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Research Article

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Kinetics of Tumor Growth and Regression in IgG Multiple Myeloma

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A B S T R A C T Studies of immunoglobulin synthesis, total body tumor cell number, and tumor kinetics were carried out in a series of patients with IgG multiple myeloma. The changes in tumor size associated with tumor growth or with regression were underestimated when the concentration of serum M-component was used as the sole index of tumor mass. Calculation of the total body M-component synthetic rate (corrected for concentration-dependent changes in IgG metabolism) and tumor cell number gave a more accurate and predictable estimate of changes in tumor size. Tumor growth and drug-induced tumor regression were found to follow Gompertzian kinetics, with progressive retardation of the rate of change of tumor size in both of these circumstances. This retardation effect, describable with a constant α, may be caused by a shift in the proportion of tumor cells in the proliferative cycle. Drug sensitivity of the tumor could be described quantitatively with a calculation of B_o, the tumor's initial sensitivity to a given drug regimen. Of particular clinical significance, the magnitude of a given patient's tumor regression could be predicted from the ratio of B_o to α. Mathematical proof was obtained that the retardation constant determined during tumor regression also applied to the earlier period of tumor growth, and this constant was used to reconstruct the preclinical history of disease. In the average patient, fewer than 5 yr elapse from the initial tumor cell doubling to its clinical presentation with from 10^{11} to more than 10^{12} myeloma cells in the body. The reduction in total body tumor mass in most patients responding to therapy ranges from less than one to almost two orders of magnitude. Application of predictive kinetic analysis to the design of sequential drug regimens may lead to further improvement in the treatment of multiple myeloma and other tumors with similar growth characteristics.

INTRODUCTION

Kinetic analysis of growth of disseminated malignancies in man is a subject of major importance, both for understanding the pathophysiology of cancer and for planning optimal therapy. Convincing evidence of the therapeutic ramifications of such kinetic analysis is found in the series of dramatic demonstrations with various animal tumors; knowledge of the cell population and cell-cycle kinetics of certain tumors has provided the necessary foundation for designing curative chemotherapy (1–4). Animal tumors, which arise from—and can be transplanted with—a single neoplastic cell, can be assessed serially, using measurements of tumor mass; by further transplantation studies, the population and cellular kinetics of viable tumor stem cells can be studied during growth and during drug-induced regression of the neoplasm (5).

Although measurements of tumor size can sometimes be obtained in man, transplantation studies cannot be done, and other methods of serially estimating the number of viable tumor cells must be found. Multiple

myeloma may represent an "ideal" disseminated neoplasm for kinetic study, as the population of tumor cells can be readily sampled, the cells almost always produce a marker immunoglobulin (M-component)[1] whose metabolism has been well characterized, and the tumor appears to arise from a single neoplastic cell clone.

Previous studies from this laboratory (6, 7) established that measurements of synthesis of M-component in vivo and in vitro could be used to determine the total number of tumor cells present in patients with multiple myeloma. In those patients, clinical manifestations of disease did not become apparent until more than 10^{11} myeloma cells were present in the body. Our present investigations were designed to provide a firm mathematical basis for the application of predictive kinetic analysis to the design and evaluation of treatment of multiple myeloma, and to provide new insights into its natural history.

METHODS

Patient studies. Patients with well-documented IgG multiple myeloma (subclasses IgG_1 and IgG_2) were selected for study. Clinical and immunologic criteria used for diagnosis and staging of these patients were detailed previously, as were data on metabolism in vivo of [^{125}I-labeled]IgG and on total body tumor cell number in 10 of these patients (6).

Bone marrow aspirates were collected in heparin; short-term tissue cultures were prepared for studies of cellular synthesis of myeloma protein (IgG_{mp}) (8). Newly synthesized IgG was measured with the "sandwich" radioimmunoassay technique (9). Cellulose acetate electrophoresis and densitometry[2] were performed at least monthly. The serum concentration of IgG_{mp} was determined by careful analysis of the electrophoretic patterns. Plasma volume was measured by the Nuclear Medicine division of this institution every 3 months with the [^{125}I-labeled] albumin technique. The total body synthetic rate of IgG_{mp} was calculated from the concentration of IgG_{mp} in serum, the plasma volume, and the body weight as described below.

In addition to the cases studied at this institution, records on 19 patients treated in cooperating institutions of the Southwest Cancer Chemotherapy Study Group (SWCCSG) were also analyzed. Plasma volumes for these patients were estimated on the basis of height and weight, using the technique of Fairbanks and Tauxe (10). Storage of patient records and computations of metabolic turnover data, IgG_{mp} synthetic rates and total body tumor cell number, as well as curve fitting and tumor kinetic studies, were carried out with a General Electric 260 digital computer via the GE Mark I Time Sharing Service (General Electric Co., Information Services Business Div., Schenectady, N. Y.). Remission of myeloma was defined as at least halving of the total body tumor cell number, and significant regression was defined as an approximate 1-log reduction in the total number of tumor cells in the body.

Calculation of the total body IgG synthetic rate and tumor cell number. The total body synthetic rate for IgG_{mp} was calcu-

lated with the equation for the fractional catabolic rate of IgG developed by Waldmann and Strober (11). A detailed description of their derivation and its assumptions appears in their work. Their equation is:

$$f = a - \frac{D}{Vc + mD} \tag{1}$$

where: f = fractional catabolic rate, a = the fraction of the plasma pool isolated per day (the fraction of the intravascular pool catabolized per day at infinite IgG concentration), D = the number of molecules protected from catabolism per unit time at full saturation, Vc = the total circulating IgG pool (the product of the serum IgG concentration and the plasma volume), and m = the slope of a plot of the reciprocal of the milligrams of IgG protected $\left(\frac{1}{Vc(a-f)}\right)$ against the reciprocal of the plasma pool of IgG, $\frac{1}{Vc}$.

From our studies in man, we have estimated 0.16 as the value for a and, as did Waldmann and Strober (11), we used 147 mg of IgG/kg per day as the value for D. In the steady state, the total IgG catabolized by the body ($f \cdot Vc$) equals the total IgG synthesized by the body. It should be noted that f is governed by the total serum IgG concentration, not just the concentration of the M-component. As a result of the severe deficiency in normal immunoglobulins which occurs in myeloma, the concentration of M-component alone is usually satisfactory; in the presence of a significant amount of normal IgG in addition to the IgG_{mp}, total IgG must be measured, and the calculated f must be used to determine the turnover rate of the M-component.

As described previously (6), IgG myeloma cell number can then be derived if the average rate of IgG synthesis per myeloma cell is measured. Thus:

Total body myeloma cell number
$$\frac{\text{Total body myeloma cell number}}{\text{Number of myeloma cells in vitro}}$$
$$= \frac{\text{Rate of total body myeloma IgG synthesis in vivo}}{\text{Rate of total myeloma IgG synthesis in vitro}}$$

Correcting to the average IgG synthetic rate per myeloma cell,

$$N = \frac{f \cdot Vc}{r} \tag{2}$$

where: N = total number of myeloma cells in the body, r = rate of IgG synthesis per myeloma cell per day, and Vc and f are as defined above.

A first order differential equation was developed to provide a more general solution for myeloma cell number in the non-steady state (during tumor growth or response):

Change = Production − Destruction

$$\frac{d}{dt} Vc = (r \cdot N) - (f \cdot Vc).$$

Solving for tumor cell number ·

$$N = \frac{\left(f \cdot Vc + \frac{d}{dt} Vc\right)}{r} \tag{3}$$

where: $\frac{d}{dt}$ = time rate of change.

Clinical data including serum IgG_{mp}, plasma volume, and body weight were used to calculate f from equation 1, and the value for f was substituted into equation 3 for determination of the total number of myeloma cells in the body. The value for r was determined for each of our cases by in vitro measurement of IgG synthesis on fresh marrow aspirates (6, 8). Measurements of r were not carried out for the cases provided by the SWCCSG, and assumed reasonable values for r were used in the analysis of those cases. Solution of equation 3 with the approximation, $(d/dt)Vc \cong \Delta Vc/\Delta t$, yielded curves of the log of tumor cell number vs. time such as those seen in Fig. 1.

THEORETICAL BACKGROUND

The curves (in Fig. 1) demonstrate that neither tumor growth nor response to treatment was exponential, but rather that the rate of these processes appears to be continuously changing. As extensively formulated first by Laird, embryonic tissues (12) and a variety of animal tumors (13, 14) have a similar growth pattern which can be described by a Gompertzian function. Subsequent work at the Southern Research Institute has extended this concept (4, 15, 16).

Growth, which occurs in Gompertzian fashion, could be due to any of the following situations: (a) a decreasing proliferative fraction with an increasing number of resting cells (G_o), an increasing number of nonclonogenic cells, or both; (b) an increase in the mean cell-cycle time; or (c) a continuous increase in the fractional cell loss. Griswold, Simpson-Herren, and Schabel (17), working with the metastatic plasmacytoma of the hamster (PLA-1), have shown that the slowing of tumor growth is due primarily to an increasingly large fraction of the cells in a nonproliferative state, with a possible increase in the fraction of cells lost. Measurements of the cell-cycle time for the hamster plasmacytoma by Griswold et al. (17) and for human myeloma by Killmann, Cronkite, Fliedner, and Bond (18) have yielded quantitatively similar results.

Characteristics of the Gompertz equation. Gompertz first developed his equation for actuarial purposes (19). Rewritten in terms of tumor cell number (12), we have:

$$N = N_o e^{(A_o/\alpha)(1-e^{-\alpha t})} \qquad (4)$$

where: N = the dependent variable, t = the independent variable, and N_o, A_o, and α = constants for any particular system. Equation 4 is also the solution of the differential equations:

$$\frac{dN}{dt} = \gamma N$$
$$\frac{d\gamma}{dt} = -\alpha\gamma \qquad (5)$$

where: $\gamma = Ae^{-\alpha t}$. Equation 5 describes a system of exponentially increasing or decreasing N where the rate of exponential change is continuously decreasing at the constant rate α. When $t = 0$, $N = N_o$; and when
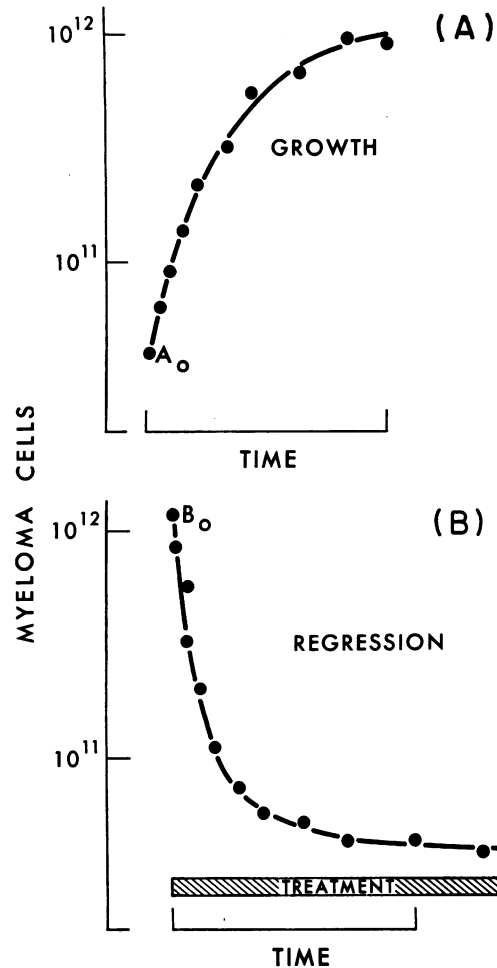

FIGURE 1 Log of tumor cell number vs. linear plot of time in an untreated case (A) and a treated case (B). Neither growth nor regression is linear.

$t = \infty$, $N = N_o e^{A_o/\alpha}$. Equation 4 may be rewritten as:

$$\frac{1}{N} \cdot \frac{dN}{dt} = A_o e^{-\alpha t}$$

and $\ln \left(\frac{1}{N} \cdot \frac{dN}{dt} \right) = \ln A_o - \alpha t$.

The term $\left(\frac{1}{N} \cdot \frac{dN}{dt} \right)$ is referred to as the *specific growth rate* and is estimated from data as $\frac{1}{N} \cdot \frac{\Delta N}{\Delta t}$. When α is constant this equation yields a straight line with slope of $-\alpha$ and y intercept at $\ln A_o$ (Fig. 2), and the Gompertz equation provides a workable model of the tumor's growth. When a tumor yields data that approximate a straight line on this graph, more precise curve-fitting techniques (Appendix I) are used. Apply-
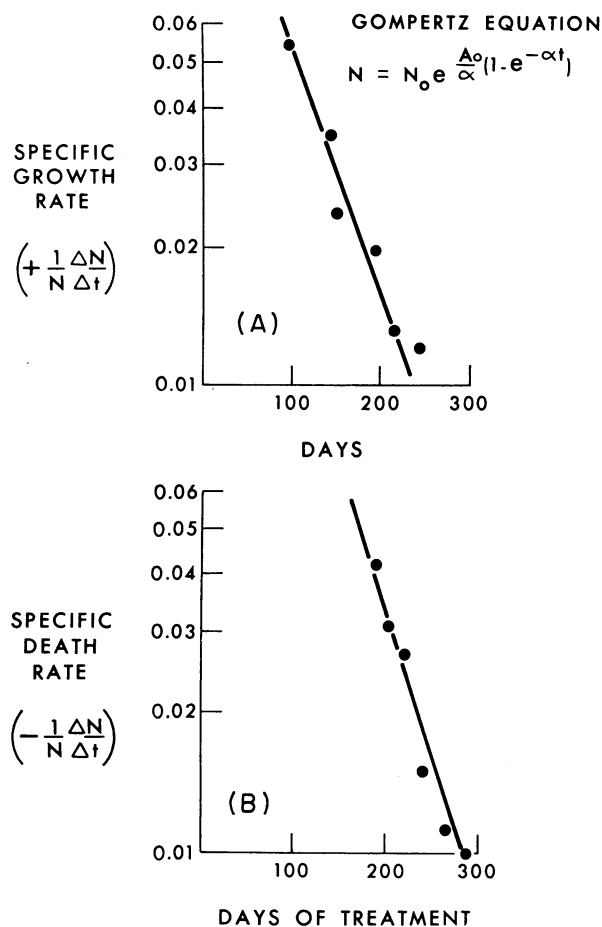
GOMPERTZ EQUATION

$$N = N_o e^{\frac{A_o}{\alpha}(1-e^{-\alpha t})}$$

SPECIFIC
GROWTH
RATE

$\left(+\dfrac{1}{N}\dfrac{\Delta N}{\Delta t}\right)$

(A)

DAYS

SPECIFIC
DEATH
RATE

$\left(-\dfrac{1}{N}\dfrac{\Delta N}{\Delta t}\right)$

(B)

DAYS OF TREATMENT

FIGURE 2 (A) Derivation of the instantaneous growth rate (A_i) and α for myeloma cells from serial measurements of the specific growth rate during the period of observation in an untreated myeloma patient. A_o, the instantaneous growth rate at the time of first observation, is the intercept of the line at time 0, while α is the slope. (B) Use of clinical data obtained during a period of consistent drug treatment for approximation of α and the instantaneous regression rate (B_o) for patient W. W. The specific death rate of tumor cells is a term comparable with the specific growth rate shown in (A), but is negative in sign. α is the slope of the line on this plot, while B_o, considered to reflect the sensitivity of the tumor to the drug, is the intercept of the line at time 0. Plots such as those shown in (A) and (B) are screening techniques to determine whether Gompertzian conditions prevail. When they do, and a straight-line relationship such as those shown here is seen, the more precise curve fitting computer relationships (Appendix I) are used.

ing the Gompertz equation to tumor regression (see below and Appendix II), a new comparable term— *specific death rate* $\left(-\dfrac{1}{N}\cdot\dfrac{dN}{dt}\right)$—yields the same linear relationship but with a negative A_o, which we define as B_o. B_o is thus the instantaneous regression rate at the start of treatment and is an index of drug sensitivity of the tumor.

Some of the implications of Gompertzian growth are detailed by Laird, Tyler, and Barton (20), including the following useful concepts: (a) The time axis may be easily shifted T units, permitting a new equation to be written,

$$N = N_1 e^{(A_1/\alpha)(1-e^{-\alpha t})}$$

where: $A_1 = A_o e^{-\alpha T}$, $N = N_1$ when $t = 0$, and $N = N_1 e^{A_1/\alpha}$ when $t = \infty$; (b) the instantaneous rate of growth at any t_i is A_i, where

$$A_i = A_o e^{-\alpha t_i}$$

(A_i is the slope of the line tangent to the growth curve at t_i); (c) the theoretical time required to grow from one cell to N_o cells is

$$T = \frac{\ln\left[1-\left(\dfrac{\alpha}{A_o}\right)\ln N_o\right]}{-\alpha};$$

(d) the theoretical doubling time (generation time, t_g) from one cell to two cells is

$$t_g = \frac{\ln 2}{A_s}$$

where $A_s = A_o e^{\alpha T}$; and (e) the theoretical proliferative fraction (p) at t_i, if t_g is assumed to be constant, is

$$p_i = A_i \cdot t_g \%.$$

Application of the Gompertz equation to tumor regression data and derivation of α for tumor growth. As developed by Dr. Hugo M. Martinez of this institution and detailed in Appendix II, mathematical derivation shows that the retardation constant α applies to both tumor regression and tumor growth. Thus, in circumstances where a consistent treatment program was used for a given myeloma patient, it was possible to derive the retardation constant necessary to describe the dynamics of the disease. With certain assumptions, stated below, the derived α could also be used for reconstruction of the subclinical growth phase of the tumor clone from its initial doubling.

Estimation of the initial growth rate (A_s). Values for A_s, the instantaneous growth rate at the time of the tumor's initial doubling (start of growth), cannot be derived from tumor regression data. Its approximation can be made only by analysis of progressive tumor growth in untreated cases, or by addition of several constraints to the Gompertz function which are based on generally accepted survival data. The constraints added for this purpose were an average survival time from diagnosis in untreated or drug-resistant cases of less than 24 months (median 18 months) (21), a

threshold tumor cell number at diagnosis of at least 2.5×10^{11} myeloma cells in the body (6) and an average tumor doubling time of 4–6 months during the clinical phase of disease (6, 22, 23). These three constraints, plus a value for α, provide all the data necessary for a solution of the Gompertz equation.

RESULTS

Cellular immunoglobulin synthetic rates. Measurements of the cellular IgG synthetic rate in vitro were made in a total of 36 patients with IgG multiple myeloma. 22 of these patients have been studied serially during their clinical course, and a total of 67 studies (range, 2–8 per patient) have been performed. Synthetic rates were determined at the time of presentation and during subsequent tumor regression or remission. Bone marrow specimens for culture were obtained when the patients were receiving no therapy (e.g., before treatment was begun; off treatment; or immediately before the beginning of an intermittent course of melphalan, prednisone, or both) or while they were receiving treatment with low daily doses of melphalan. Synthetic rates among these patients ranged from 1.5 to 34 pg/myeloma cell per day (mean, 14.5 ± 9.6). The cellular IgG synthetic rates have remained constant $\pm10\%$ for as long as 3 yr for all patients studied serially under these circumstances, whether or not the patient was receiving therapy and independent of the number of tumor cells in the body. Additional studies were performed during 4 day, high-dose, "pulse" chemotherapy; however, these data are not included as they gave disparate results, probably because of the acute effects of intensive therapy. None of the in vitro studies reported here was carried out during relapse on continued chemotherapy.

Comparison of predicted and measured IgG metabolic parameters. Data on 10 patients on whom metabolic turnover studies had been done were processed with the computer program to calculate the fractional catabolic rate (f) by means of the metabolic equation. Table I summarizes this comparison; the metabolic equation provides an excellent approximation of the f measured for IgG by metabolic turnover study. The average deviation from the predicted value was 8% ($\pm3.9\%$ SEM). Predicted plasma volumes for these 10 patients (based on height, weight, and hematocrit) also agreed well with the measured plasma volumes. One patient (E. M.) had an initial plasma volume more than 25% greater than predicted. However, it is important to note that the expanded plasma volume in this patient contracted during chemotherapy even though there was no significant change in hematocrit.

Relationship of concentration-dependent changes in IgG metabolism to tumor regression. Data were analyzed on 15 SWCCSG patients with IgG myeloma, who were

TABLE I
Comparison of Predicted and Measured IgG Fractional Catabolic Rate (f) in 10 Patients with IgG Multiple Myeloma

Patient	f		Difference
	Predicted	Measured*	
	%	%	%
L. M.	9.0	9.3	0.3
J. H.	9.1	9.6	0.5
J. U.	13.7	10.9	2.8
A. V.	12.2	12.0	0.2
R. T.	12.7	12.1	0.6
R. W.	10.8	12.9	2.1
M. P.	12.1	11.9	0.2
W. W.	12.1	12.5	0.4
L. F.	11.8	13.8	2.0
D. R.	12.5	12.2	0.3

* Data previously reported (6).

considered to be "nonresponders" on the basis of less than a 50% reduction in the concentration of M-component. Eight of these patients had an actual reduction in synthetic rate that was greater than 50% (mean 53%), even though electrophoretic data had suggested a significantly lesser degree of response to treatment (mean 39%). Seven of these eight patients who were reclassified as responders had initial concentrations of M-component of 3.7 g/100 ml or less, thus falling into that portion of the curve of IgG catabolism most affected by changes in IgG concentration (11). Survival data on these patients who were reclassified as partial responders showed them to have a significantly longer mean and median survival than those patients who had less than a 50% reduction in the M-component synthetic rate. For the entire group of 15 patients, the mean calculated reduction in the total body IgG synthetic rate was $34.9\pm4.8\%$ greater than the change in the serum concentration of M-component. Data on the patients analyzed in Table II showed a similar phenomenon and demonstrated that uncorrected electrophoretic measurements underestimate the magnitude of tumor response to chemotherapy.

Analysis of tumor regression. Studies of measured and calculated immunoglobulin synthetic rate and tumor cell number (Table II), and regression and growth kinetics (Table III) were carried out in 11 patients. These 11 patients were selected because (*a*) they had manifested objective response to treatment, (*b*) serial electrophoretic patterns and detailed clinical data were available for analysis, and (*c*) each had received consistent patterns of treatment with minimal deviation in drug dosage or schedule. Serial measurements of IgG synthetic rate were done on the first seven patients and plasma volumes were measured with [^{125}I-labeled]

TABLE II

Calculations of Immunoglobulin Synthesis, Tumor Cell Number, and Tumor Kinetics in Patients with IgG Multiple Myeloma

Patient	Sex	Age	Body weight	Light chain type	Treatment	Serum IgG$_{mp}$		Total body IgG$_{mp}$ synthesis		Cellular IgG synthesis	Total number of myeloma cells		Total body tumor regression	Survival from onset of therapy
						Initial	Plateau	Initial	Plateau		Initial	Plateau		
		yr	*kg*			*g/100 ml*		*g/24 hr*		*pg/cell per 24 hr*	*cells × 10^{12}*		*%*	*days*
L. M.	F	54	53	λ	Melphalan 0.05 mg/kg per day	2.8	1.3	5.6	2.9	12.0	0.77	0.23	70.0	1175
J. H.	M	58	56	κ	Melphalan 1 mg/kg per 4 wk*	2.7	0.1	5.7	0.1	11.0	0.55	0.01	98.2	560+
W. W.	M	67	50	κ	Melphalan 0.05 mg/kg per day Prednisone 80 mg/48 hr‡	6.5	0.7	17.1	0.7	6.0	2.70	0.12	95.6	1045+
E. M.	M	73	103	κ	Melphalan 1 mg/kg per 6 wk* Prednisone 80 mg/48 hr‡	8.0	3.8	68.6	22.7	23.7	2.70	0.96	64.4	570+
D. M.	F	67	79	λ	Melphalan 1 mg/kg per 6 wk* Prednisone 10 mg/kg per 6 wk*	3.1	1.4	11.9	4.0	17.5	0.68	0.22	67.1	220+
H. J.	F	80	92	λ	Melphalan 1 mg/kg per 6 wk* Prednisone 10 mg/kg per 6 wk*	2.5	1.0	9.5	3.1	7.9	1.10	0.49	55.5	557+
L. W.	F	53	50	κ	Melphalan 0.05 mg/kg per day Prednisone 80 mg/48 hr‡	7.0	1.7	17.3	2.4	13.0	1.20	0.20	83.5	1470+
N. S.	F	52	55	κ	Melphalan 1 mg/kg per 6 wk*	9.6	1.7	33.3	3.7	22.0§	1.40	0.17	87.9	750
S. A.	F	70	43	κ	Melphalan 1 mg/kg per 6 wk*	4.4	0.7	9.3	0.8	8.0§	0.92	0.10	89.1	1550
T. D.	F	68	70	κ	Melphalan 1 mg/kg per 6 wk* Prednisone 80 mg 3 X/wk	3.8	1.0	10.4	1.7	6.0§	1.70	0.29	82.9	1040
K. L.	F	76	65	κ	Melphalan 1 mg/kg per 6 wk* Prednisone 50 mg 3 X/wk	2.7	0.4‖	5.0	0.4‖	6.0§	0.83	0.03	96.4‖	1095

* Given in divided doses over 4 consecutive days; repeated at time interval specified.
‡ Given as a single dose at time interval specified.
§ Cellular IgG synthetic rate was estimated from the patient's serum concentration of IgG$_{mp}$, body weight, and radiographic extent of disease (estimates based on matched comparisons with more than 40 patients in whom these measurements were made).
‖ M-component peak no longer detectable by electrophoresis; value shown was calculated by Gompertzian analysis of regression curve.

TABLE III
Calculated Gompertz Constants and Growth Parameters in Patients with IgG Multiple Myeloma

Patient	α	Instantaneous regression rate (B_o)	Initial growth rate (A_s)	Myeloma cell generation time	Instantaneous growth rate at 2.5×10^{11} cells	Duration of subclinical disease to 2.5×10^{11} cells
				days		est. days
L. M.	0.011	−0.013	0.313	2.20	0.030	217
J. H.	0.010	−0.040	0.287	2.41	0.027	237
W. W.	0.015	−0.047	0.395	1.55	0.038	153
E. M.	0.018	−0.020	0.513	1.35	0.049	133
D. M.	0.026	−0.028	0.751	0.92	0.072	91
H. J.	0.010	−0.008	0.296	2.34	0.028	230
L. W.	0.013	−0.023	0.368	1.88	0.035	184
N. S.	0.013	−0.028	0.380	1.82	0.036	179
S. A.	0.004	−0.009	0.117	5.94	0.012	575
T. D.	0.022	−0.039	0.624	1.11	0.060	109
K. L.	0.007	−0.024	0.195	3.56	0.019	349

albumin. In four patients, data were supplied by the SWCCSG and fixed cellular IgG synthetic rates and plasma volumes were estimated with the techniques described above.

Kinetics of tumor regression. A characteristic mode of tumor regression is observed when myeloma patients are treated with alkylating agents alone or combined with prednisone. The tumor regresses rapidly at first, then slows and reaches a plateau size which is stable for a period of months or years. The rapidity with which myeloma cell number initially decreased with treatment was dependent upon B_o, the sensitivity of the tumor to drug. With a large B_o, the initial rate of tumor regression was rapid. This effect occurred in three patients (J. H., W. W., T. D.); each had a B_o of −0.039 or greater. In these patients, the tumor cell number halved in less than 30 days.

The retardation constants α for these 11 patients were all between 0.004 and 0.022, suggesting a narrow range for this constant for myeloma cells. The total degree of tumor regression is not dependent solely upon either B_o or α but, rather, upon their ratio. Optimal tumor response thus occurs when B_o is large and α is small; that is, when the ratio of these two is large. It follows from the Gompertz equation that a ratio of B_o:α of greater than 2.4 predicts a tumor regression of greater than 1 log. Regressions which approximated this magnitude were induced in 5 of the 11 patients studied (87.9–98.2% regression). Three of the five patients who had significant tumor regressions (N. S., S. A., K. L.) had tumors less sensitive to drug than the three patients discussed above (B_o values of −0.009 to −0.028), but their values for α were also extremely small. The predictive capability of kinetic analysis is illustrated in Fig. 3, wherein final measured and predicted tumor cell numbers are shown for patient W. W. In this instance, the plateau of tumor during treatment was predicted many months in advance.

Calculated theoretical growth characteristics of myeloma. Using the measured values for α and the constraints to the Gompertz equation indicated by clinical observations (see above), the initial growth rate (A_s), myeloma cell generation time (t_g), proliferative fraction at
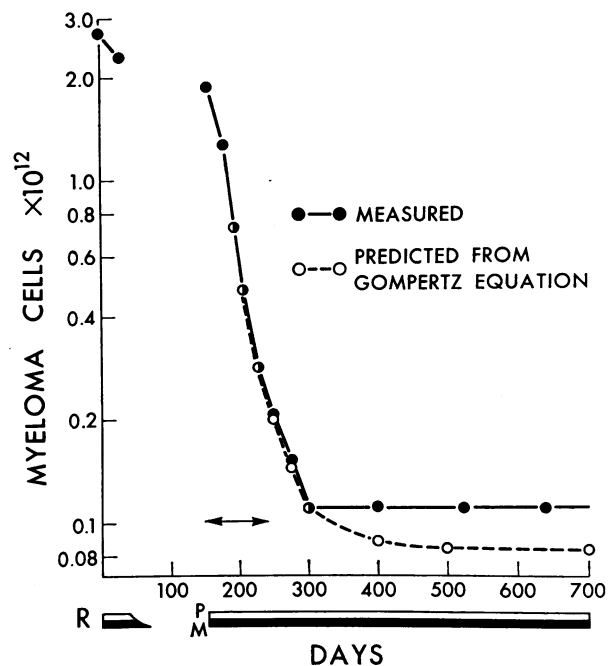

FIGURE 3 Measured and predicted response to treatment in patient W. W. who had IgG multiple myeloma. The patient's course of treatment with low-dose melphalan and prednisone is shown. The initial IgGmp concentration was 6.5 g; the final, 0.7 g/100 ml. Data points obtained in the period designated with the horizontal arrow could be used to predict the subsequent plateau of the patient's tumor. At the low concentrations of M-component observed at the time of remission, the computer estimation of cell number may be more accurate than that derived from electrophoretic data.

2.5 × 10^{11} cells ($p_{2.5×10^{11}}$), and subclinical duration of disease were calculated (Table III). Myeloma cell generation time varied from 22 to 142 hr (mean, 55 hr). The calculated fraction of cells in the proliferative compartment at the threshold of clinical detectability ranged from 5.9 to 7.1%. The tumor appears to have a subclinical period of growth of approximately 100 times the value for t_g before a clinical threshold of 2.5 × 10^{11} cells is reached. For the patients studied, this period ranged from 91 to 575 days. Since only 38 doublings are required to reach this threshold from a single malignant cell, the degree of retardation of tumor growth is readily apparent, as exponential growth of tumors with similar values for t_g would result in a much shorter subclinical phase of disease.

DISCUSSION

Unlike the techniques of measurement of tumor volume which have been widely applied in previous studies of animal and human tumors, derivation of serial tumor mass from measurements of immunoglobulin synthesis is not subject to the difficulties of measuring tumor nodules which may contain variable but significant numbers of nonviable cells, normal tissue elements, and interstitial fluid. Our studies do entail a concept of cell death which is somewhat different from that generally used by the radiobiologist, who defines death as the loss of a cell's capability to serve as a stem cell (i.e., it becomes sterile, or non-clonogenic) (24). In our kinetic studies, myeloma cells that we call dead on the basis of their loss of capability to synthesize an M-component are most likely "dead and gone," rather than simply nonclonogenic. Substantiation of this impression was recently provided when we analyzed electrophoretic data obtained by Bergsagel on a patient whom he treated with total body irradiation (25). In this patient, the half-life of irradiated myeloma cells was at least 2 wk, during which time they continued to secrete the M-component.

Recognition that changes in IgG metabolism, which occur as a concentration-dependent phenomenon, could lead to significant underestimation of the degree of change in total body M-component synthesis (and tumor mass) prompted us to develop a technique to correct for these metabolic effects. IgG clones account for slightly more than half of all cases of myeloma (26–28); errors in interpretation of response in IgG myeloma could significantly alter response data for myeloma in general. Analysis of so-called "nonresponders" in the SWCCSG experience indicated that some of these patients had actually had a significant (50%) reduction in the amount of tumor and that this degree of response was associated with prolongation of survival. Such findings indicate that we may be underestimating the effects of conventional treatment and,

perhaps, abandoning it prematurely (especially in patients with an initial serum concentration of IgG of less than 3.5 g/100 ml).

Study of the regression and growth of the neoplasm by means of serial estimates of the total number of tumor cells in the body unearthed a number of features of tumor kinetics which may have major significance. Use of the Gompertz equation to analyze growth of the neoplasm in a responding patient whose treatment was stopped (because of marrow toxicity rather than drug resistance) indicated that, in this patient, tumor growth during the period of relapse was Gompertzian and had a very clear plateauing phenomenon. Of particular interest was the unexpected observation that the curves of tumor regression on continuous therapy and of regrowth on its cessation had similar retardation (Fig. 4). Deductive analysis, which proved that an α determined during tumor regression also applied to the earlier period of tumor growth, confirmed the validity of use of regression data to determine growth characteristics (see Appendix II).

This approach broadens the applicability of growth analysis in human tumors and provides a more representative sample of patients than could ethically be obtained in untreated patients. Study of an untreated patient group would be prejudiced toward the selection of patients with slowly growing tumors because beneficial therapy cannot be justifiably withheld from patients with progressive myeloma (e.g., 29). Additionally, analysis of the process of tumor relapse during continued therapy may not yield information which is representative of the growth of the untreated or drug-responsive neoplasm; "subcloning phenomena," as described by Coffino, Laskov, and Scharff for mouse myeloma (30) and by Hobbs for myeloma in man (31), do appear to occur (we have observed this feature in several of our patients). The measurements of immunoglobulin synthesis in vitro reported here were obtained by sampling 10^7–10^8 bone marrow cells at the time of initial presentation and during regression and plateau phases of myeloma; we have not observed significant changes in the rate or type of cellular M-component synthesis. We recognize that subcloning does occur during relapse in at least 5% of cases, and serial measurements of M-component synthesis in vitro will be needed to characterize cellular function in the resistant phase of the disease. We are investigating this phenomenon but have, as yet, relatively few measurements. However, analysis of some electrophoretic data from patients in relapse suggests that a drug-resistant clone appearing during relapse may have a much smaller value for α.

Our interpretation of the cellular and population events which account for these growth and regression phenomena must be considered tentative; however, the

demonstration that the identical α applies to both circumstances strongly supports the concept that reciprocal changes are occurring in the size of the proliferating or growth fraction of the tumor. During the period of progressive growth of the tumor the proliferating fraction appears to decrease, while during the period of regression during treatment a larger fraction of the remaining tumor mass appears to shift into the proliferative compartment. This form of retarded regression could be anticipated whenever a treatment (e.g., an alkylating agent) produces a constant fractional kill of tumor cells. Such therapy could be considered as an additional environmental force favoring cell death; it would force the tumor to seek a new (and lower) population size, with the resulting regression and plateau in size representing a newly established balance between increased cell death due to treatment and increased tumor cell birth rate resulting from an expanded proliferative fraction. The plateau phenomenon during remission probably does not reflect the acquisition of drug resistance; a prolonged plateau phase (several years in some of our patients) could not be anticipated in a drug-resistant tumor—the tumor mass would increase progressively even with continued therapy.

The capability to predict the maximum degree of regression and time until plateau of tumor size for any form of therapy should be clinically important. If the plateauing phenomenon during therapy is caused by an expansion of the proliferative fraction of the tumor as the total population is reduced, or if the residual cells are mostly nonclonogenic, use of antimetabolites that attack the tumor stem cells during the proliferative cycle would be most rational after the tumor had first been reduced significantly in size by a noncycle-specific agent such as an alkylating agent. The plasmacytoma of the hamster, which also grows in Gompertzian fashion, appears to display precisely this phenomenon (17). It cannot be cured with a potent cycle-active agent (cytosine arabinoside) when the tumor is extensive, but it can be cured with the same drug if the total mass is first reduced by several logs with alkylating agents (reversal of this sequence is ineffective).

Our estimated generation times for myeloma cells are similar to those measured in man by Killmann et al. (18). Their tritiated thymidine-labeling studies in vivo showed that the proliferative fraction is very small at the clinical stage of disease. Analysis of our kinetic data on the theoretical proliferative fraction (Theoretical Background) suggests that, in most patients, approximately 6.5% of myeloma cells are in the proliferative fraction at the clinical threshold of 2.5×10^{11} tumor cells. Flash-labeling studies with tritiated thymidine in vitro suggest that the labeling index is extremely low (less than 3.5%) when measured at high total body
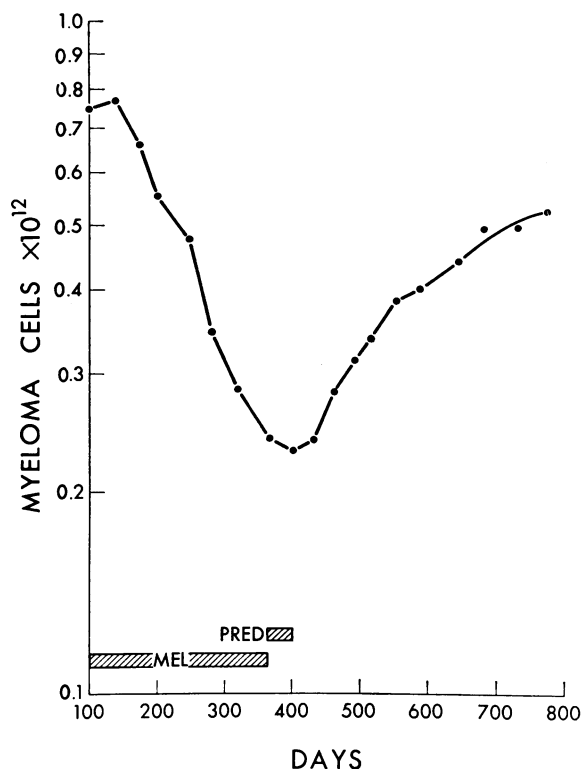

FIGURE 4 Log of tumor cell number vs. time in patient L. M., who had a drug-induced regression and subsequent relapse after cessation of therapy. The regression and relapse curves are not exponential.

densities of tumor cell number (7), although it remains to be established whether the labeling index increases significantly during remission. Study of the labeling index at low tumor cell density presents substantial problems due to the difficulties inherent in identifying as myeloma cells a small percentage of all marrow cells and in identifying an even smaller fraction of these as being labeled. However, the possibility of an expanded proliferative fraction during regression can be tested with the use of clinical trials of cycle-active agents in patients who have had very large tumor regressions. For evaluation of such a trial, an extremely sensitive indicator of the myeloma protein would be required once the M-component was no longer detectable by electrophoresis.

Reconstructing the natural history of myeloma, it appears that its duration is shorter than had previously been considered (6, 22). Our calculations on the subclinical phase of disease (up to 2.5×10^{11} cells) suggest that this initial period of tumor growth occurs in from less than 4 months to approximately 18 months. However, most patients are not recognized at this threshold point; diagnosis usually occurs after the tumor has undergone further but ever slowing growth for several
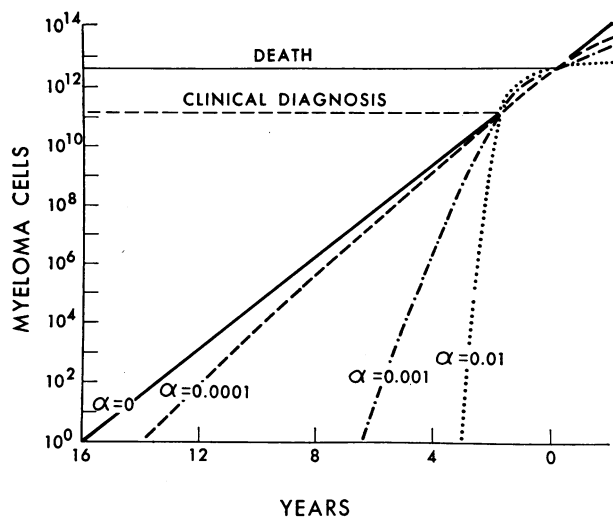
FIGURE 5 Hypothetical models of the natural history of untreated multiple myeloma. The curves plotted are those predicted by various values of α, assuming the clinical constraint that the tumor must double four times in 2 yr between diagnosis and death. Calculated values for α in the patients studied ranged between 0.004 and 0.022, suggesting a preclinical period of less than 5 yr.

additional years. Various models of myeloma can be constructed within the constraints dictated by the clinical phase of disease (Fig. 5). Our estimates of α in myeloma have all been in the range of 0.004–0.022, indicating that the tumor grows rapidly at the subclinical level and likely is manifest in most patients within 5 yr of its initial doubling. Assuming that so-called "benign monoclonal gammopathies" (32) are also subject to similar growth regulation, we would propose that such tumors may be quite similar to the malignant variant; however, such clones may undergo a greater degree of growth retardation or have a smaller initial growth rate, and consequently plateau at a level which does not lead to lethal complications.

A central enigma persists in identifying the biochemical basis for the retardation constant, α. One appealing possibility is that a feedback inhibition system may control the growth of both normal and neoplastic antibody-producing clones. Viewed in this context, myeloma cells would not appear to have the same degree of sensitivity to this inhibition as normal clones; however, their proliferation would also be inhibited progressively with increasing tumor cell number.

Should a feedback inhibitor produced by myeloma cells also affect normal antibody-producing clones, then one major clinical feature of myeloma may be clarified. An antibody-deficiency syndrome is a constant feature of this disorder; it appears to be due to a reduction in

the number of normal immunoglobulin-producing clones rather than a change in immunoglobulin catabolism (33–35). Extrapolating from our measurements in myeloma, we calculate that the mass of all antibody-producing clones in normal man is some 3×10^{11} cells (about 300 g). Based on our measurements, patients with myeloma have at least a 1 log reduction in the number of normal antibody-producing clones in the body. In the mouse, recent studies by Zolla (36) present strong evidence that myeloma cells do have depressive effects on normal immune cell proliferation. Her studies demonstrate that subcutaneously transplanted myelomas suppress the proliferation of antibody-producing cells progressively as they grow.

Investigations of the growth of myeloma may thus not only clarify the natural history of the disorder and provide an approach to predictive cancer chemotherapy, but may also provide some insight into the processes that regulate neoplastic and normal cell growth.

APPENDIX I.
CURVE–FITTING TECHNIQUES
FOR THE GOMPERTZ EQUATION

When a specific growth or death rate implies Gompertzian kinetics, a best-fit curve can be generated using the method developed by Simpson-Herren and Lloyd (16). A computer was programmed to evaluate N_o, A_o, and α from the experimental data by an iterative one-dimensional search on α.

If equation 4 is rewritten as

$$\ln N = \ln N_o + \frac{A_o}{\alpha}(1 - e^{-\alpha t}),$$

it may be expressed as

$$u = a_o + a_1 v$$

where:

$$u = \ln N$$

$$a_o = \ln N_o + \frac{A_o}{\alpha}$$

$$a_1 = -\frac{A_o}{\alpha}$$

$$v = e^{-\alpha t}.$$

This is a linear equation in u and v with slope a_1 and intercept a_o. The parameters a_1 and a_o are evaluated by the method of least squares for each estimate of α and the variance is calculated. The value of α is then systematically incremented or decremented until the variance about the straight line converges to a minimum. Upon convergence, the best α is known, and A_o and N_o are readily calculated:

$$A_o = - \alpha \cdot a_1$$
$$N_o = e^{(a_o + a_1)}.$$

The equation is then fully described.

APPENDIX II.
RESPONSE IN A GOMPERTZIAN SYSTEM OF A TUMOR TREATED WITH A CYCLE–NONSPECIFIC DRUG: MATHEMATICAL DERIVATION

Assumptions. (a) The tumor's growth can be described with Gompertzian kinetics, i.e., at any time t, the growth of the tumor may be described as:

$$N(t) = N(0)e^{(A_0/\alpha)(1-e^{-\alpha t})}.$$

(b) The tumor is treated with a fixed dose and schedule of a cycle-nonspecific drug (e.g., an alkylating agent such as melphalan) that causes a fixed percentage kill independent of the total number of cells or their position in the cell cycle.

Then, if $N(t)$ is the number of viable tumor cells at time t of drug administration, and $N(t+)$ is the remaining number of viable cells just after the drug is given,

$$\ln\left[\frac{N(t+)}{N(t)}\right] = R,$$

that is, the fraction e^R survive treatment.

Problem. If T is the time interval between successive drug doses, then find values for

$$\ln N(T), \ln N(2T) \cdots \ln N(nT)$$

given $\ln N(0)$.

Solution. From the Gompertz relationship

$$\ln N(t) = \ln N(0) + \frac{A_0}{\alpha}(1 - e^{-\alpha t})$$

we see that

$$\frac{A_0}{\alpha} = \ln N(\infty) - \ln N(0)$$

in which $N(\infty)$ is the normal terminal number of tumor cells if the system is allowed to proceed without interruption. Denoting this normal terminal value as a constant, $\hat{N}$, then

$$\ln N(t) = e^{-\alpha t} \ln N(0) + (1 - e^{-\alpha t}) \ln \hat{N}.$$

If the drug is first administered at time $t=0$, so that

$$\ln N(0+) = \ln N(0) + R,$$

then

$$\begin{aligned}\ln N(t) &= \ln N(0+)e^{-\alpha t} + \ln \hat{N} \cdot (1 - e^{-\alpha t})\\ &= [\ln N(0) + R]e^{-\alpha t} + \ln \hat{N} \cdot (1 - e^{-\alpha t}).\end{aligned}$$

Hence

$$\ln N(T) = e^{-\alpha T} \ln N(0) + Re^{-\alpha T} + \ln \hat{N} \cdot (1 - e^{-\alpha T})$$

and, in general,

$$\begin{aligned}\ln N[(n + 1)T] &= e^{-\alpha T} \ln N(nT)\\ &\quad + Re^{-\alpha T} + \ln \hat{N} \cdot (1 - e^{-\alpha T})\end{aligned}$$

or

$$\ln N_{n+1} = a \cdot \ln N_n + b$$

where:

$$\begin{aligned}\ln N_k &= \ln N[kT]\\ a &= e^{-\alpha T}\\ b &= Re^{-\alpha T} + \ln \hat{N} \cdot (1 - e^{-\alpha T})\\ &= Ra + \ln \hat{N} \cdot (1 - a).\end{aligned}$$

The following observations may be made about this relationship:

(a) The terminal value for $\ln N(nT)$ is $\dfrac{b}{1-a}$ which is independent of $N(0)$.

(b) $\ln N[(n + 1)T]$ vs. $\ln N(nT)$ is a straight line from which we can estimate a and b.

(c) This relationship is itself a Gompertzian equation of the form

$$N = N_o e^{(B_o/\alpha)(1-e^{-\alpha t})}$$

where B_o is negative and α is the α of the growth equation. Formal proof of this is as follows:

$$\ln N_{n+1} = a \cdot \ln N_n + b$$

let:

$$a = e^{-\alpha T}$$

$$b = \left(\ln N_o + \frac{B_o}{\alpha}\right) \cdot (1 - e^{-\alpha T})$$

$$\begin{aligned}\ln N_{n+1} &= e^{-\alpha T} \cdot \ln N_n + \left(\ln N_o + \frac{B_o}{\alpha}\right)(1 - e^{-\alpha T})\\ &= e^{-\alpha T}\left[\ln N_o + \frac{B_o}{\alpha}(1 - e^{-\alpha nT})\right]\\ &\quad + \ln N_o(1 - e^{-\alpha T}) + \frac{B_o}{\alpha}(1 - e^{-\alpha T})\\ &= e^{-\alpha T}\ln N_o + e^{-\alpha T}\frac{B_o}{\alpha} - e^{-\alpha T}\frac{B_o}{\alpha}e^{-\alpha nT}\\ &\quad + \ln N_o - \ln N_o e^{-\alpha T} + \frac{B_o}{\alpha} - \frac{B_o}{\alpha}e^{-\alpha T}\\ &= \ln N_o + \frac{B_o}{\alpha} - \frac{B_o}{\alpha}e^{-\alpha T - \alpha nT}\\ &= \ln N_o + \frac{B_o}{\alpha}(1 - e^{-\alpha T(n+1)})\\ &= \ln N_{n+1}.\end{aligned}$$

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