Cytogenetic Studies in Multiple Myeloma: A Study of Fourteen Cases

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SUMMARY

The bone marrows of fourteen patients with multiple myeloma containing a total of more than twenty scorable metaphase plates in all samples collected were subjected to cytogenetic study. In five of these an abnormal acrocentric marker chromosome with long arms the length of those of the B group was found in a number of the hyperdiploid cells. In two additional patients, this same abnormality may have been present. A single patient had an abnormal submetacentric marker instead of the acrocentric one. Six patients had no abnormality. The incidence of abnormal karyotypes in the marrow of this series of myeloma patients is higher than previously reported and may reflect the result of evaluating a larger sample of cells in metaphase.

INTRODUCTION

Cytogenetic data of bone marrow or blood cultures from patients with plasma cell myeloma have been reported in twenty-seven cases (2, 4-6, 12-17). In approximately 60% of these cases, chromosomal abnormalities, either in number or in morphology, were found (6). As in most other malignancies, these have been variable from case to case. Hyperdiploidy, with chromosomal number ranging more often between fifty and sixty, has been much more common than hypodiploidy, in which cell lines with chromosomal modes of 40, 44, and 46 chromosomes have been documented in three instances (4, 5, 13). Occasionally, an abnormal cell line with a distinctive abnormal or marker chromosome has been reported (5). Unlike the Ph1 marker chromosome, which, with few exceptions, is present in marrow cells of patients with chronic myelocytic leukemia, no marker chromosome similar from case to case has been reported in any series of patients with plasma cell myeloma.

The purpose of this report is to present chromosome analysis of bone marrow cells of a series of fourteen patients with multiple myeloma. Although an attempt was made to study all patients with myeloma seen at our institution, we have arbitrarily included in this report only those who had a total of twenty or more scorable metaphase plates in the sum of all their marrows available to us for study. A marker chromosome, morphologically similar in five of the fourteen cases, is reported.

MATERIALS AND METHODS

The diagnosis of plasma cell myeloma was established in each case by finding typical infiltration of abnormal plasma cells in marrow or soft tissue and the presence of a paraprotein in the serum or urine. The clinical details of these patients will be presented in a subsequent publication.

Bone marrow was aspirated three hours after a single intravenous dose of vincaleukoblastine (kindly provided by Eli Lilly & Co.), 0.05 mg per kilogram body weight, in ten of the fourteen cases. In these, the bone-marrow sample, usually less than 1 ml volume, was promptly transferred to a Vacutainer heparinized tube containing 10 ml of Hanks' balanced salt solution. The suspension was then transferred to siliconized conic centrifuge tubes and centrifuged at 1500 rpm in a centrifuge (approximately 380 × g), for three minutes; the supernate was then discarded. Warm (37°C) hypotonic bovine serum-water (1 part serum, 4 parts water) was added to approximately ten times the volume of the cell button in the centrifuge tube; the button was suspended in the hypotonic solution and incubated at 37°C for 15 minutes. The tube was centrifuged more gently at 800 rpm (approximately 110 × g) for five minutes; the supernate was discarded. Freshly made Carnoy's fixative was added drop-wise until a standard turbidity was achieved. Fixation proceeded overnight in the refrigerator. Next morning, the supernate was discarded and new fixative was added until the standard turbidity was reached. Chromosome spreads were made by dropping the suspension on cold, wet, clean slides and flaming gently over an alcohol lamp. Aceto-orcein and Giemsa stain were used. In four patients, no vincaleukoblastine was administered; the aspirated marrow was processed according to the method of Tjio and Whang (20). No significant differences were noted due to the use of the two technics except for an increased mitotic index with the vincaleukoblastine technic.

Consecutive metaphase plates were scored directly under microscopy. A sustained effort was made not to reject for scoring those metaphase plates which, although as well spread as others, were not as darkly stained or as pleasing to the eye. Leukemic cell metaphase plates are frequently poorly stained when compared to those of normal cells. Cells were rejected from scoring at first sight if they were too poorly spread for accurate counts (score) of the chromosome number or if the cell was obviously ruptured. In each case where the number of cells permitted, photographs and karyotypic analysis was made of the visually scored cells as follows: 10 of the euploid, 5 of the those with a number of 45, all of the hyperdiploid, and, in Case I.B., suitable tetraploid cells. In this latter case, good, clearly spread, tetraploid metaphase plates were difficult to score consistently; yet a marker was clearly visible in the periphery of the cell where overlap was minimal.

The percent incidence of plasma cells in the bone marrow aspirate was scored by one of the authors (J. R. D.) on Wright-stained preparations made from an aliquot of the same marrow aspirate from which the cytogenetic sample was also made. It

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2 Advanced Clinical Fellow of the American Cancer Society.

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will be noted that in Cases I. B., E. B., and F. H., the percent of plasma cells was insufficient to make a certain diagnosis of myeloma. In these instances, marrow obtained previously contained large numbers of myeloma cells.

RESULTS AND DISCUSSION

The cytogenetic data based on chromosome analysis of bone-marrow cells is summarized in Table 1.

Aneuploidy was observed in the marrows of all but two patients (H. S. and R. W.) and was characterized by hyperdiploidy (above a chromosome number of 47) and in one instance (I. B.) by tetraploidy or hypotetraploidy. No instance of a pseudodiploid or hypodiploid clone of cells was observed. The incidence of aneuploidy in this series (87 percent) is higher than that reported in previous series with the possible exception of that reported by Levin et al. (12). The higher detection rate may well reflect the result of scoring, where possible, at least one hundred metaphase plates per marrow.

Oft times, despite the systematic scoring of one hundred to two hundred metaphase plates, only two or three clearly abnormal hyperdiploid cells were encountered, even in the presence of as many as 80 percent plasma cells in such a marrow (e.g., Patient T. S.). It is puzzling to find so few cells with abnormal karyotypes in a marrow overrun by presumably malignant plasma cells. Other investigations have indicated that the neoplastic plasma cell is long-lived and that the overpopulation of bone marrow by these cells is due at least in part to an increase in their longevity. Astaldi et al. (1) provided insight into this problem by short-term cultural studies. They exposed normal and malignant hematopoietic cells to optimum doses of colchicine in vitro in order to induce metaphase arrest. Surprisingly, the mitoses achieved in a given time were greater for normal than for leukemic myelocytes. The proliferative activity of plasmablasts from plasmacytoma was, in this way, found to be remarkably low when compared to that of normal myelocytes. Bond et al. (3) provided confirmation of this by means of radioautographic technic employing tritiated thymidine. It is quite likely, therefore, that because of this differ-

TABLE 1
Composite of Chromosome Counts in All Patients Studied

Reading from the top down, the first five patients are marker positive, the next two (*) are equivocally marker positive. Patient O.S. has a different marker, and the bottom six are marker negative. The presence of the marker is indicated by the superscript M, and the number of marker positive plates found in plates with a particular chromosome number is indicated by the numeral preceding the M. Since not all the hyperdiploid plates were sufficiently clear to karyotype, the last column indicates the number of clear hyperdiploid plates which were marker positive over the total number of clear hyperdiploid plates. The percentage of plasma cells in the marrow studied is also given. It should be noted that Case I.B., E.B., and F.H. had an insufficient percentage to merit a diagnosis of myeloma. In each instance, another marrow or tissue section was diagnostic.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chromosome number</th>
<th>% Plasma cells</th>
<th>Cells counted</th>
<th>No. of clear hyperdiploid plates M+</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. S.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11-12-64</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4-28-65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>I. B.</td>
<td></td>
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<tr>
<td>F. G.</td>
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<tr>
<td>A. W. a</td>
<td></td>
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<tr>
<td>3-16-65</td>
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<tr>
<td>B. N. a</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O. S. a</td>
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<tr>
<td>E. B.</td>
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<td>F. H.</td>
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<tr>
<td>H. S.</td>
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<tr>
<td>H. W.</td>
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<td>R. W.</td>
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<tr>
<td>J. B.</td>
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</tbody>
</table>

* Presence of marker equivocal (see text).
* Marker somewhat different from cases (1-5).
Ernest M. Tassoni, John R. Durant, Sharon Becker, and Barbara Kravitz

ence in mitotic index, the vast majority of bone marrow cells karyotyped in the present series represents normal myeloid rather than plasma cells in mitosis. Thus, paucity of cells with chromosome abnormalities may be explained.

Analysis of the aneuploid cells in each marrow revealed a variable pattern of chromosome abnormalities from which no characteristic chromosome pattern could be established with the exception of six cases in which a distinct marker chromosome was present (Fig. 1) and of two additional cases in which a similar but less distinct marker was observed.

The marker chromosome was morphologically similar in the hyperdiploid cells of five patients (T. S., E. L., M. L., I. B., F. G.; Table 1). It was in the size range of the C group chromosomes but was acrocentric with long arms approximately the size of those of a B group chromosome. It did not replace a member of the B group, since these were usually present in full complement. In one metaphase plate, satellites were noted on the short arms of the marker, suggesting in that case at least a possible relationship to either D or G group chromosomes. No claim is made, however, that the marker is, in the remaining cases, of D group origin. This abnormal chromosome, although occasionally found in close association with a normal D chromosome, was not found thus frequently enough to suggest satellite association. Tritiated thymidine labeling was not attempted in these cases due to our previous failures to carry bone-marrow cells in vitro sufficiently long to permit successful autoradiographic analysis. In vivo studies are planned.

In two additional patients (A. W., B. N.) the acrocentric marker was downgraded to equivocal status. The long arms, although longer than the long arms of a D group chromosome and approximating in size those of a B group chromosome, were despiralized and stained less darkly than the long arms of either B or D. If skeptically viewed, it is possible to interpret these supernumerary chromosomes as despiralized normal D group chromosomes. It may be, of course, that despiralization represents a part of the chromosomal abnormality associated with the presence of the marker.

Another, but morphologically different, marker was observed in two hyperdiploid cells of Patient O. S. It, too, was in the size range of the C group but was submetacentric rather than acrocentric. Chromosomal fragments and translocations were noted in these cells, rendering speculation hazardous as to the derivation of the marker.

The presence of the long acrocentric marker chromosome in marrow cells of this series of patients is between 36 and 50 per cent, depending of whether the two equivocal cases are included. This frequency suggests that it should be found by other investigators with as extensive a search as was made in this study. It is our experience that this marker is relatively easy to find on visual scanning.

The significance of the finding remains to be assessed. That it is not due to therapy is clear, because it was found prior to chemotherapy and/or radiotherapy in 4 of the 6 patients (T. S., M. L., F. G., O. S.). However, the relationship of the abnormally long, acrocentric chromosome to morphologically similar ones in other malignancies requires investigation. For instance, Stewart et al. (19) and Jacobs et al. (10) reported a number of cases of Burkitt's lymphoma with pseudodiploid cells containing a chromosome resembling the one seen in our cases of myeloma. However, a missing A and C group chromosome and the consistent presence of another larger marker clearly suggests a translocation as the genesis of the abnormal chromosome. DeGrouchy (7) reported a similar marker in a patient with ovarian cancer. Ishihara et al. (9) reported chromosome analysis of several cases of malignant effusion due to ovarian or colonic carcinoma in which abnormal acrocentric chromosomes were identified. The large acrocentric marker identified by Hungerford and Nowell (8) in cultured peripheral blood in a case (189 T) of acute granulocytic leukemia is significantly larger than those reported in our series of myeloma patients. The published metaphase plates of the unselected series of 27 acute leukemia patients reported by Sandberg et al. (18) were reviewed. In only one (Case 61) was an acrocentric marker chromosome identified, but unlike that in the myeloma series, it was larger than a Group B chromosome. It is possible that the morphologically similar chromosomes mentioned above are unrelated in terms of genome to those found in multiple myeloma.

In addition, there may be possible clinical significance associated with the presence or absence of this marker in multiple myeloma as there is in the case of the Ph 1 chromosome in chronic myelogenous leukemia (11). An additional report is planned, detailing the relationships sought between marker-positive patients and characteristics of the associated paraproteinemias, response to therapy, and nature of bone lesions, but at this writing no consistent correlation is found.

ADDENDUM

Since this paper was accepted for publication, we have found another patient with myeloma characterized by the presence of the large acrocentric chromosome. In this case the cells studied were obtained from pleural fluid proven at autopsy one day later to be due to myelomatous pleural involvement. All of the metaphase plates obtained after two days of culture without phytohemagglutinin were approximately 3N and the marker could be clearly identified in many of these.

ACKNOWLEDGMENTS

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REFERENCES

Cytogenetic Studies in Multiple Myeloma

Fig. 1. Typical metaphase plate with karyotype of a marker-positive patient (M. L.). The arrow points to the large acrocentric chromosome in the metaphase plate. This is shown at the top right in the karyotype. The other abnormalities were not consistent from one case to the next, but the acrocentric chromosome seen here was similar to those present in the other cases.
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