

Phenotypic Heterogeneity in Aneuploid Multiple Myeloma Indicates Pre-B Cell Involvement

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The expression of early and mature B cell markers, surface β_2 -microglobulin (B_2M) and cytoplasmic immunoglobulin (cIg) by aneuploid tumor cells in bone marrow aspirates from 44 patients with multiple myeloma was evaluated by correlated DNA immunofluorescence flow cytometry. Myeloma tumor cells of almost 90% of the patients contained monoclonal cIg and expressed the mature plasma cell antigen R1-3 as well as surface B_2M ; common acute lymphoblastic leukemia antigen (CALLA) was present in 55%, B2 in 17%, and B4 in 23% of samples studied.

MULTIPLE MYELOMA is a B cell malignancy typically associated with mature plasma cell morphology and monoclonal immunoglobulin secretion. Patient survival is inversely related to the tumor mass at diagnosis, which can be estimated from a number of laboratory parameters¹ or, as reported more recently, from the serum level of β_2 -microglobulin (B_2M).² The neoplastic involvement of earlier B cells has been suggested by the presence of idiotype-concordant and common acute lymphoblastic leukemia antigen (CALLA)-positive lymphoid cells in the blood of patients with multiple myeloma.³⁻⁵ CALLA+ lymphocytes from blood and bone marrow could be differentiated in vitro to mature plasma cells with concordant monoclonal immunoglobulin isotype expression and secretion.⁶ Recently, an aggressive clinical course has been described in myeloma with CALLA+ plasma cells.⁷

Our laboratory has investigated myeloma cellular features as a means of understanding the biology and the clinical course of the disease. Methods were developed for quantitative assessment of B cell and other cellular markers in relationship to DNA aneuploidy, which serves as an unequivocal tumor cell marker present in about 80% of patients with plasma cell myeloma.⁸ We now report our studies on the expression of early and mature B cell markers by tumor cells in the bone marrow from 44 patients with aneuploid myeloma.

MATERIALS AND METHODS

Bone marrow aspirates were obtained from 44 patients with DNA-aneuploid myeloma in various stages of disease; ten of the patients were previously untreated. Informed consent was obtained from the patients before bone marrow aspiration. After Ficoll-Hypaque separation (density, 1.077 g/mL), interphase cells were washed in phosphate-buffered saline and reacted with a panel of monoclonal antibodies to surface antigens by using either direct or indirect immunofluorescence assays. After immunostaining, the cells were fixed in ice-cold 70% ethanol and counterstained with propidium iodide at 20 μ g/mL for correlated DNA phenotypic analysis, which was carried out with an EPICS V flow cytometer (Coulter Electronics, Hialeah, FL).⁹ The investigated surface markers included the pre-B cell antigen CALLA, which was studied by using the monoclonal antibody J5¹⁰; B2, which is expressed by early to mature nonactivated B cells¹¹; B4, which is expressed by all B cells but not by mature plasma cells^{12,13} (Coulter); and the plasma cell marker R1-3, which was kindly provided by Dr J.A. Katzmman (Mayo Clinic, Rochester, MN).^{5,14} In addition, cytoplasmic immunoglobulin (cIg) was also analyzed by using both anti- κ and anti- λ

Coexpression of CALLA and cIg in 46% of all patients identified a novel myeloma phenotype without known counterpart in the normal differentiation of B cells. CALLA and cIg were independently expressed and gave rise to CALLA+ /cIg-, CALLA+ /cIg+, and CALLA- /cIg+ cells. The association of CALLA and mature plasma cell markers may define discrete stages of neoplastic plasma cell differentiation.

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light chain antisera [F(ab')₂ fragments; Cappel Laboratories, West Chester, PA].¹⁵ We also studied surface expression of B_2M (Dako, Santa Barbara, CA), which is secreted in proportion to tumor mass.² Separate aliquots of all samples were also subjected to acridine orange staining for correlated analysis of DNA and RNA content as previously described.¹⁶ Routinely, 10,000 cells were analyzed for each marker. Quantitative analysis was carried out with a Terak computer (Scottsdale, AZ) using cells stained with nonreactive antibodies as matching controls.

RESULTS

An example of DNA phenotype analysis in a patient with a hyperdiploid myeloma is presented in Fig 1. Hyperdiploid tumor cells showed a high RNA content; reacted positively with anti- B_2M , J5, and R1-3; and contained cIgA with monoclonal κ light chain reactivity. Cells in the diploid cell compartment had a low RNA content, did not express cIg, but were positive for B_2M and J5. Because of scarcity of marrow samples, complete studies could be performed in 65% of the cases. Monoclonal cIg and R1-3 were present in aneuploid tumor cells of 88% and 89% of the patients, respectively. The pre-B cell antigen CALLA was expressed by aneuploid myeloma tumor cells in 55% of 43 samples studied for this marker (Table 1). Phenotype coexpression by aneuploid tumor cells was observed in 80% of patients for monoclonal cIg, R1-3, and B_2M , and in 45% of the patients, the pre-B cell antigen CALLA was present together with monoclonal cIg and the mature plasma cell antigen R1-3 (Fig 1 and Table 2). Only a few patients (13% and 15%, respectively) demonstrated coexpression of CALLA with B2 and B4 antigens. There was no correlation between the

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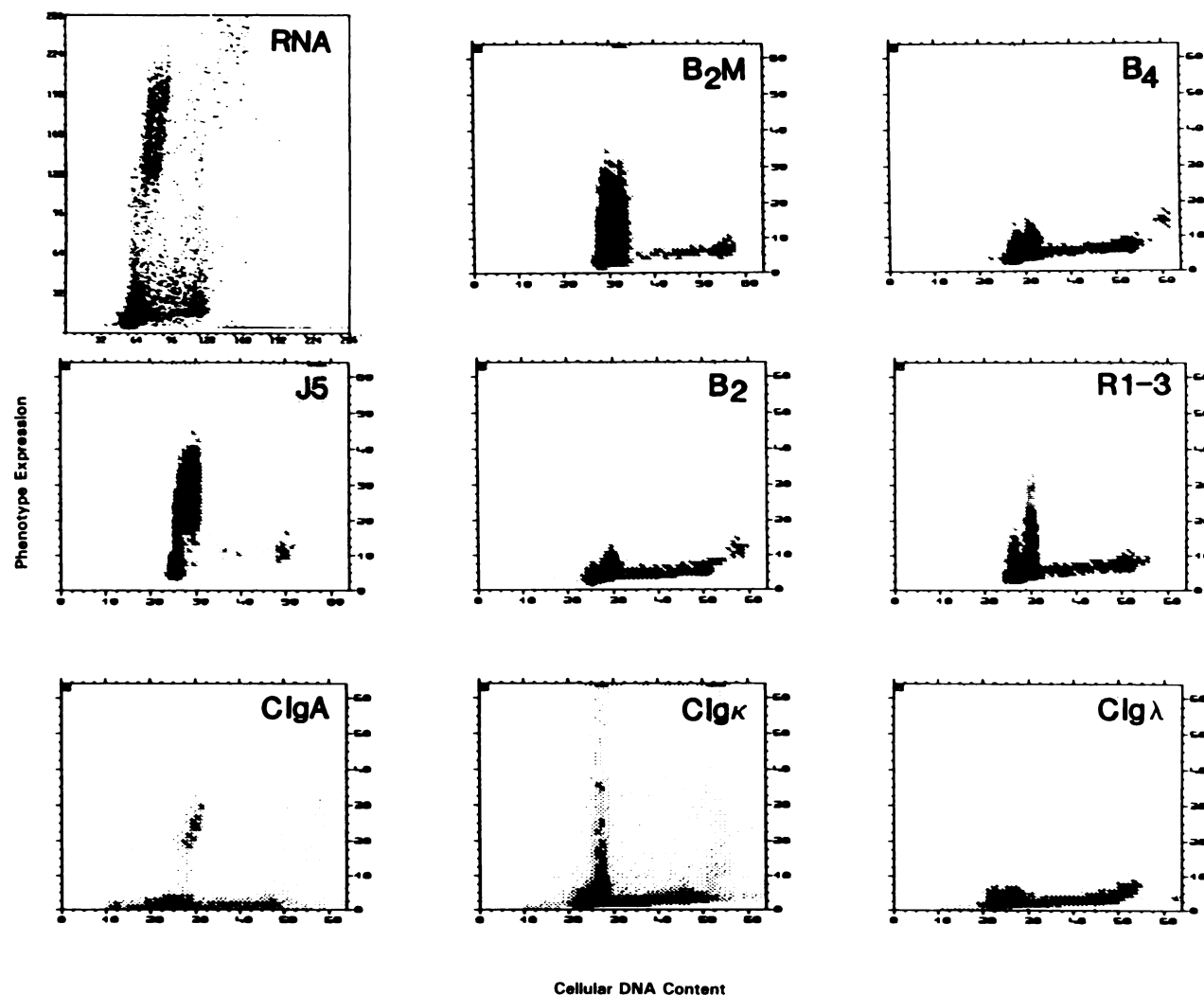


Fig 1. Phenotypic heterogeneity in a patient with hyperdiploid multiple myeloma. Hyperdiploid cells show a high RNA content; react with anti-B₂M, J5, and R1-3; and show monoclonal cIgA κ expression. Diploid cells have low RNA contents without monoclonal cIg reactivity but express B₂M and CALLA. Abscissa, DNA content; ordinate, fluorescence intensity of cellular feature.

proportions of tumor cells that stained positively for CALLA, R1-3, B₂M, or cIg in patients whose cells were positive for these markers, thus indicating their independent expression in plasma cell myeloma (Fig 2). Seven of the 44 patients displayed an additional diploid tumor DNA stem-line on cIg analysis, a representative example of which is presented in Fig 3. Hyperdiploid cells with a high RNA content and monoclonal cIg κ were positive for CALLA (J5) and R1-3; diploid cells also contained cIg κ , were CALLA-positive, but had a low RNA content and did not express R1-3. Interestingly, in all patients with CALLA+ aneuploid cells, the diploid cells also expressed CALLA. CALLA+ diploid cells were only seen in association with CALLA+

aneuploid cells. Further examination of CALLA/cIg expression revealed three different phenotypes, CALLA+/cIg-, CALLA+/cIg+, and CALLA-/cIg+, that are present alone or in combination, at times with different ploidy levels (ie, diploid and hyperdiploid) (Figs 3 and 4). It has been reported that CALLA-positive myeloma has a particularly aggressive course with a short median survival of 6 months.⁷ The median survival from diagnosis of 21 patients with

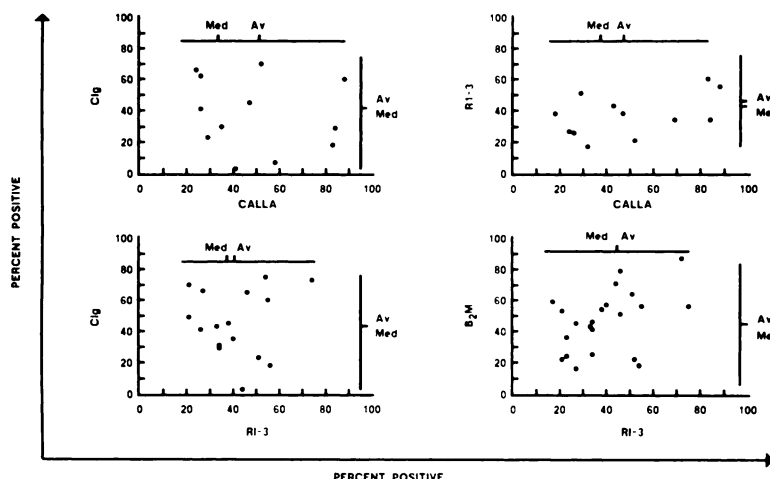
Table 1. CALLA Expression in Aneuploid Myeloma Tumor Cells

	B4	CALLA	B2	R1-3	B ₂ M	cIg
Number of patients studied	35	43	33	40	44	44
Percentage of positive patients	23	55	17	89	90	88

Table 2. Coexpression of Early and Late B Cell Markers by Aneuploid Myeloma Plasma Cells (Percentage of Positive Patients)

	CALLA	B2	R1-3	B ₂ M	cIg
B4	15	4	19	19	14
CALLA		13	44	45	46
B2			13	17	20
R1-3				78	81
B ₂ M					79

Fig 2. Independent expression of cellular markers by myeloma tumor cells. The percentages of cells positive for each marker in bone marrow samples coexpressing these markers are plotted against each other. The correlation coefficient for each plot was $<.5$.



CALLA-positive myeloma was significantly longer than that of 23 patients with CALLA-negative disease (>80 v 24 months; $P = .004$) (Fig 5).

DISCUSSION

Expression of several differentiation-associated B cell markers and surface B_2M by tumor cells was evaluated in the bone marrow of 44 patients with DNA-an euploid multiple myeloma by using correlated DNA and immunofluorescence flow cytometry. Tumor cells are unequivocally identified by DNA aneuploidy, which is present in more than 80% of all myeloma patients.⁸ The tumor cells of about 90% of the patients reacted with the mature plasma cell marker R1-3,

expressed surface B_2M , and contained cIg. Unexpectedly, however, tumor cells of one half of the patients expressed the pre-B cell antigen CALLA together with the mature plasma cell features R1-3 and cIg, recently also reported by Grogan et al.¹⁷ Coexpression of CALLA and cIg has no known counterpart in normal B cell differentiation and could represent a unique tumor cell phenotype in myeloma.

In cases of concurrent CALLA and cIg or R1-3 reactivity, these markers were expressed independently of each other and represented tumor cell subpopulations that were either CALLA+/cIg-, CALLA+/cIg+, or CALLA-/cIg+. Although none of the CALLA-negative aneuploid cases showed J5 reactivity in DNA-diploid cells, all cases with

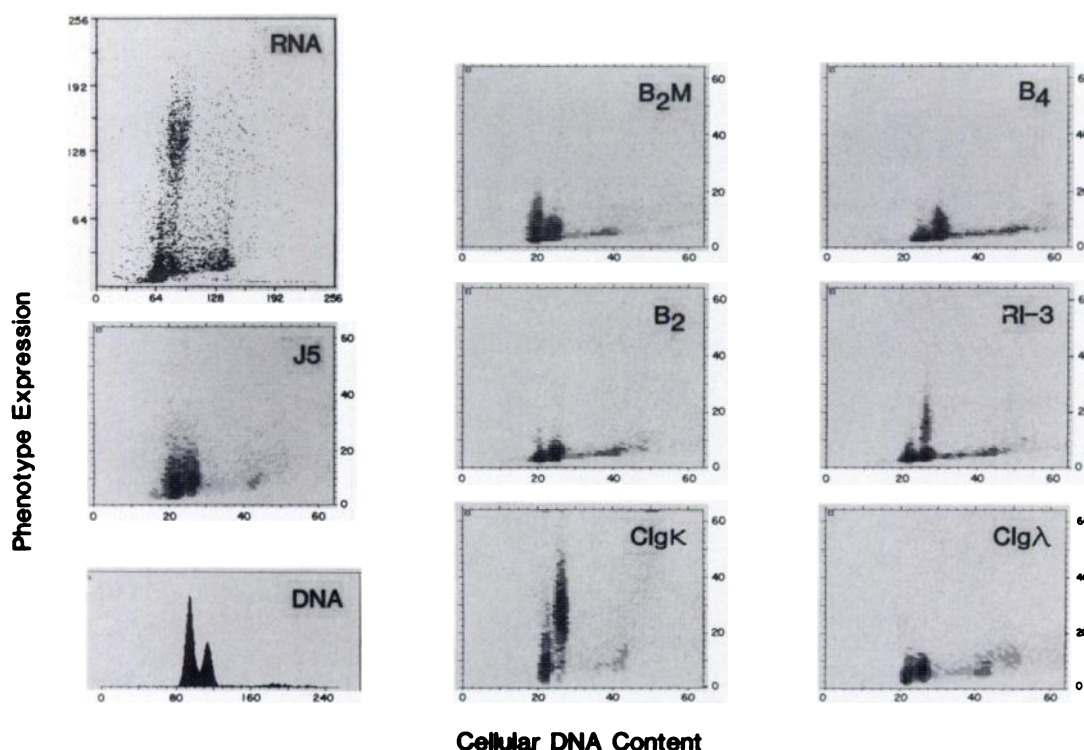


Fig 3. Phenotypic heterogeneity in a biclonal myeloma with two DNA stemlines. Both diploid and hyperdiploid cells contained cIg and expressed CALLA (J5). Hyperdiploid cells also reacted with R1-3, whereas diploid cell expressed surface B_2M .

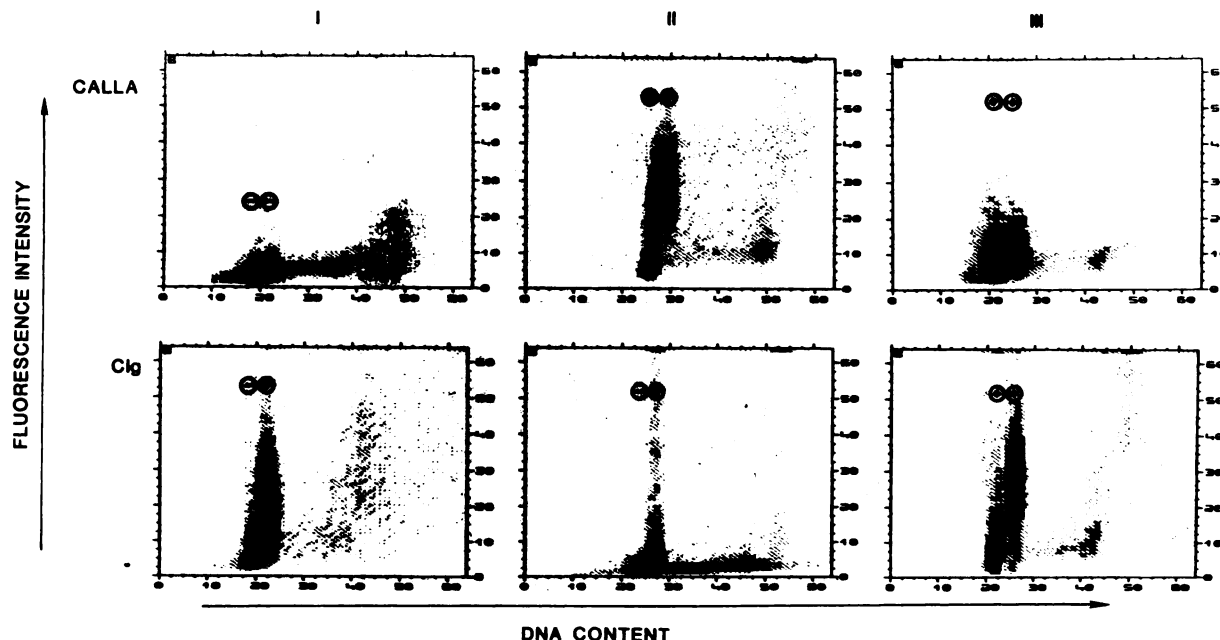


Fig 4. Heterogeneity of CALLA expression in aneuploid myeloma. Three phenotypes were observed: (a) CALLA- / cIg- +; (b) CALLA++ / cIg- +; (c) CALLA++ / cIg++. +, indicates the presence of cellular marker; -, the absence of marker for the diploid and hyperdiploid cells, respectively.

CALLA expression by aneuploid tumor cells also showed expression of this pre-B cell marker by DNA-diploid cells, but usually without cIg or R1-3. This phenotypic heterogeneity with the coexistence of CALLA+ / cIg-, CALLA+ / cIg+, and CALLA- / cIg+ aneuploid tumor cell compartments and the additional presence of a CALLA+ / cIg- diploid compartment may reflect discrete stages in the development of multiple myeloma: CALLA+ / cIg- precursor cells mature progressively to CALLA+ / cIg+ and then to the expected mature plasma cell phenotype CALLA- / cIg+. The presence of a diploid CALLA+ / cIg- cell popu-

lation in all and only in aneuploid CALLA+ myelomas suggests the existence of a diploid tumor progenitor cell compartment. DNA aneuploidy may therefore be distal to more subtle genetic changes associated with malignant transformation. Such a model could explain the emergence, in some patients, of new ploidy levels (eg, hypodiploidy in a hyperdiploid myeloma) as the disease advances¹⁸ and would imply that clonal changes during the course of the disease result from adaptation in response to selective pressures exerted by chemotherapy given at subcurative dose.

The concept of pre-B cell involvement in the development of myeloma is supported by cytogenetic as well as molecular genetic studies: lymphomalike translocations [t(8;14) and t(11;14)] were found in patients with multiple myeloma¹⁹; and *c-myc* and *BclII* involvement have also been recently reported.^{20,21} These findings suggest that the malignant transformation in multiple myeloma occurs at an early stage of B cell maturation. The recently reported finding of T cell receptor γ gene rearrangements in three patients with plasma cell myeloma²² also raises the possibility that the malignant transformation in multiple myeloma occurs even proximal to the commitment to lymphoid lineage differentiation.

CALLA expression by myeloma cells has been noted in association with an aggressive course and short survival.⁷ Even though obtained from patients at different stages of their diseases, our results are at variance with this report and, in fact, seem to indicate a more favorable clinical course in CALLA+ myeloma, perhaps as a result of different chemotherapy (vincristine, Adriamycin, dexamethasone), with marked efficacy also in CALLA-positive acute lymphoblastic leukemia in adults.²³

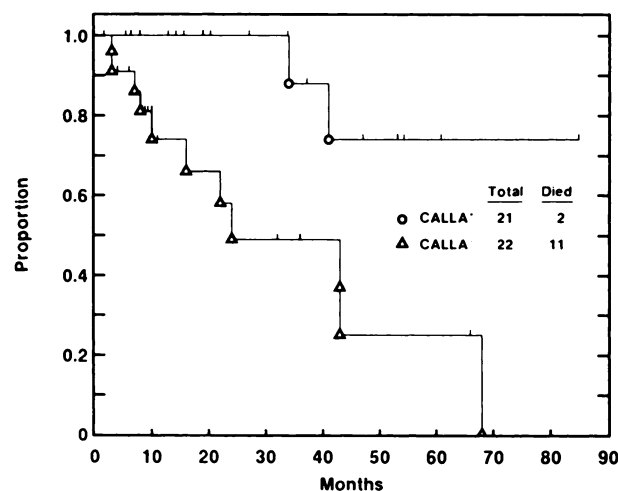


Fig 5. Superior survival of CALLA+ myeloma patients compared with CALLA- patients.

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