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DNA Content of Human Myeloma Cells*

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Abstract—*DNA flow cytofluorometry of bone marrow cells from 29 patients with multiple myeloma disclosed a 55% incidence of aneuploid cell populations with a DNA index between the diploid and the tetraploid regions (2–4 C) and a mean value of 2.5 C. The bone marrow of these patients demonstrated a correlation between the proportion of aneuploid and morphologically identifiable myeloma cells, which indicates a monoclonal pattern in the aneuploid cases without additional diploid myeloma cell clones. In the present patient material no statistically significant relation was found between diploid and aneuploid cases as regards the type of monoclonal immunoglobulin. Further, there was no difference between the diploid and aneuploid cases as regards myeloma cell differentiation, myeloma cell* [^{3}H]*-thymidine labeling index or response to melphalan treatment.*

INTRODUCTION

IN CONTRAST to karyotypic analysis, flow cytofluorometric analysis of cellular DNA content may provide a means for ploidy determination in cell populations with no or very low proliferative activity. In multiple myeloma the relative slow proliferation rate of the myeloma cells [1, 2] and their mixture with normal bone marrow cells has limited the number of cases in which it has been possible to perform karyotypic analyses [3]. In the present investigation flow cytofluorometric measurements were performed on the bone marrow of previously untreated cases of multiple myeloma. Comparisons were made with serum and urinary protein abnormalities, the differentiation and proliferative activity of the myeloma cells and response to melphalan treatment.

MATERIALS AND METHODS

Bone marrow specimens from 29 patients with multiple myeloma were investigated. The bone marrow was obtained at the time of diagnosis, suspended in heparinized phosphate-buffered saline and subjected to differential centrifugation in Ficoll-Isopaque (density 1.077, 750 *g* for 20 min). Bone marrow cells of all kinds were separated from matured erythrocytes, granulocytes and lipids and collected from the interphase. That part of the specimen intended for morphological analysis was cytocentrifuged onto gelatinized glass slides and stained by the May-Grünewald Giemsa technique. Another part of the specimen was fixed in ethanol and analysed for cellular DNA content by the rapid flow cytofluorometric method described previously [4]. In brief, interphase cells were washed in Tris-EDTA buffer together with 1 mg/ml RNase in order to remove RNA. Suspensions of single-cell nuclei were obtained by pepsine treatment. After washing in the buffer, the nuclei were stained using 2.5×10^{-5} M ethidium bromide in Tris-EDTA buffer with a molarity of 285 mmol/l. The risk of unspecific binding of ethidium bromide is further reduced when this high molarity is used. The DNA content of the cell nuclei were analysed using a rapid flow cytofluorometer ICP11 (Phyve W. Germany) with a flow rate of up to 1000 cells/sec. The excitation and emission wavelengths were 455–490 nm and 390–630 nm respectively. The output was sorted with a 256 multichannel analyser. After correction for the background the proportion of aneuploid cells was determined from the integrated cell numbers under the part of the DNA histogram considered to represent cells with an aneuploid DNA content. The DNA values of the analysed cells were calculated in relation to the DNA content of normal human lymphocytes.

For autoradiographic analyses of myeloma cell [^{3}H]-thymidine labeling index (LI) and [^{3}H]-uridine incorporation, interphase cells were

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suspended at a density of 10^6 cells/ml in Parker medium 199 (Flow laboratories) supplemented with 125 IU of benzylpenicillin and 125 μg streptomycin/ml. For [^{3}H]-thymidine labeling index, 10 μCi of [^{3}H]-thymidine (5 μCi/mmol) was added/ml of medium, after which the cell suspensions were incubated for 2 hr in a humidified 5% CO_2 atmosphere at 37°C. For the assessment of [^{3}H]-uridine incorporation an aliquot of the interphase cells was incubated with 10 μCi [^{3}H]-uridine (47 μCi/mmol)/ml of medium for 45 min. After incubation the cell suspensions were cytocentrifuged onto gelatinized glass slides. The slides were then treated for cell fixation with 70% ethanol for 10 min. Free radioactivity was removed by washing in 2% perchloric acid for 45 min. This was followed by dehydration with ascending strengths of ethanol for 10 min at 70%, 5 min at 96% and 15 min at 99.5% ethanol. The slides were then coated with Kodak AR10 stripping film and exposed for 24 hr. After development the cells were stained by the May–Grünewald Giemsa Technique. The autoradiographs prepared in this fashion were of high quality. In the experiments with [^{3}H]-thymidine the background grain count did not exceed 5 grains/cell. Cell nuclei considered to be labeled were covered with at least 20 grains. Surprisingly, there were no cell nuclei that would fit into the grain range of 5–20 grains. Five hundred myeloma cells were counted in order to determine the LI, which was expressed as the percentage of myeloma cells labeled by [^{3}H]-thymidine. In the experiments with [^{3}H]-uridine the grain counting was performed on 50 myeloma cell nuclei, and a mean grain count was calculated.

The myeloma cells were subdivided into 3 groups according to morphological characteristics. Large cells with a nuclear diameter greater than 17 μm and having nucleoli were classified as poorly differentiated. Cells with a nuclear diameter of less than 17 μm and having nucleoli were classified as moderately differentiated. Cells bearing no evident nucleoli (usually with a nuclear diameter of less than 13 μm) were classified as well-differentiated myeloma cells and were also easily distinguishable from the moderately differentiated cell type by the more pyknotic nuclei located peripherally. This subdivision of myeloma cells into three degrees of differentiation is similar to that suggested by Azar and Potter [5].

Patients were randomized to treatment with either melphalan and prednisolone, given in an intermittent fashion (22 patients) or with interferon (7 patients). The influence of aneuploidy on the response to treatment was evaluated only for the patients treated with melphalan and prednisolone. Response to therapy was evaluated by the criteria described by Alexanian *et al.* [6].

RESULTS

The flow cytofluorometric analyses of bone marrow from 29 myeloma patients disclosed an aneuploid population of cells in 16 cases. A typical example of such an analysis is shown in Fig. 1. The position of the initial larger peak corresponds to the position of the peaks observed when normal human lymphocytes are analysed. The adjacent peak on the right contains $G_{1/0}$ cells, corresponding to a hyperdiploid abnormality. The proportion of cells in this hyperdiploid peak (48%) is about the same as the number of myeloma cells identified on cytocentrifuged slides (52%). When all cases with an aneuploid abnormality were analysed for the percentage of aneuploid cells vs the percentage of morphologically identifiable myeloma cells, a linear correlation was established ($r = 0.68$, $P = 0.001$). The range of the percentage of myeloma cells and aneuploid cells was 3–60 and 4–70 respectively. The mean difference between the percentage of myeloma cells and aneuploid cells was 1.6, with 95% confidence limits of −5.3 and +8.5. This observation, which is consistent with the findings of other investigators [7], indicates that the DNA-content abnormalities originate from the myeloma cells.

In the 16 cases with aneuploid cell populations, 13 were hyperdiploid, with a C-value ranging between 2.2 and 2.6 (Fig. 2). None of the cell populations exceeded 4.0 C.

Retests of patients during therapy with melphalan and interferon revealed an almost complete stability of the myeloma cell clone as regards DNA content. Retests were performed up to three times. In 5 cases treated with melphalan and 3 cases treated with interferon no change of DNA content was noted. Only in 1 case treated

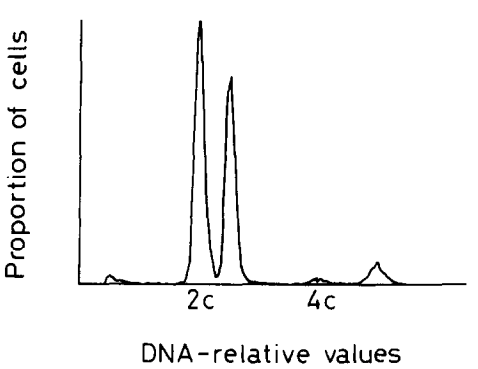

Fig. 1. DNA histogram in a patient with multiple myeloma. The cell material was obtained by aspiration biopsy of bone marrow.

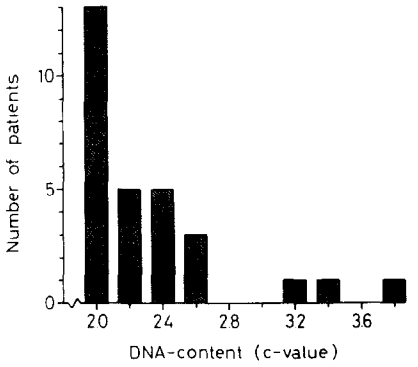

Fig. 2. Frequency distribution of 29 patients by DNA index of their myeloma cell-infiltrated bone marrow. DNA content (C-value) is related to normal diploid (2.0 C) lymphocytes used as controls.

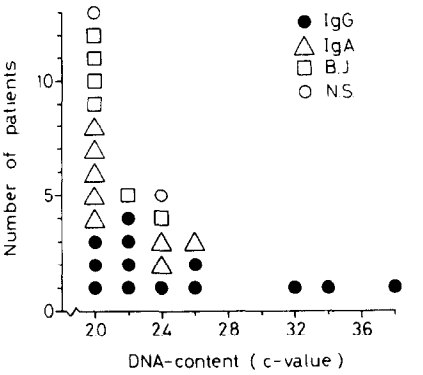

Fig. 3. Frequency distribution of monoclonal immunoglobulin types by DNA index of patients with multiple myeloma.

with interferon was an alteration of the pretreatment DNA content of 2.2–2.0 C seen.

A comparison was made between the degree of ploidy and type of myeloma, as characterized by immunoglobulin type, degree of myeloma cell differentiation and the [^{3}H]-thymidine labeling index. In Fig. 3 the breakdown by monoclonal immunoglobulin component type vs DNA content is given. The different immunoglobulin types were found in diploid as well as aneuploid myelomas.

In some human tumor diseases an abnormal DNA content is more frequently found in poorly than well-differentiated tumors [8–10]. In the present investigation the myeloma cells were subdivided on the basis of the cytomorphological characteristics. The validity of such a subdivision is further supported by a relationship between the stage of differentiation and the rate of RNA-synthesis. In 10 cases of human myeloma the highest incorporation of [^{3}H]-uridine was consistently found in the cells defined as poorly differentiated (mean grain count = 57). A progressive decrease of [^{3}H]-uridine incorporation was found parallel to further differentiation, with the lowest incorporations being found in the cells defined as well-differentiated (mean grain count = 15). The findings are in agreement with those of Storti and Torelli [11]. No difference in the cellular differentiation, i.e. the composition of myeloma cell types, was found when diploid and aneuploid myeloma cell populations were compared (Table 1).

The [^{3}H]-thymidine labeling index (LI) of bone marrow myeloma cells has been identified as a prognostic factor in human myeloma [12]. In the present study LI was compared in aneuploid and diploid myeloma cell populations. No difference was found between the groups (Table 2).

Patients receiving melphalan and prednisolone therapy were evaluated for therapy response

Table 1. Percentage of myeloma cells of various stages of differentiation in 10 diploid and 13 aneuploid cases of multiple myeloma analysed before treatment

	DIPLOID	ANEUPLOID
Myeloma cells	mean ± SD	mean ± SD
Poorly differentiated	2.8 ± 2.7	2.8 ± 1.6
Moderately differentiated	17.3 ± 15.1	20.4 ± 12.3
Well differentiated	80.0 ± 16.3	76.8 ± 13.7

Table 2. [^{3}H]-thymidine labeling index in the myeloma cell population of non-treated cases of myeloma with a diploid or aneuploid DNA content

DIPLOID		ANEUPLOID	
No. of cases	mean ± SD	No. of cases	mean ± SD
7	8.3 ± 9.7	9	6.9 ± 7.7

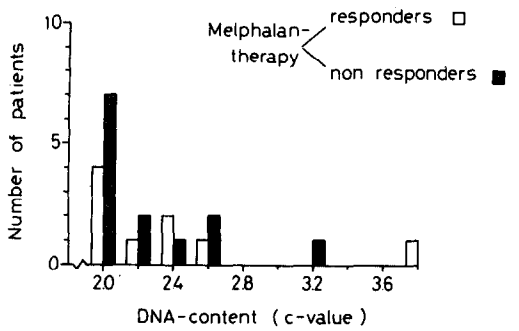

Fig. 4. Distribution of melphalan responders and non-responders by DNA index of patients with multiple myeloma.

according to the criteria described by Alexanian *et al.* [6]. In Fig. 4 the number of responding and non-responding cases are plotted against the DNA-content of their myeloma cell population. As can be seen, there is no relation between response to treatment and DNA content.

DISCUSSION

The present study, as well as that by Latreille *et al.* [7], indicates that human myeloma cells may be diploid or aneuploid in DNA content. The incidence of aneuploid cells among the cases of the present investigation was 55%. In the subgroup of patients with more than 10% of myeloma cells in the bone marrow the incidence was 64%. In the investigation by Latreille *et al.* the incidence figures were 65 and 85% respectively. The different results may be explained by a difference in the number of patients investigated and/or the number of diploid cases with less than 10% of myeloma cells. Before the introduction of cytofluorometry for the measurement of the DNA content, conclusive evidence for the existence of diploid myeloma cell populations was not obtainable by studies of karyotype due to the uncertainty with which karyotypes were to be regarded as belonging to myeloma cells [13]. The presence of 10–50% of myeloma cells in 9/13 cases registered as diploid gives support for the existence of myelomas in the diploid range. In the group of aneuploid cases we found only cell populations characterized by a single DNA index. Further, the analyses of the relationship between the percentage of morphologically identifiable myeloma cells and aneuploid cells makes the presence of a diploid cell population in addition to the aneuploid population unlikely in the cases registered as aneuploid. Absence of diploid myeloma cells in aneuploid cases is further supported by microscopic cytofluorometry of myeloma cells [14].

Multiple myeloma is traditionally classified according to serum and urinary protein abnormalities. In the present investigation no relationship was found between DNA content and type of immunoglobulin M-component or κ/λ -subtype. Even though IgG myelomas seem to be found in aneuploid myelomas more frequently than other immunoglobulin types, such a statistical difference could not be confirmed in the present material. These results are in agreement with those of karyotypic studies, in which no characteristic numerical or structural chromosomal abnormality was established [15].

While the prognostic significance of protein abnormalities in multiple myeloma is still doubtful and cytological characteristics have not been evaluated, the [^{3}H]-thymidine labeling index has been found to correlate with prognosis [12]. In the present investigation there was neither a correlation between DNA content and LI nor a difference in LI between diploid and aneuploid cell populations. Thus a different prognosis for diploid and aneuploid cases would not be expected. In some malignancies a relationship between cellular DNA content determined by flow cytofluorometry and clinical prognosis has been established [8, 16, 17]. The follow-up of the patients included in this study is at present too short to provide survival data. It has been shown, however, that patients that respond to therapy live longer than those who do not [18 19]. The random distribution of DNA content among responders as well as non-responders to melphalan–prednisolone therapy makes it doubtful whether measurement of DNA content is a useful pretreatment indicator of response for this type of treatment.

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