 and a 16, while one 13 and one 16 are missing. The result is the gain of a dicentric marker, whereas the acentric fragments are lost.

Patient 2

Thirty-two mitoses were analyzed from two marrow samples obtained November 6, 1974 and January 3, 1975. Four different cell types were identified. The chromosomal compositions are given in Table 2.

Type K is entirely normal. The chromatin is distinct without the blurring and difference in contraction seen in the aneuploid karyotypes. Type L is characterized by missing a chromosome 1, 3, and Y, and by having one extra 11 and an extra segment on the long arms of one 14. Most of the missing chromosomes 1 and 3 are refound in the marker t(1; 3). The origin of the extra segment on chromosome 14 could not be established. The 14q+ marker is about the length of the long arms of chromosome 2. Type M has no normal chromosome 1, only one 3 and no Y. The missing Is are refound however in the two t(1; 1) so that the final result will be tetrasomy for the 1q2?–1q3? part of 1, disomy for the rest. The D group chromosomes of these three cells were too blurred to allow recognition of the 14q+ marker possibly present. The chromosomes of the

Table 3. Designation of Marker Chromosomes

<table>
<thead>
<tr>
<th>Patient</th>
<th>Marker Chromosomes</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>t(1;15)(1qter–1q12::15p13–15qter)</td>
</tr>
<tr>
<td>1</td>
<td>t(1;16)(1qter–1p36::16p13–16qter)</td>
</tr>
<tr>
<td>1</td>
<td>rcp(3;5)(3pter–3q2(789);–5q13–5qter)</td>
</tr>
<tr>
<td>1</td>
<td>rcp(3;5)(5pter–5q13::3q2(789));3pter</td>
</tr>
<tr>
<td>1</td>
<td>t(13;16)(13pter–13q2;16p13–16qter)</td>
</tr>
<tr>
<td>2</td>
<td>t(1;1)(1pter–1q3?::1q2?–1qter)</td>
</tr>
<tr>
<td>2</td>
<td>t(1;3)(1pter–1q3?::3q2(789);–3pter)</td>
</tr>
<tr>
<td>2</td>
<td>14q+</td>
</tr>
</tbody>
</table>
three N type mitoses were too blurred and irregularly contracted to allow full identification. However, the A group chromosomes and the t(1;1) were easily recognized, and the presence of the t(1;3) could be ruled out. Two normal chromosome 1, three normal 3, and two t(1;1) were found in all mitoses of this type.

The Marker Chromosomes

Eight different marker chromosomes were found in these two patients. Their designations are given in Table 3. In Figs. 2 and 3 each individual marker chro-
Fig. 3. Five marker chromosomes (M), rcp(3; 5)', rcp(3; 5)'', t(1; 3), t(13; 16), and t(1; 16), are shown paired with the normal chromosomes participating in the translocations. The breaking points of the chromosomes are marked by the horizontal lines a, b, and c.
Fig. 4. The breaking points of the chromosomes participating in the translocations leading to the eight identified marker chromosomes are plotted in a diagram as given by the Paris conference.\textsuperscript{15} o, patient 1; □, patient 2.

...
However, under the circumstances they do not give much evidence for a close relationship of the clones. Both miss one chromosome 1, 3, and Y. However, the missing Y is not an infrequent finding in bone marrow cells from aging persons, and the missing A group chromosomes have participated in different translocations.

If we are operating with the cell types of proven existence, the N type seems to have evolved by fusion of the types K and M, since this is the only reasonable way to explain the very exceptional chromosome composition concerning chromosomes 1 and 3: two 1s, two t(1; 1), three 3s. The so-formed precursor of the N type has then in turn lost several chromosomes, the identities of which are obscure because of the pronounced blurring of the chromosomes from this type. The event of fusion between malignant and nonmalignant cells followed by chromosome loss and regaining of malignant growth properties is in accordance with tumor cell experiments of Klein et al.

Even if only two patients have been investigated, the eight different marker chromosomes characterized in Table 3 provide strong evidence for a nonrandom chromosomal rearrangement in MM. Thus five of the 13 identified breaking points are located on chromosome 1 (Fig. 4). Furthermore, chromosomes 1, 3, and 16 appear to have broken twice in the same region, suggesting the existence of certain excessively vulnerable points on these chromosomes. The identities of these breaking points indicate that in looking for specific chromosomal rearrangements the occurrence of morphologically different markers such as the MG markers in monoclonal gammopathies does not rule out the existence of specific changes. This situation is parallel to that in chronic myeloid leukemia where the essential change seems to be the break in a chromosome 22. The translocation of the missing part occurs most often to chromosome 9, but may also happen to chromosomes 2, 19, or 22, or the missing part may not be found translocated at all. The result is different marker chromosomes besides the Ph'. The essential event might well consist of some injury to a specific point of a chromosome. The lesion may or may not give rise to gross chromosomal changes, and if a break occurs the broken parts may be transferred to different chromosomes.

The only marker in our patients in which the chromosomes 1 or 3 cannot be proven to participate or in which none of the suggested vulnerable points are
affected is the 14q+. This marker, however, can hardly be considered accidental since it has been reported in three of the four cases of MM (including the present cases) studied thus far by banding analysis.

REFERENCES

G-banding analysis of complex aneuploidy in multiple myeloma bone marrow cells

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