

Plasma Cell Karyotype in Multiple Myeloma

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Karyotypic abnormalities were studied in multiple myeloma and were correlated with clinical features. Among 115 evaluable patients, 46% had an abnormal karyotype. Trisomy 3, 5, 9, and 15 and monosomy 13 and 16 were the most common clonal abnormalities. Translocations described previously in other B cell malignancies occurred in nine patients, including four with t(8;14)(q24;q32) translocations. The association of all t(8;14) abnormalities with

IgA protein type suggested a pathogenetic relationship between a specific karyotypic abnormality and myeloma protein type. Hypodiploidy occurred mainly in patients with only Bence Jones protein, was associated with resistance to therapy, and justified the early consideration of investigational therapies.

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MULTIPLE MYELOMA is a malignant disorder of plasma cells that secrete monoclonal immunoglobulin. Cytogenetic studies in this disease have been largely unsuccessful because of low tumor proliferative activity.¹ Most commonly reported abnormalities were structural changes of chromosomes 1 and 14, as well as a variety of monosomies and trisomies.²⁻¹¹ Translocations of t(11;14) have also been reported,^{3,9,10} as has one patient each with t(8;14) and t(14;18) translocation.^{12,13} This report describes the cytogenetic findings in a large number of patients with plasma cell myeloma and reveals an association of certain chromosomal anomalies with myeloma protein type.

METHODS

Between February 1985 and December 1986, 153 bone marrow samples from 140 patients with unequivocal plasma cell myeloma were submitted for both cytogenetic and flow cytometric analysis. Cytogenetic studies were conducted on marrow aspirates collected in RPMI 1640 growth medium with heparin and colcemid (0.04 µg/mL) without mitogens. Following 20 minutes of hypotonic treatment in 0.06 mol/L KCL, several changes of 3:1 methanol:glacial acetic acid fixative were used to fix and to eliminate RBCs. Air-dried slides were prepared using the cell pellet resuspended in methanol:glacial acetic acid mixture (1:1 by volume). Q-, G-, and/or C-bands were performed according to standard methods.¹⁴ Identical abnormalities in two or more cells defined a clonal population, except for monosomies or deletions, where three or more cells with identical aberrations were required.¹⁵ In samples showing karyotypic heterogeneity, the reported karyotype included all clonal abnormalities detected. A normal karyotype was confirmed when no clonal abnormality was detected among 15 metaphases examined. Excluded from the analysis were 13 patients studied during remission with less than 1% monoclonal plasma cells in the marrow on flow cytometry and a normal karyotype; 12 patients with less than 15 metaphases and no clonal abnormality were also excluded.

Flow cytometric analyses were conducted of cellular DNA, RNA, and cytoplasmic immunoglobulin content.^{16,17} The DNA index was defined from the ratio of fluorescence intensities of tumor G1/0 cells to normal peripheral blood lymphocytes.¹⁸ Tumor mass and response to therapy were defined by standard criteria.^{19,20} Statistical comparisons were conducted by chi-square tests.

RESULTS

Of 115 patients with evaluable metaphases, 46% showed an abnormal karyotype, which was more likely in patients with IgA or relapsing myeloma (Table 1). The frequency of abnormalities was unrelated to age, sex, tumor mass, or the degree of marrow plasmacytosis (Fig 1).

Chromosomal abnormalities were usually complex, with multiple structural changes (translocations, derivatives, deletions) occurring in 90% and numerical deviations in 86% of

Table 1. Patient Characteristics

	No. Patients 115	% Abnormal Karyotype 46
Disease status at cytogenetic study		
At diagnosis	31	36
Unresponsive	44	45
Relapsing	30	60 (P = .12)
Remission	10	40
Protein type		
IgG	60	42
IgA	30	63 (P = .05)
Light chain only	22	41
Nonsecretory	3	0
DNA ploidy		
Hypodiploid	5	40
Diploid	25	40
Hyperdiploid	85	48

patients. Hyperdiploid samples commonly showed multiple trisomies and tetrasomies of chromosomes 3, 5, 7, 9, 11, 15, 18, 19, and 21; monosomies typically involved chromosomes 8, 13, 16, 20, or 22 (Fig 2). Structural changes of chromosome 1 were found in 49% of patients but without a consistent breakpoint or a common region of deletion (Fig 3). In contrast, 13 of 18 patients with structural anomalies of chromosome 14 had a breakpoint at q32; the remaining five patients showed breakpoints at sites between q22 and q31 (Fig 3). One individual with a prior history of large cell lymphoma showed both t(8;14)(q24;q32) and t(11;14)(q13;q32) in the same metaphases.

On flow cytometry studies, 78% of the patients had hyperdiploidy (Table 1). There was a linear, statistical

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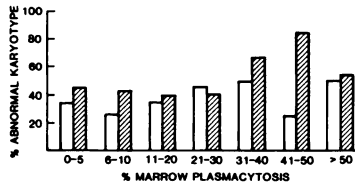


Fig 1. Similar frequency of abnormal karyotype with increasing marrow plasmacytosis defined either morphologically or by abnormal DNA/RNA content. □ Marrow differential; ▨ Flow Cytometry.

correlation between DNA-derived ploidy (DNA index) and modal chromosome number expressed as a karyotype index (modal chromosome number divided by 46; Fig 4). Among 53 patients with evaluable karyotypes, chromosomal hypodiploidy occurred in 11%, but low DNA content was observed in only 4% ($P = .01$). Two patients showed concordant DNA indices and karyotypes in the tetraploid range with some chromosome rearrangements present in both copies. One patient showed a near haploid chromosome number.

Of four patients with $t(8;14)(q24;q32)$, all produced IgA myeloma protein (Table 2, Fig 5). Other translocations associated with B cell malignancy were also found, including four patients with $t(11;14)(q13;q32)$. A hypodiploid karyotype or a $t(11;14)$ translocation was present in eight of nine patients with only light chain production. Only three of 13 hypodiploid patients (23%) responded to chemotherapy either prior or subsequent to study, in comparison with responses in 29 of 40 other patients with abnormal cytogenetics (73%, $P = .01$).

No apparent relationship was noted between specific chromosomal abnormalities and disease manifestations, such as bone disease or tumor mass. In contrast to the report by Durie et al,²¹ only four patients showed a deletion of the long arm of chromosome 6, and all showed bone destruction. Monosomy 13 occurred in 19 of 43 patients who had received prior chemotherapy but not in any of ten previously untreated patients ($P = .02$). No difference was evident in chromosome number between untreated patients and those who had prior chemotherapy.

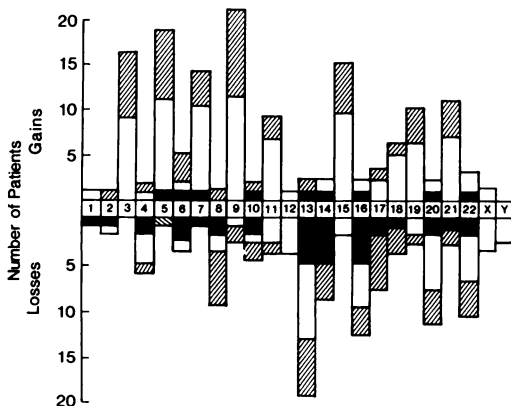


Fig 2. Frequency and distribution of numeric abnormalities in patients with abnormal karyotype. ▨ IgA; □ IgG; ■ light chain only.

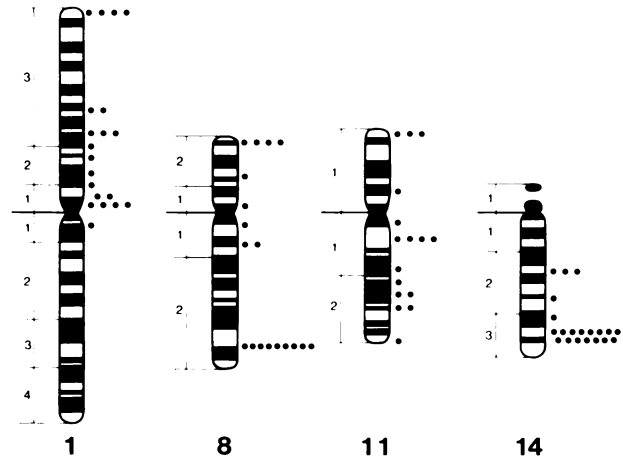


Fig 3. Composite ideogram showing distribution of clonal breakpoints on the chromosomes affected mostly with structural aberrations. Each symbol represents one patient with a breakpoint at this locus.

DISCUSSION

Cytogenetic studies of myeloma have been difficult to perform, probably due to low tumor-proliferative activity. Prior flow cytometric analyses of myeloma marrow have revealed aneuploidy in about 80% of patients,¹ suggesting that karyotypic abnormalities should be identified more frequently than the 30% to 50% incidence found in this and other studies.²⁻¹¹ This discrepancy suggested that normal marrow cells accounted for the normal karyotype found in patients with cytometric aneuploidy. These findings highlight the greater sensitivity of flow cytometry in assessing ploidy in tumors with low proliferative activity.

Among patients with an abnormal karyotype, a close relationship was usually observed between DNA content as determined by cytogenetics (modal chromosome number) and flow cytometry (DNA ploidy). This correlation indicates that the identified karyotype was indeed that of the abnormal cell population. Hypodiploidy was found more than twice as frequently by cytogenetics than by flow cytometry. This discrepancy can be attributed to the insensitivity of DNA flow cytometry in detecting deletions of small chromosomes and/or the presence of high DNA complement despite low

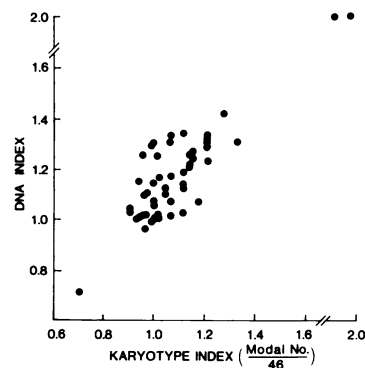


Fig 4. Direct relationship between DNA and karyotype index in myeloma patients with abnormal karyotype.

Table 2. Myeloma Karyotype and Ig Phenotype

	No.	IgG	IgA	Only BJP
B cell translocations				
(8;14) (q24;q32)*†	4	0	4	0 $P = .001$
(11;14) (q13;q32)*‡	4	1	1	2
(14;18) (q32;q21)	1	1	0	0
Hypodiploidy†‡	13	3	4	6 $P = .001$
Other anomalies	34	21	12	1

*.†.‡. Two abnormalities were present in two patients with IgA gammopathy and one patient with only BJP.

chromosome number.²² These findings stress the complementary power of the two techniques in tumor cell analysis, with cytogenetics assessing dividing cells and flow cytometry describing the entire cell population.

This study confirms prior reports of complex numerical and structural karyotype abnormalities in multiple myeloma, especially gains of chromosomes 3, 5, and 9; loss of chromosome 13; and rearrangement of chromosome 1.²³ However, no consistent structural rearrangements were identified other than those associated with B cell neoplasms. Translocations (11;14) have been described,^{3,9,10} as have single patients with t(8;14) or t(14;18)(12,13). In addition, important associations with immunoglobulin phenotype were observed, such as the exclusive association of t(8;14) with IgA myeloma protein and the prevalence of hypodiploidy in patients with only Bence Jones proteinuria. The lower response rate among patients with chromosomal hypodiploidy was consistent with the drug resistance described previously in patients with low DNA content.¹ The presence of hypodiploidy by either technique should help identify patients who might benefit from the early application of innovative therapies, such as high-dose melphalan, which has been effective in many patients resistant to standard treatment.^{24,25}

Specific translocations have been linked to oncogene activation in certain human malignancies.²⁶⁻²⁸ Thus the t(8;14)(q24;q32) translocation, typically associated with Burkitt's lymphoma, dysregulates *myc* gene expression by its juxtaposition to the immunoglobulin heavy chain gene.²⁹

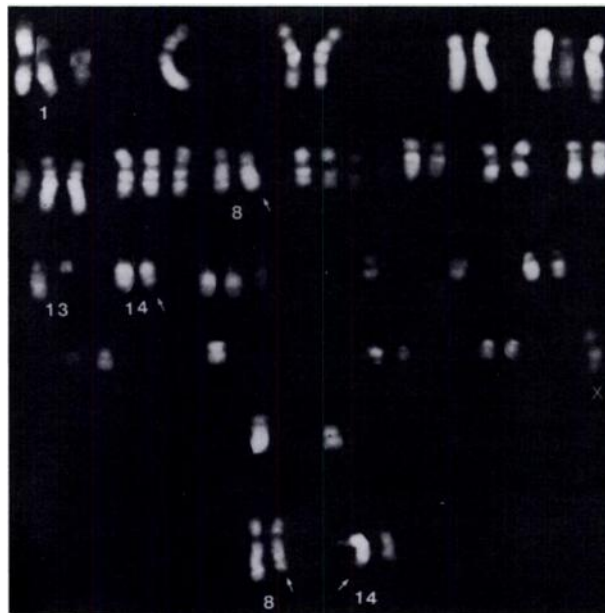


Fig 5. Q-banded karyotype of a marrow cell showing multiple trisomies and t(8;14)(q24;q32). A typical Burkitt's lymphoma-like translocation, t(8;14), is marked by arrows.

Enhanced *myc* RNA expression was present in about one fourth of our patients with advanced myeloma studied by Northern analysis, two of whom showed rearrangement of the *myc* gene.³⁰ Both t(8;14) and *myc* gene anomalies have occurred preferentially with IgA myeloma. Despite seemingly identical cytogenetic aberrations and *myc* involvement in Burkitt's lymphoma and IgA myeloma, molecular differences must be postulated to explain the different clinical features of these B cell malignancies. Thus different sites of the *myc* gene locus may be affected, depending on whether the translocation occurred at an early phase of B cell commitment (ie, during immunoglobulin V-D-J joining) and leading to endemic Burkitt's lymphoma or at a later phase (ie, during isotype switching) and leading to sporadic Burkitt's lymphoma or IgA myeloma.³¹

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