Cellular DNA Content as a Marker of Human Multiple Myeloma

By Jean Latreille, Barthel Barlogie, Gary Dosik, Dennis A. Johnston, Benjamin Drewinko, and Raymond Alexanian

DNA flow cytometry of bone marrow cells from 61 patients with multiple myeloma in various stages of severity and treatment revealed a 65% incidence of aneuploidy. In the subgroup of 34 patients with plasmacytosis, 85% had aneuploid abnormalities. Of 50 patients with active disease, 76% had aneuploid bone marrow cells; conversely, 95% of patients with aneuploid abnormality had active multiple myeloma. The degree of ploidy abnormality was expressed as DNA index and ranged from 0.85 to 2.1. Except for one case of hypodiploidy in a patient with a previous history of malignant lymphoma, the remainder of patients showed hyperdiploid abnormalities with a modal DNA index value of 1.1. Thirty-eight of 40 patients with ploidy abnormality had unimodal DNA distributions; 2 patients had heteroploid abnormalities with 2 and 3 discrete subpopulations, respectively. Except for two patients, there was an excellent correlation between the percentage of cells with abnormal DNA content and the proportion of morphologically identifiable plasma cells (r = 0.91). Lack of ploidy abnormality in 15 patients with less than 10% bone marrow plasma cells may reflect a problem of recognition of small-degree aneuploidy rather than true absence, due to limits of resolution. The high incidence of DNA flow cytometrically detectable aneuploidy in patients with active disease makes DNA content a significant cellular marker of multiple myeloma.

CELLULAR DNA content is not only a discriminator of cell cycle stage, but also of ploidy, and can be rapidly assessed by flow cytometry after quantitative DNA fluorochromation. While the diagnostic yield of conventional cytogenetic studies of mitotic karyotypes depends on the proliferative activity of target cells, ploidy determination on the basis of cellular DNA content can also be conducted on interphase cells, which usually represent as many as 90% of any given cell population. We have already documented the validity of such measurements in acute leukemia and more recently in solid tumors. The reported low incidence (17%) of DNA flow cytometrically detectable aneuploidy in leukemic bone marrow cells reflects the generally small degree of numeric chromosomal aberrations. This contrasts with recent findings on solid tumor tissue from more than 100 patients with various malignancies, demonstrating an incidence of aneuploid abnormality in excess of 90%. With few exceptions and at variance with karyotype results, ploidy abnormalities were characterized by unimodal DNA distributions of high resolution with little dispersion. The vast majority of solid tumors were hyperdiploid and, with one exception, showed identical ploidy abnormalities upon reexamination. In contrast, 121 benign tumors all had a normal DNA content.

We have recently extended such DNA flow cytometric investigations to determine ploidy characteristics in patients with multiple myeloma. Using human granulocytes as diploid reference standard, flow cytometric studies of bone marrow cells revealed DNA content abnormalities in 76% of patients with active disease and in 85% of individuals harboring more than 10% plasma cells.

MATERIALS AND METHODS

Sixty-one consecutive patients with multiple myeloma seen between June and December 1978 in the Hematology Section of the Department of Medicine, at M. D. Anderson Hospital and Tumor Institute, form the basis of this report. Consent to bone marrow aspiration was the only selection process in this otherwise heterogeneous group of patients with varying tumor burden and treatment history. Fourteen patients were studied at diagnosis, 11 patients at the time of response to induction therapy, 13 at the time of primary treatment failure, and 23 in relapse. Fourteen patients underwent repeat marrow examinations at time intervals of up to 6 mo following initial evaluation.

The response criteria used are those described by Alexanian et al. Heparinized bone marrow was subjected to Hypaque-Ficoll gradient separation (density, 1.078 g/ml). Interphase cells were washed once with 0.9% NaCl. Part of the specimen was used for morphological analysis of May-Grünwald-Giemsa stained cytocentrifuge slides. The remainder of the cell suspension was fixed in 70% ethanol and stored at 4°C. Granulocytes, obtained by leukapheresis from normal donors, served as diploid reference standard. These cells were subjected to the same initial processing technique and then stored in 70% ethanol at 4°C. For flow cytometric analysis, aliquots of each sample were mixed with granulocyte standard at 2 different ratios to determine the presence and degree of aneuploidy, as previously described. Briefly, the target cell/granulocyte mixture was treated with 0.5% pepsin (Serva, Heidelberg, Germany) for 5 min at room temperature. DNA-specific fluorochromation was conducted with ethidium bromide (Serva) and mithramycin (Charles Pfizer & Co., Inc., New York, N.Y.) at final concentrations of 12.5 and 25 μg/ml, respectively. Maximal dye uptake was routinely accomplished within 5 min. At this time, RNase treatment (0.2 ml; 0.1%, 1 min, room temperature) was performed. Each sample was then measured in an ICP-11 pulse cytometer (Phywe, Göttingen, Germany) at a flow rate of 200 cells/sec for a total of 20,000–30,000 cells. The degree of ploidy...
abnormality was expressed as DNA index, representing the ratio of modal channel number of tumor G10 to normal G10 cells.4 Hence, the abnormal population was identified as the G10 population that progressively decreases with increasing granulocyte admixture (Fig. 1). Quantitation of the fraction of cells with abnormal DNA content in samples displaying bi- or multimodal DNA distribution is carried out with restriction to G10 populations, as outlined schematically in Fig. 2. Thus, the usually small fractions of cells in S and G2 + M phases are not accounted for.

RESULTS

Review of monoclonal protein type, stage of disease, age, sex, and race of the 61 patients under study revealed that the study group was quite representative of the total population of multiple myeloma patients seen at M. D. Anderson Hospital over the past 5 yr (33 males, 50 whites, and 33 patients >60 yr). Table 1 summarizes the diagnostic breakdown by monoclonal component type versus clinical status at time of study. The majority of patients (67%) had IgG monoclonal gammopathy.

Table 1. Patient Characteristics by M-Component Versus Clinical Status

<table>
<thead>
<tr>
<th>M-Component</th>
<th>Clinical Status</th>
<th>At Diagnosis</th>
<th>First Failure</th>
<th>Relapse</th>
<th>Response</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>IgG</td>
<td>At Time of Study</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>γ</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
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<tr>
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<td>1</td>
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<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NS*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>13</td>
<td>23</td>
<td>11</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

*Nonsecretor.

53 % Plasma Cells
43 % Abnormal DNA
(DI = 1.05)

Normal G10

Abnormal G10

Fig. 3. Bone marrow DNA histogram in patient with multiple myeloma with 53% plasma cells on differential.

For the entire population, bone marrow samples from 40 patients were shown to harbor subpopulations with distinctly abnormal DNA content for a 65% incidence of aneuploidy. Considering only patients with ≥10% plasmacytosis, 29 of 34 patients (85%) had aneuploid abnormalities. Figure 4 illustrates the frequency distribution of aneuploidy by DNA index in histogram form. The DNA index varied between 0.85 and 1.05.
and 2.1, with a mean value of 1.15, and modal value of 1.1 for patients with ≥10% plasma cells. The one patient with hypodiploid abnormality developed multiple myeloma 3 yr following cytotoxic chemotherapy for nodular poorly differentiated lymphocytic lymphoma associated with an IgG λ M-component. While the bone marrow biopsy contained lymphoma cells at the time of initial diagnosis, the recent bone marrow demonstrated only malignant plasma cell involvement, with a 14% plasmacytosis. There was one patient each with two (Fig. 5) and three subpopulations with distinctly abnormal DNA content. The limit of sensitivity with regard to difference in DNA content is apparent from Fig. 3 (DI = 1.05). On the other hand, Fig. 6 demonstrates an example of hyperdiploid abnormality (DI = 1.10) in a case of minimal plasmacytosis (6% plasma cells, 7% abnormal DNA content). It is apparent that the detection of aneuploidy is thus a function of both the proportion of plasma cells and the magnitude of the DNA index. We have therefore considered the relationship between the percentage of morphologically identifiable plasma cells to DNA index (Fig. 7). With five exceptions, specimens from patients with more than 10% plasma cells on bone marrow differential were aneuploid. Within the group of 22 patients with less than 10% plasma cells, only 7 were hyperdiploid; the remaining 15 were classified as having a normal...
however, may have made recognition of a small-degree DNA content abnormality impossible.

To lend further support to our hypothesis that DNA content abnormalities originate from myeloma plasma cells, we have investigated the relationship between the percentage of morphologically identifiable plasma cells and the proportion of cells with abnormal DNA content (Fig. 8). Except for two patients with disproportionately excessive fractions of cells with abnormal DNA content, an excellent linear correlation was established \( r = 0.91 \). Repeat investigations in these two patients show persistence of the aforementioned discrepancy between morphological and flow cytometric findings.

In order to investigate the clinical significance of ploidy status in this heterogeneous population, the data were analyzed for 4 patient categories (Table 2): (1) at diagnosis prior to any chemotherapy, (2) at the time of primary treatment failure, (3) at the time of relapse after earlier remission, and (4) during response or in remission. Of 50 patients in categories 1, 2, and 3 ("active disease"), 76% had aneuploidy. Conversely, of 40 patients with aneuploidy, 38 (95%) had active disease. Of 50 patients with active disease, 12 had less than 10% plasma cells; 6 of them had ploidy abnormalities, so that 50% of patients with active disease and less than 10% plasma cells could be identified on the basis of DNA content abnormality. Of 2 patients with aneuploidy in the response category, both had significant bone marrow plasmacytosis (8% and 30%).

1 had been in remission for 2 yr, while the other was entering the responsive category at the time of study. All 9 responsive patients with normal DNA content had less than 10% plasma cells.

Repeat examination was conducted in 16 patients at intervals of up to 6 mo after initial evaluation. There was no change in DNA index values with time for patients with persistent active disease. The transition into clinical remission in two patients was associated with disappearance of the aneuploid abnormality. No correlation was found between the magnitude of DNA index and the type of monoclonal protein, age, sex, previous therapy, or severity of disease, as defined by the clinical stage at diagnosis.\textsuperscript{14}

**DISCUSSION**

The limited number of cytogenetic studies in multiple myeloma indicate a varying incidence of karyotype abnormalities, including marker chromosomes (for review, see ref. 15). All of these studies emphasize the

**Table 2. Patient Characteristics by DNA Content Versus Clinical Status**

<table>
<thead>
<tr>
<th>Clinical Status at Time of Study</th>
<th>DNA Content</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Diagnosis</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>First Failure</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Relapse</td>
<td>6</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>All Active Disease</td>
<td>12</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Response</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

| Total                           | 61          |
ANEUPLOIDY IN MULTIPLE MYELOMA

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difficult task of obtaining sufficient numbers of eval-

uable metaphases, compared to the rather high evalu-

ability rate in the leukemias.16 This difference is best

explained by the generally low proliferative activity in

myeloma cells, as confirmed by the in vitro pulse

tritiated thymidine labeling index method.17 Utilizing

high resolution flow cytometry of DNA content with

normal human granulocytes as reference standard, we

have found a high incidence of predominantly hyperdi-

ploid abnormalities in bone marrow cells from patients

with active multiple myeloma.

We have further demonstrated that the abnormal

cell population of cells indentified by DNA flow cytometry

generally correlated with the percentage of morpho-

logically identifiable plasma cells in the bone marrow.

The only two exceptions had an excess number of cells

with abnormal DNA content in relation to the number

of plasma cells identified microscopically on the bone

marrow smear. This suggests that the malignant cells

in myeloma may not always be recognized morpholo-

gically and may involve either lymphocytoid precursors

or other hemopoietic elements, as has been shown

for Philadelphia chromosome positive chronic granulo-

cytic leukemia.18 Direct evidence for this hypothesis

might be provided through flow-sorting experiments,

which are presently underway. DNA histogram reso-

lution, however, was inferior to that obtained with the

flow system utilized for this study. Therefore, two-

parameter analysis of DNA and protein content or cell

volume may be necessary to provide sufficient resolu-

tion for cell-sorting experiments.

There were 2 responsive patients who were found to

have aneuploidy and bone marrow plasma cells of 8%

and 30%. For the majority of patients in this study,

however, clinical response was associated with less

than 10% plasma cell involvement in the bone marrow

diploidy. It is conceivable that in those clinical

responders, a finding of plasmacytosis and aneuploid

abnormality indicates that the cellular substrate lags

behind or precedes monoclonal protein secretion and

thus can be an indicator of early relapse. This would

also indicate that in spite of the focal distribution of

bone marrow infiltration by multiple myeloma, the

proportion of plasma cells with an abnormal DNA

content provides a good reflection of disease activity.

A small fraction of low-degree DNA content abnor-

mality may have escaped recognition in spite of high

resolution performance.

Although previously shown for acute leukemia, a

linear correlation between karyotype index (defined as

the modal chromosome number of target cells divided

by 46) and DNA index should not be expected.9 The

existence of such correlation would require that only

chromosomes with an average DNA content are

involved in the aneuploid abnormality. The terms

hypo- and hyperdiploidy in the cytogenetic literature

refer to a minus or plus in chromosome number, while

we have adopted these terms solely to indicate defi-

ciency or excess in cellular DNA content. Preliminary

karyotype data in breast cancer suggest a 30% inci-

dence of hypodiploidy, contrasting with less than 10%

hypodiploid abnormalities in 24 patients studied by

DNA flow cytometry.19 Such discordant findings

could be explained by the presence of one or two large

marker chromosomes with an excess DNA content.

Review of the available cytogenetic literature

reveals that among 94 patients with multiple

myeloma, 36 had hypodiploidy, 29 hyperdiploidy.20–23

Of 38 patients examined by Andray et al., 11 had

marker chromosomes.15 The finding of a substantial

proportion of hypodiploid karyotype (38%) is in

marked contrast to the preponderance of hyperdiploid

DNA content abnormalities presented in this study.

Provided that comparable patient populations were

examined in our and the cited studies, an excess

amount of DNA content appears to be associated with

a reduced number of chromosomes.

In view of the high incidence of aneuploidy detecta-
le by DNA flow cytometry, we suggest that DNA

content is a useful marker of the cellular substrate of

multiple myeloma, the humoral product of which is

commonly employed for its diagnosis. The magnitude

of the DNA index appears stable for up to 6 mo of

observation, but may conceivably undergo changes

with the development of drug-resistant tumor cell

clones. In contrast to our findings in solid tumors, with

3 modal DNA values at 1.1, 1.6, and 2.0, ploidy

abnormalities in multiple myeloma were scattered

around a modal value of 1.1.5 Whether the degree of

ploidy abnormality relates to the natural history and

response of multiple myeloma to chemotherapy

requires further study. Cellular DNA content may

provide a useful marker in the absence of monoclonal

globulin secretion and for the distinction of truly

benign monoclonal gammopathies from those gammo-

pathies preceding the neoplastic disorder of multiple

myeloma.

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