

EFFECT OF CHEMOTHERAPY ON THE LABELLING INDEX OF MYELOMA CELLS

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The labelling index, namely the percentage of bone marrow plasma cells incorporating tritiated thymidine after short in vitro exposures, was correlated with tumor mass reduction following chemotherapy in 37 patients with multiple myeloma. Tumor mass reduction was assessed from changes in myeloma protein production rate. Patients untreated or not responding to treatment had the lowest labelling index values. The median labelling index increased more than six-fold when tumor mass was reduced by more than 50%. These findings support the concept that an increased fraction of myeloma cells proliferates during remission and justify clinical trials with cell cycle-active drugs in selected patients with a high labelling index.

Cancer 34:526-531, 1974.

THE GROWTH KINETICS OF DIFFERENT NEOPLASTIC cell populations have been investigated in attempts to define superior treatment regimens.^{8,14,25} The present study was conducted in order to clarify some aspects of growth kinetics of neoplastic plasma cells and the influence thereupon of chemotherapeutic drugs. Plasma cell tumors are accessible to repeated bone marrow aspirations. The neoplastic cells composing the tumor produce a specific protein (myeloma protein), the fluctuations of which provide an index of tumor mass change.^{1,28} The labelling index (LI) indicates the fraction of cells in DNA synthesis and provides an estimate of the growth fraction of cell populations. This study correlated changes in the LI of neoplastic plasma cells with different degrees of tumor mass reduction following chemotherapy. Results indicated

that a reduction in myeloma tumor mass is associated with a marked increase in the fraction of proliferating plasma cells. These data justify clinical trials with cell cycle-active drugs in myeloma patients during remission in attempts to achieve a maximal tumor reduction.

MATERIALS AND METHODS

Patient Population

Thirty-seven patients with multiple myeloma were studied. All had bone marrow plasmacytosis and a monoclonal globulin peak on serum or urine electrophoresis. Twenty-four patients had IgG peaks; 5 had IgA peaks; 8 excreted only Bence Jones protein. Twenty-four patients were male; the median age was 58 years. Thirteen patients were studied before any chemotherapy, and the remainder from 1-16 months after the institution of intermittent courses of melphalan-prednisone combination chemotherapy in accordance with specific treatment protocols.^{2,3} The degree of remission was evaluated in all patients from the ratio between the myeloma protein production rate at the time of study and the pretreatment value considered as 100%.^{2,22,28} Patients with disappearance of serum or urine peaks were considered to have a reduction in tumor mass to less than 10% of the pretreatment value. At least three simultaneous bone marrow aspirations were obtained from different anatomical sites in 4 patients. In 8 other patients, serial samples were obtained at in-

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The authors thank Mrs. Elvi Fewell, Mrs. Judith Buckingham, and Ms. Cynthia George for assistance in these studies.

Received for publication November 2, 1973.

tervals of 1 to 16 months to define serial changes in LI as tumor mass was modified by chemotherapy. In addition, samples from 6 patients with other malignancies and without bone marrow disease were studied to assess the LI of non-malignant plasma cells.

Labelling Procedures

Marrow aspirates were placed in a centrifuge tube containing 5 ml of McCoy's medium anticoagulated with Versene. Tritiated thymidine (TdR-³H), 1 μ Ci/ml (S.A. 3.0 Ci/mmol) was added, and the marrow particles were mixed by repeated pipette aspirations. Marrow suspensions were incubated in a 5% CO₂ atmosphere at 37°C for 30 minutes. Smears were prepared from centrifuged concentrates or directly centrifuged onto slides by means of a cytocentrifuge⁹ and processed for radioautography by the liquid emulsion technique using Ilford K₅ emulsion. After an exposure time interval of 1 to 2 weeks, the smears were developed, fixed, and stained either with a modified Wright's stain at pH 5.8 or with acid Giemsa. Differential counts were made, and at least 500 plasma cells were examined. Particular attention was given to the differentiation of plasma cells from other hemopoietic elements, especially red blood precursors. With rare exceptions, this was not a major technical problem. Cells were considered labelled when they exhibited at least five grains overlying the nucleus. Multinucleated plasma cells were counted as one cell since the nuclei were either all labelled or unlabelled. The peripheral blood of two patients with plasma cell leukemia with white blood cell counts of 17,600/mm³ and 37,000/mm³ respectively were processed in an identical fashion.

Correlations of Tumor Mass

Correlations were made between the percentage of labelled plasma cells and: 1) the degree of remission expressed as a percent of the pretreatment mass level considered as 100%, and 2) the percent of plasma cells in the marrow suspension. Myeloma protein production rate was calculated in each patient from the serum concentration of the myeloma protein, the catabolic rate for the specific serum concentration, and the estimated plasma volume derived from the hematocrit and body weight.^{2,22} For the eight patients with only Bence Jones protein, changes in the daily excretion of Bence Jones protein provided an index of changes in tumor production rate.

RESULTS

Reproducibility of LI

Comparisons were made of the differential count and the LI of plasma cells obtained simultaneously from different marrow sites. The differential of plasma cells varied considerably in a given patient (Table 1). The most marked range was 5–14% (a three-fold difference) in patient W.S. However, the LI of plasma cells was virtually identical from site to site in all patients. Thus, the LI of the plasma cells evaluated in each patient was considered representative of the LI of the total tumor. In one patient (C.C.V.), the LI was determined twice during a 1-month interval when no reduction in tumor mass was observed. The LIs were 1.4 and 1.2 respectively.

Multinucleated Plasma Cells

Some patients had many multinucleated plasma cells, ranging up to 25% of the plasma cell population. All of the nuclei of multinucleated plasma cells were either labelled or unlabelled. The proportion of labelled multinucleated cells was compared with the fraction of labelled mononuclear plasma cells in one patient (N.H.). There were 32% plasma cells in the marrow aspirate with a LI of 11 for the combined population of mono- and multinucleated cells. Twenty-one percent of the plasma cells were multinucleated. A LI of 4 was measured for mononuclear cells and of 36 for multinucleated cells. The χ^2 test for

TABLE 1. Percentage of Plasma Cells and Labelled Plasma Cells from Different Anatomical Sites

Patient	Anatomical site	% Plasma cells in marrow aspirate	% Labelled plasma cells
M.M.	MS	8	5
	LPIC	12	8
	RPIC	11	*
V.F.C.	US	41	1
	LS	30	1
	RPIC	35	2
W.S.	MS	5	14
	LPIC	8	*
	RPIC	14	15
F.J.	US	20	*
	MS	24	11
	LS	21	7
	LPIC	36	11

US = upper sternum, MS = mid-sternum, LS = lower sternum, LPIC = left posterior iliac crest, RPIC = right posterior iliac crest, * = no data.

TABLE 2. Labelling Index and Bone Marrow Differential of 13 Untreated Patients with Multiple Myeloma

Tumor mass	Patient	L.I.	Percent plasma cells
High	M.L.	0.1	98
	W.G.	0.4	36
	D.J.	0.6	27
	V.C.	1.0	35
	C.V.	1.4	38
	W.T.	2.0	15
	A.T.	2.4	54
	M.M.	4.0	10
Low	B.R.	4	5
	E.O.	5.2	44
	G.T.	8.5	30
	L.C.	11	7
	E.B.	18	15

equality of the LI for mono- and multinuclear cells indicated a significant difference between these values ($p < 0.01$). Although not quantified, a higher LI was always observed for multinucleated cells in other patients.

Plasma Cell LI of Patients with other Malignancies

The LI of bone marrow plasma cells was evaluated in six patients with other malignancies not involving the bone marrow. Plasma cells constituted 0.2–0.8% of the differential count. Two patients had an LI of 1

and 2 respectively, while the other four did not demonstrate a single labelled plasma cell among at least 200 cells examined.

Plasma Cell Leukemia or Pleural Effusion

The blood of one patient with plasma cell leukemia (λ Bence Jones Protein), with 17,600 WBC/mm³ and 62% plasma cells, exhibited a LI of 0.4. A second patient with plasma cell leukemia (IgGK) with 37,000 WBC/mm³ and 98% plasma cells, had a LI of 0.1. A third patient with 12,600 WBC/mm³ (68% plasma cells) in a pleural effusion from a large rib plasmacytoma showed a LI of 6.8 in pleural plasma cells and a LI of 18 for bone marrow plasma cells (16% plasma cells).

Correlation of LI with Tumor Mass

Patients were considered to have either "high" or "low" tumor mass depending on certain laboratory features. The patients were assigned to the "low" tumor mass group after all of the following features were confirmed before the institution of chemotherapy: 1) hemoglobin concentration in excess of 11 g/100 ml; 2) serum peak of gamma globulin less than 4 g/100 ml; 3) Bence Jones protein excretion less than 3 g/day; 4) no evidence of hypercalcemia; and 5) minimal bone destruction. When one or more of these disease features was present, patients were considered to have "high" tumor mass.

Patients without previous chemotherapy (13 patients) showed a LI ranging from 0.1 to 18 (median of 2.4). Five untreated patients in the "low" tumor mass category had a median LI of 8.4 (range 4 to 18), while the median LI of 8 untreated patients in the "high" tumor mass group was 1.2 (range 0.1 to 4). This difference was statistically significant ($p < 0.02$) (Table 2).

The LI of bone marrow plasma cells was correlated with changes in tumor mass in treated patients, as evaluated from the myeloma protein production rate and degree of bone marrow plasmacytosis (Figs. 1 and 2). Eleven patients with no reduction in tumor mass from chemotherapy had a LI ranging from 0.5 to 11 (median of 6.5) (Fig. 1). There was no significant difference between the LIs of untreated and treated patients who did not show a reduction in tumor mass. The LI usually increased as tumor mass was reduced by chemotherapy, assessed either in terms of myeloma protein production rate (Fig. 1), or of the plasma cell differential (Fig. 2). The Spear-

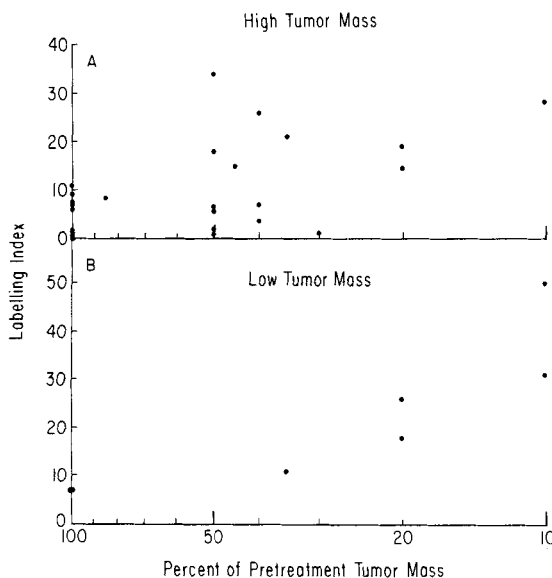


FIG. 1. The labelling index of bone marrow plasma cells from treated patients correlated with tumor mass, expressed as a percentage of the pretreatment value.

man rank correlation coefficient was higher (0.619) when the LI values were compared with tumor mass change than when compared with the plasma cell differential count (0.495), but this difference was not statistically significant. There was marked variability in LI values after tumor mass reduction. The median LI was 18.5 for patients with a reduction in tumor mass of more than 50%. When the reduction in tumor mass exceeded 50%, the median LI was 15 (range 1–28) for nine patients in the “high” tumor mass category (Fig. 1A); such changes produced a median LI of 26 (range 11–50) in five patients in the “low” tumor mass group (Fig. 1B). This difference although suggestive of a higher LI for the “low” tumor mass group after tumor reduction, was not statistically significant.

Sequential determinations of the LI in eight individual patients (Table 3) indicated an inverse relationship between the degree of tumor mass reduction and the LI. All five patients with reductions in myeloma protein production rate showed increments in the LI, while two patients with progressive disease demonstrated decrements in the LI. No change occurred in the LI of one patient who had no reduction in tumor mass.

DISCUSSION

The labelling index of plasma cells in patients with myeloma was evaluated in order to clarify the effect of chemotherapy on plasma cell tumor kinetics in man. Plasma cell tumors were considered to provide a model for neoplasms with low growth rate. The fraction of proliferating neoplastic plasma cells was evaluated by assessing the proportion of cells undergoing DNA synthesis as indicated by the incorporation of tritiated thymidine after short in vitro exposures. Adequate samples of plasma cells were procured from bone marrow

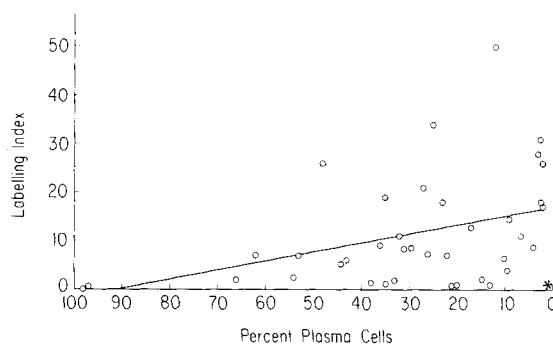


FIG. 2. The LI of bone marrow plasma cells from treated patients correlated with the plasma cell differential count. The large asterisk (*) represents the plasma cell LI of six patients with a normal bone marrow.

aspirates in a large number of patients with different degrees of tumor mass change. The reproducibility of this technique was confirmed in individual patients by assessing simultaneous marrow aspirates from different parts of the skeleton. Previous studies had confirmed the superiority of evaluating plasma cell differential counts in marrow suspensions rather than in direct smears. In each patient, the LI was correlated both with the magnitude of tumor mass change assessed from changes in myeloma protein production rate and the plasma cell differential count of bone marrow suspensions. Other studies have demonstrated the utility of evaluating changes in tumor mass from changes in myeloma protein production rate.^{2,22}

The nuclei of multinucleated cells were either all labelled or unlabelled, suggesting synchronized transit by all nuclei through the cell cycle. The proportion of plasma cells labelled with tritiated thymidine was much higher for multinucleated cells than for mononuclear cells. Apparently, a larger proportion of multinucleated cells was proliferating. In

TABLE 3. Sequential LI in Patients with Multiple Myeloma

Tumor mass	Initial study		Tumor mass	Time interval between studies (months)	Second study	
	Patient	LI			LI	Tumor mass
Decreasing	M.R.M.	4.0	100%	5	6.4	50%
	M.A.M.	1.0	51%	3	7	41%
	D.R.R.	6.0	100%	1	8.4	81%
	A.S.T.	2.4	100%	2	5.8	51%
	J.G.S.	0.8	100%	2	15	46%
Unchanged	C.C.V.	1.4	100%	1	1.2	100%
Increasing	G.P.L.	26	18%	8	11	39%
	J.T.G.	2	35%	16	1.6	49%

vitro studies on immunoglobulin-producing cells suggest that multinucleated cells may arise from the fusion of separate mononuclear cells.¹⁰ The higher frequency of proliferating multinuclear cells could be explained by the transfer of information initiating DNA synthesis from a proliferating cell to a non-cycling one at the moment of cell fusion. Alternatively, multinucleated cells arising from endomitosis may have a greater ability to remain in cycle than mononuclear cells.

Plasma cells in the blood of two patients with plasma cell leukemia had a LI less than 1%. These findings differ from those reported by Salmon et al.²¹ in one patient with IgE plasma cell leukemia who had a peripheral blood plasma cell LI of 40, but are similar to the low values for peripheral blood LIs reported in patients with other leukemias.^{6,19} Our data indicate that the plasma cells present in the peripheral blood of our patients with plasma cell leukemia were either terminal elements incapable of further proliferation or resting cells in a G_0 phase of their cycle.

The LI of normal bone marrow plasma cells from patients with malignancies other than multiple myeloma was always less than 2. This demonstrates that most normal plasma cells are either resting (G_0 pool) or in a terminal stage incapable of further proliferation.

The fraction of proliferating plasma cells was low in untreated patients with multiple myeloma. Our median LI of 2.4 recorded before the institution of chemotherapy was similar to results of Killman¹² and to those reported by Salmon.²³ These findings support the concept that progressive tumor growth is associated with a steadily declining proliferating fraction. Following reductions in tumor mass to less than 50% of the pretreatment level with chemotherapy, the LI increased to a median of 18.5. Our finding of a higher LI following tumor reduction is similar to that reported by Salmon in patients with multiple myeloma,²³ and conforms with similar findings in patients with acute leukemia.^{15,19} The wide variability in measured LI for a specific degree of tumor mass reduction could be attributed to the marked differences among our patients in absolute cell numbers before the institution of chemotherapy. This was supported by our observations that patients with the most extensive disease before treatment had a lower LI, both before and after tumor reduction than other patients with a similar degree of tumor reduction but with less disease involvement.

The inverse relationship between LI and tumor mass was further supported by serial studies in eight patients. Our observations imply that a reduction in myeloma tumor mass by alkylating agents shifts large numbers of resting plasma cells into a proliferative pool and that the magnitude of this conversion depends both on the degree of tumor reduction and the initial tumor mass.

Kinetic studies have shown that, in most tumors, a large fraction of viable neoplastic cells proliferates very slowly.^{18,24,30} As tumor growth progresses, the fraction of slowly proliferating cells increases, the proportion of cells initiating DNA synthesis per unit time decreases, and the rate of growth declines. Thus, tumors expand in an exponentially retarded exponential fashion, best described by a Gompertzian curve.¹³ The short-term behavior of untreated tumors is determined by the cell cycle time, the length of the phases of the cycle, the growth fraction, and the cell loss factor.²⁷ When a single course of treatment is given, cycling cells are particularly sensitive.^{15,20,31} Following the death of cycling cells, some nonproliferating cells return to the proliferative pool and become vulnerable to the next treatment.^{4,16,17} Thus, the long-term outcome of treatment depends upon the number of tumor cells eliminated by each course of therapy, the fraction of noncycling cells which return to the proliferative pool, and the viability and drug sensitivity of these cells.

Similar considerations apply to plasma cell myeloma. Griswold et al. have demonstrated,¹¹ in experimental hamster plasmacytomas, that long-term remissions occur when a cell cycle-active agent is given after treatment with alkylating agents, but not before. The major factor in accounting for the effectiveness of such a drug (arabinosylcytosine) is the increased growth fraction following the initial course of alkylating agents. Our studies on the growth kinetics of human plasma cell tumors confirm substantial increments in the proportion of proliferating cells after tumor reduction with alkylating agent chemotherapy. These observations justify the systematic evaluation of cell cycle-active drugs in the treatment of patients with plasma cell tumors after remissions have been induced with alkylating agent combinations. Since superior degrees of remission are associated with longer remission and survival times,² this approach may improve substantially the prognosis for a large fraction of patients with multiple myeloma.

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