Immunoglobulin Synthesis and Total Body Tumor Cell Number in IgG Multiple Myeloma

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ABSTRACT Studies of synthesis of IgG paraproteins were performed in 10 patients who had IgG myeloma in order to quantitate cellular immunosynthetic functions and derive estimates of the number of tumor cells present in such patients. Serial in vitro studies demonstrated constancy in the cellular rate of IgG paraprotein secretion for up to 8 months. Average molecular synthesis rates in different patients ranged from 12,500 to 85,000 molecules of IgG per minute per myeloma cell. Estimated total body tumor cell number ranged from 0.5×10^{12} to 3.1×10^{12} myeloma cells, and could be correlated with the degree of skeletal damage observed on roentgenograms ($P = < 0.01$). Serial measurements of tumor cell number may prove useful in characterizing the growth rate and natural history of multiple myeloma. Myeloma is the first metastatic human malignancy in which quantitative measurements of the body's burden of malignant cells have been obtained.

INTRODUCTION

Multiple myeloma has served as a model of neoplastic disease in both mouse and man. Investigations of mouse myeloma first demonstrated the quantitative relationship between concentration of serum myeloma proteins and tumor weight (1). Studies of the pathologic immunoglobulins of myeloma (paraproteins) have added substantially to the understanding of the structure, function, and metabolism of normal immunoglobulins and antibodies. Immunoglobulin G (IgG) represents the most abundant normal serum immunoglobulin, and paraproteins of the IgG class occur in over 50% of patients with myeloma (2, 3). The extreme homogeneity of these paraproteins provides evidence that these products are secreted by the progeny of a single neoplastic cell clone ("monoclonal gammopathy") (4).

Detailed studies of the metabolism of IgG in vivo (5–7) provide useful background for the present investigations, in which quantitative measurements of the total body IgG synthesis rate in vivo and the average myeloma cell IgG synthesis rate in vitro were used to estimate numbers of tumor cells in patients with IgG myeloma. Tumor cell number was then related to the clinical evaluation of extent of disease, and was also used to estimate the average rate of growth of this disseminated human malignancy.

METHODS

Patients. 10 patients with IgG multiple myeloma were selected for study. The diagnosis was established by (*a*) the presence of an electrophoretically homogeneous serum paraprotein in a concentration greater than 2.0 g/100 ml, which could be typed immunologically as IgG with a single light-chain type, in association with markedly reduced levels of normal immunoglobulin components; (*b*) the clinical findings of a marrow plasmacytosis of greater than 20%, often with atypical plasma cells; and (*c*) one or more additional signs of myeloma, such as hypercalcemia, lytic bone lesions, soft tissue plasmacytomas, anemia, Bence Jones proteinuria, renal insufficiency, or amyloidosis. At the time the metabolic studies were done, skeletal roentgenograms were obtained and the extent of bone damage was determined by the Department of Radiology at this institution; involvement was classified in order of increasing severity as: normal bone, osteoporosis, lytic lesions, or extensive skeletal destruction and major fractures. Clinical and laboratory data on the patients selected for these initial studies are shown in Table I. Severity ranged from those who were newly diagnosed to those with far advanced myelomatosis. Patients L. M., J. H., J. U., A. V., and W. W. had never received chemotherapy before the metabolic studies.

Characterization of immunoglobulins. Serum paraprotein concentrations were measured by cellulose acetate electrophoresis and quantitative densitometry with the Microzone system (Beckman Instruments, Palo Alto, Calif.). Pathologic immunoglobulins were characterized by Ouchterlony gel diffusion, immunoelectrophoresis, and radioimmunoassay.

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Antisera employed for gel diffusion and immunoelectrophoresis included horse anti-human serum (Hyland Laboratories, Los Angeles, Calif.), as well as anti-IgG, -IgA, -IgM, -kappa, and -lambda raised in rabbits (8). Primate antisera specific for the four IgG subclasses were kindly provided by Dr. John Leddy, Rochester, N. Y. Urinary light-chain concentrations were determined by measurement of 24 hr urinary protein excretion in conjunction with immunoelectrophoresis and cellulose acetate electrophoresis of urine concentrates.

Preparation of labeled IgG. IgG from normal human donors was purified by DEAE-cellulose chromatography by a modification of the method of Fahey and Horbett (9). Serum was dialyzed against phosphate buffer (pH 8.0) and chromatographed on DEAE-cellulose which was equilibrated with the same buffer. The eluted IgG was then subjected to gel filtration on a Sephadex G-200 column. The 7S peak was pure IgG by Ouchterlony analysis and contained no detectable contaminants.

The purified IgG was labeled with ^{125}I by two different techniques. For in vivo metabolic studies, the iodine monochloride method of McFarlane was used (10); the preparations made with this method were dialyzed against pH 7.3 PBS[1] at 10°C, normal human albumin was added to prevent damage due to self-irradiation, and the preparations were sterilized by filtration. The preparations were over 97% precipitable, were calculated to have less than one atom of iodine per molecule of protein, and were pyrogen free.

For the in vitro inhibition radioimmunoassay procedure, higher specific activity (over 10 μCi/μg) IgG-^{125}I was prepared with a modification of the chloramine-T method of Hunter and Greenwood (11).

In vivo studies. Informed consent for the metabolic investigations was obtained from all patients studied. IgG turnover studies were performed while the patients were hospitalized in the Clinical Cancer Research Center or the General Clinical Research Center of this institution. Normal volunteers and patients with neoplastic disease and normal serum immunoglobulin levels served as controls for the IgG turnover studies. Patients were not acutely ill, and repeated determinations of serum paraproteins were constant during the period of study.

All subjects received a saturated solution of potassium iodide to block the thyroidal uptake of ^{125}I. IgG turnover studies were initiated with the intravenous injection of 30 μCi of IgG-^{125}I (approximately 5 million cpm) with a calibrated syringe. A 10 min plasma sample was obtained, as were daily samples and 24 hr urine collections for 14–21 days. Plasma samples and urine were counted in an automatic gamma ray well-type scintillation counter with a 2 inch sodium iodide crystal. Total body IgG synthesis in vivo was calculated from the urine and plasma radioactivity by the metabolic clearance method (7, 12).

Calculation of total body IgG myeloma protein synthesis. The in vivo synthetic rate for IgG myeloma protein (IgG_{mp}) was calculated as follows:

$$FCR = \frac{\text{radioacitvity in 24 hr sample of urine}}{\text{radioactivity remaining in plasma}}$$

$$= \frac{\%\text{ of injected dose in 24 hr urine collection}}{\text{mean daily plasma activity as \% of 10 min sample}}$$

$$PV = \frac{\text{total cpm injected}}{\text{cpm/ml of plasma in 10 min sample}}$$

Total intravascular IgG_{mp} = serum concentration (mg/ml) of $IgG_{mp} \times PV$.

Total IgG_{mp} catabolic rate = total intravascular $IgG_{mp} \times$ FCR.

The several assumptions of the metabolic clearance technique have recently been summarized by Waldmann and Strober (7). In addition, the catabolic rate of normal IgG is assumed to be the same as the catabolic rate for the IgG paraproteins of the patients studied.

In vitro studies. In vitro studies of plasma cell IgG synthesis were performed with methods that we have detailed elsewhere (13). All tissue culture media and supplements were obtained from Grand Island Biological Co., Berkeley, Calif. A 10 ml specimen of bone marrow was aspirated aseptically into a syringe containing heparin. The red cells were sedimented with 3.0 ml of 3% dextran, and the supernatant plasma, which contained the marrow cells, was transferred to a sterile, 50 cc graduated centrifuge tube. The leukocyte-rich plasma was diluted to 45 ml with HBSS-FCS and centrifuged for 10 min at 600 *g* at room temperature. The cell button was then suspended in 45 ml of HBSS-FCS and centrifuged again under the same conditions. This washing procedure was done six times to reduce the quantity of human IgG in the supernatant plasma to less than 100 ng/ml. After the sixth wash, the cell button was suspended in 3 ml of HBSS-FCS, white blood cells were counted, and viability was assessed (trypan blue exclusion). The cell preparations were free of clumping and viability at the completion of the washings was from 98 to 100%. Slides for microscopic examination were prepared with the Shandon cytocentrifuge and were stained with Wright's stain for a 600-cell differential count. All marrow cells which were morphologically in the lymphoid-plasma cell series were defined as myeloma cells (13).

After the marrow cells were counted, they were centrifuged one last time to establish a "zero time," and resuspended in minimal essential medium (Spinner's) at a concentration of from 0.5 to 3.0×10^6 cells per ml. The medium was supplemented with 15% FCS, and combined with final concentrations of 50 U of penicillin, 50 μg of streptomycin, 2 U of heparin, 0.29 mg of glutamine, and 2.2 mg of sodium bicarbonate per ml of medium. Duplicate 3.0-ml cultures were prepared in 16- × 125-mm screw-cap culture tubes, treated briefly with 5% CO_2 in air, and incubated at 37°C. Multiple samples were harvested at intervals up to 20 hr by centrifugation at 600 *g* for 10 min at 10°C. Initial viability was 95 ±2%; after 18 hr it was 73 ±6%. The supernatant medium was decanted and frozen for radioimmunoassay of IgG. The cell pellet was washed twice by resuspension in 10 ml of 0.15 M NaCl followed by centrifugation, and then it was frozen. Before immunoassay, the pellet was suspended in 3.0 ml of 0.15 M saline and sonicated.

IgG radioimmunoassay. An inhibition radioimmunoassay (the "sandwich" solid phase radioimmunoassay), which employs specific antibody bound to a disposable plastic tube (14), was used for measurement of IgG in the time-culture supernatants and cell sonicates. This assay technique is specific for the heavy-chain determinants of IgG; the sensitivity range is from 10 to 500 ng/ml. In these studies, the patient's own paraprotein was used as the quantitative reference standard for the assay. The characteristics of this assay system render it suitable for recognition of partial identity

[1] The following abbreviations are used: PBS, phosphate-buffered saline; FCR, fractional catabolic rate; PV, plasma volume; mp, myeloma protein; HBSS-FCS, Hanks' balanced salt solution with 10% fetal calf serum; and TB, total body.

TABLE I

Summary of Clinical and Laboratory Data and Calculations of Immunoglobulin

Patient, sex, and age (yr)	Time from diagnosis	Signs and symptoms	Treatment	Bone lesions	Paraprotein subclass; light-chain	Bone marrow
L. M., F, 52	6 months	Backache, alopecia, amyloidosis	None	0	IgG_1; λ	55% plasma cells
J. H., M, 56	2 wk	Laryngeal paralysis, hiccups, dysphagia	None	+	IgG_1; κ	25% multinucleated plasma cells
J. U., F, 40	1 wk	Hyperviscosity syndrome (1 yr)	None	+	IgG_1; λ	50% plasma cells
A. V., F, 77	2 months	20 lb weight loss, backache	None	+	IgG_1; κ	39% plasma cells
R. T., F, 60	3 yr	Recurrent pneumonia	Prednisone	+++	IgG_1; κ	60% lymphoid plasma cells
R. W., F, 62	1½ yr	Fracture of humerus	Local X-ray	+++	IgG_1; λ	90% immature plasma cells
M. P., F, 35	3 months	Hypercalcemia, very rapid progression of myeloma, hip fracture	Melphalan, prednisone	+++	IgG_1; κ	80% plasma blasts
W. W., M, 65	2 wk	Hypercalcemia, pneumonia	None	++	IgG_2; κ	90% multinucleated plasma cells
L. F., M, 63	4 yr	Recurrent pneumonia	Melphalan, prednisone	+++	IgG_1; κ	80% multinucleated plasma cells
D. R., F, 45	1 month	Hypercalcemia, pancytopenia	None	+++	IgG_2; κ	65% plasma cells

Bone lesions: 0, normal bone; +, osteoporosis; ++, lytic lesions; +++, extensive skeletal destruction and major fractures. Serum IgG_{mp}, concentration of IgG myeloma protein in serum; PV, plasma volume (in liters); intravascular IgG_{mp}, total intravascular IgG myeloma protein (in grams); FCR, fractional catabolic rate; TB IgG_{mp} synthesis, total body IgG myeloma protein synthesis; cellular IgG_{mp} synthesis, cellular IgG myeloma protein synthesis (in grams per myeloma cell per 24 hr).

of certain related antigens and immunoglobulin fragments, as well as for quantitation of monomers and polymers of immunoglobulins. The controls of specificity for the present studies demonstrated immunologic identity of each patient's serum paraprotein with samples of his IgG synthesized in vitro.

Calculation of myeloma cell number. The calculation of the total body number of myeloma cells (myeloma cell mass) *depends on the following assumptions:* (*a*) the myeloma cells sampled are representative of the total body myeloma cell population; and (*b*) the average rate of IgG synthesis measured in vitro is a valid reflection of the cellular IgG synthesis rate which would be attained in vivo.

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

or

$$MC_{TB} = \frac{R_{TB}}{R_M} \times MC_{\text{in vitro}}$$

Where

MC_{TB} = total body myeloma cell number
R_{TB} = rate of total body IgG synthesis (g/24 hr) in vivo
R_M = rate of total myeloma IgG synthesis (g/24 hr) in vitro, and
$MC_{\text{in vitro}}$ = Number of myeloma cells in vitro.

RESULTS

Rate of cellular IgG synthesis in vitro. Initial results of the radioimmunoassay of the myeloma marrow culture samples showed that, for any one individual, the intra-

Synthesis and Tumor Cell Mass in 10 Patients with IgG Myeloma

Hemoglobin	Creatinine	Urinary light-chain	Serum IgG_{mp}	PV	Intravascular IgG_{mp}	FCR	TB IgG_{mp} synthesis	Cellular IgG_{mp} synthesis	Total No. of myeloma cells
g/100 ml	*mg/100 ml*	*g/24 hr*	*g/100 ml*	*liters*	*g*	*%*	*g/24 hr*	*g/myeloma cell per 24 hr*	
11.5	0.8	0.0	2.8	2.15	60	9.3	5.6	1.2×10^{-11}	0.5×10^{12}
9.6	1.5	0.0	2.8	2.23	62	9.6	5.9	1.1×10^{-11}	0.5×10^{12}
8.5	1.4	0.0	10.1	3.47	350	10.9	38.1	3.4×10^{-11}	1.1×10^{12}
10.4	1.1	0.3	7.2	2.32	167	12.0	20.0	1.4×10^{-11}	1.4×10^{12}
8.2	1.0	0.6	7.6	2.78	211	12.1	25.5	1.2×10^{-11}	2.1×10^{12}
8.4	1.1	5.5	3.7	2.71	100	12.9	12.9	0.5×10^{-11}	2.6×10^{12}
8.6	1.0	0.0	5.9	1.92	113	11.9	13.4	0.5×10^{-11}	2.7×10^{12}
8.3	1.2	0.0	6.4	2.20	141	12.5	17.6	0.6×10^{-11}	2.9×10^{12}
13.0	1.3	3.0	5.5	2.84	156	13.8	21.5	0.7×10^{-11}	3.1×10^{12}
7.8	4.5	12.8	6.3	3.19	201	12.2	24.5	0.8×10^{-11}	3.1×10^{12}

cellular IgG pool remained approximately constant in size for the culture period. In contrast, IgG secreted into the medium by the myeloma cells increased in quantity at a constant rate for periods of from 10 to 24 hr after initiation of the cultures. The rate of secretion of IgG into the medium during the period of steady-state secretion was used to determine the "effective" cellular IgG synthesis rate and will be referred to as the cellular IgG synthesis rate. The cellular rate of IgG secretion was independent of myeloma cell percentage or cell concentration in these studies (13). There was no correlation between the degree of morphologic maturity of myeloma cells and the IgG secretion rate. The rate of IgG synthesis was temperature dependent, however, with an optimal synthesis rate at a constant temperature of 37°C. A representative in vitro study is shown in Fig. 1.

Myeloma cell IgG synthesis rates in vitro varied from patient to patient, and ranged from 0.5×10^{-11} to 3.4×10^{-11} g/myeloma cell per day (Table I). Expressed in molecular terms, each myeloma cell secreted approximately 12,500–85,000 molecules of IgG per min. Measurements of the myeloma cell IgG synthetic rate in vitro were reproducible. Cells from one patient (L. M.) were studied four times during an 8 month period (both before and after initiation of systemic chemotherapy). The standard deviation of the rate of synthesis for these serial studies was ±12% of the mean value. Samples of her paraprotein synthesized in vitro were compared to her serum paraprotein by chromatography on a cali-

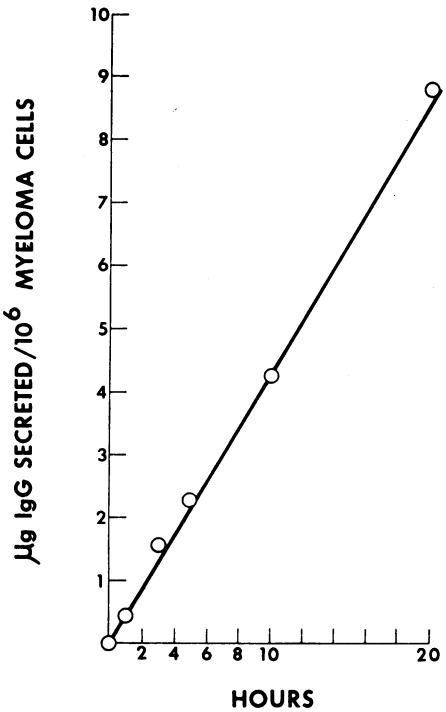

FIGURE 1 Secretion of IgG by myeloma cells obtained from patient A. V. In this experiment, a constant secretion rate was maintained for 20 hr in vitro. Multiple samples were obtained during the first 10 hr of culture because in some instances the rate of IgG synthesis declines after that time. The quantity of IgG present in the medium in the zero time sample was subtracted from all experimental points.

brated Sephadex G-200 column and the fractions were then studied by radioimmunoassay. These studies showed that the IgG synthesized in vitro was identical with that synthesized in vivo both in size and in immunologic characteristics.

Patient J. U., who had the highest rate of synthesis of cellular IgG, also had the highest serum paraprotein concentration; the patient had a hyperviscosity syndrome (relative serum viscosity, 8.5; normal range, 1.4–1.8 relative units), retinal "sausage veins," and epistaxis.

Although in this patient there appeared to be an impressive relationship between the high cellular IgG synthetic rate and the unusually high serum IgG_{mp} concentration, in the entire patient group this correlation approached but did not reach statistical significance ($r = 0.61$, $t = 2.17$, $P = < 0.07 > 0.05$). Three of the patients with low cellular synthetic rates for intact IgG were synthesizing a considerable excess of light-chains which could be detected as Bence Jones proteinuria. The cellular synthesis of Bence Jones proteins was not detected with the IgG assay used. Prior chemotherapy did not appear to influence the cellular IgG synthetic rate ($P > 0.1$).

Total body IgG metabolism. The results of the in vivo IgG metabolic studies, summarized in Table I, are similar to data previously reported on IgG turnover in multiple myeloma by other investigators (7, 15, 16). The half-time of disappearance ($t_{\frac{1}{2}}$) of IgG-^{125}I ranged from 11.6 to 17 days in the myeloma patients. In three control subjects with normal serum IgG levels, the $t_{\frac{1}{2}}$ ranged from 20 to 24 days, with FCR of 5.2–6.4% of the plasma compartment per day. The average IgG turnover rate in the myeloma patients was 0.34 g/kg per day, approximately 10 times the average rate observed in control subjects. As noted by other investigators (5–7, 17), the FCR for IgG was higher in subjects with higher serum IgG concentrations. The total body IgG synthetic rates of our patients could not be correlated with the individual variables of plasma cell morphology, disease duration or extent, or cellular synthetic rate.

Myeloma cell number. The total myeloma cell numbers for the 10 patients studied (Table I) fell within a relatively narrow range (0.5 to 3.1×10^{12} cells). Tumor cell number appeared to correlate better with degree of bone damage, as seen on skeletal X-rays, than with any other clinical manifestation of the disease. The four patients who had normal bones or osteoporosis had an average total body myeloma cell mass of $0.88 \pm 0.45 \times 10^{12}$ myeloma cells, while the six patients with advanced osteolytic bone lesions had $2.75 \pm 0.37 \times 10^{12}$ malignant cells ($P = < 0.01$). The two patients with soft tissue plasmacytomas (D. R. and M. P.) were both in the high tumor cell mass range.

Percentage of bone marrow myeloma cells varied in general with variation in myeloma cell mass, but was not always consistent on repeated aspirates. Inasmuch as cellular synthetic rate was calculated from the total in vitro myeloma cell number rather than from myeloma cell percentage, cell mass determinations were reproducible despite this variation in cell percentage.

The two patients with the lowest serum paraprotein concentrations had the smallest total number of myeloma cells, but in all other instances, the serum paraprotein level could not be related to the absolute size of the malignant cell mass. Patient J. U., who had a hyperviscosity syndrome, deserves specific mention in this regard; although she had the highest concentration and turnover rate of paraprotein of the entire patient group, she also had the highest cellular IgG synthetic rate. In her case, the very high cellular rate of IgG synthesis appears to explain the high serum level.

Similarly, the rates of total body and cellular IgG_{mp} synthesis taken independently did not correlate directly with extent of skeletal disease, and only the quotient of these two rates (myeloma cell number) was significantly related to this clinical manifestation. Hypercalcemia and heavy Bence Jones proteinuria were more common in

the group of patients with advanced skeletal disease and high myeloma cell number.

DISCUSSION

The calculations of human myeloma cell mass reported in this paper must be considered approximate, and must be viewed in the context of present knowledge of the myeloma cell and of IgG metabolism. The prototype myeloma cell is well differentiated with an extensive endoplasmic reticulum and a high capacity for protein synthesis. In some patients, heavy- and light-chain synthesis is "balanced" and only an intact immunoglobulin is secreted (13); in other patients, secretion of light-chains is excessive and synthesis unbalanced, leading to Bence Jones proteinuria. The serial in vitro studies reported on here indicate that the cellular synthetic rate for intact IgG by a given clone may be fixed at a certain level for extended periods of time.

The majority of the cellular IgG synthetic rates measured fell within a narrow range, with 9 of the 10 patients having in vitro synthetic rates of from 5 to 14 pg/myeloma cell per day. The range of myeloma cell IgG synthesis rates for these patients is similar to those measured by Nathans, Fahey, and Potter for mouse myeloma (1), and to those estimated by Nossal and Mäkelä for normal, antibody-producing plasma cells in the rat (18). With our present in vitro assay systems, similar studies could be carried out on myeloma cells which synthesized IgG at rates as low as 0.3×10^{-12} g/cell per day, or on bone marrow samples containing as few as 0.5–1.0% myeloma cells which synthesized at a normal rate for myeloma cells. In the latter situation, which might pertain during remission, the relative errors in differential counts of bone marrow cells would be larger, and the resultant cellular synthetic rates would be less accurate.

Recent studies by Morell, Terry, and Waldmann (17) have shown that the rate of catabolism of three of the four subclasses of IgG (IgG_1, IgG_2, and IgG_4, which comprise 93% of total normal IgG) were identical, while the minor component IgG_3 was catabolized somewhat more rapidly. Inasmuch as none of the patients in the present study had IgG_3 myeloma, the use of labeled normal IgG for the in vivo studies should give a valid measurement of the IgG_{mp} catabolic rate.

In vivo studies in animals and man have shown that the catabolic rates of normal IgG and myeloma IgG of the various subclasses are linked to the total serum IgG concentration, with increasing rates of catabolism associated with increasing serum concentrations up to approximately 30 mg/ml (5, 6, 17), above which no further increase in FCR is observed. Because of these metabolic considerations, serial paraprotein measurements in IgG myeloma may not reflect total paraprotein synthesis or tumor cell mass in a linear fashion. Despite this limitation, determinations of paraprotein concentrations remain the most valuable parameter for objective follow-up of such patients (19). In IgA myeloma, because of the relatively fixed IgA catabolic rate (7, 20, 21), changes in the serum concentration would be anticipated to reflect the changes in cell mass more directly.

It should be emphasized that the calculations of tumor cell number depend upon two critical assumptions which are intrinsically difficult to prove: the average rate of IgG synthesis in vitro is assumed to be a valid reflection of the cellular IgG synthesis rate which would be attained in vivo; in addition, the myeloma cells sampled are assumed to be representative of the total body's population of myeloma cells. The reproducibility of results from serial studies of any single patient's cells tends to support the latter assumption, as does the result of a comparative in vitro study of cells from a patient who was not included in the present series because in vivo studies were not done. Specimens were obtained from this patient with far advanced IgG myeloma 2 wk before his death; a 15% plasmacytosis was present in the peripheral blood, and these cells had virtually the same IgG synthetic rate as did his marrow myeloma cells, which were studied simultaneously.

By considering these studies of myeloma cell mass in conjunction with characteristic clinical observations, certain aspects of the natural history of IgG myeloma can be clarified. At the time of the clinical diagnosis, the myeloma cell mass appears to be at least 0.5×10^{12} cells, or 0.5 kg of tumor (assuming 10^{12} myeloma cells equal 1.0 kg), indicating that there is an enormous preclinical "iceberg," with prolonged tumor proliferation occurring asymptomatically. Recognition of paraproteins on serum electrophoresis is difficult below concentrations of 0.5 g/100 ml, which would correspond to a myeloma cell mass of approximately 5×10^{10} or about 50 g of tumor. Unless such a mass is localized and symptomatic, early diagnosis is virtually impossible in this disorder.

In our myeloma cell studies, a correlation could be made between the extent of lytic bone disease and tumor cell mass. Patients with far advanced lytic lesions and multiple pathological fractures had more myeloma cells than those who had osteoporosis or normal bones. It is recognized that this correlation may not always hold; in some cases, extraskeletal involvement may be extensive while bones are relatively spared (22). Large myeloma cell mass was associated with a poor prognosis. Four patients with large numbers of malignant cells died within 6 months of the metabolic studies. These patients were either initially unresponsive to chemotherapy (R. W. and M. P.) or had relapsed while

on chemotherapy (R. T. and L. F.). Tumor cell mass alone is not the only determinant of survival in myeloma, however, because other variables, including increased susceptibility to infection, hypercalcemia, anemia, and renal insufficiency, also affect survival.

In acute leukemia, clinical manifestations are thought to be due to the number of neoplastic cells in the host. This conclusion has been derived primarily from quantitative studies of leukemia in mice, where progression of leukemia from a single transplanted cell can be characterized with a first-order kinetic model (23). In the experimental plasmacytoma of the hamster, fatal disease has also been induced after transplantation of a single isolated malignant cell (24). In man, first-order kinetics can be used to explain the rate of leukemic cell repopulation as measured by duration of unmaintained drug-induced remission (25). In attempting to construct a hypothetical model of the natural history of human multiple myeloma, we assume first-order kinetics of proliferation for the human monoclonal myeloma cell. By comparing tumor cell mass in newly diagnosed patients with that seen in patients with advanced disease, it appears that the tumor goes through approximately four doublings in size to a mass of up to 3×10^{12} cells (about 5% of body weight) at the time of death.

Based on the average magnitude of decline in the serum concentration of IgG paraproteins in patients who show objective response to chemotherapy with alkylating agents (26), it appears that such therapy usually reduces the cell mass by less than 1 log. Such responses, when maintained with chemotherapy, are associated with an approximate doubling in life expectancy (26). The average life expectancy from time of diagnosis in untreated myeloma patients (27) or in patients refractory to chemotherapy is about 2 yr (26). Assuming that our patients are typical, this would suggest that the average doubling time of myeloma cells is 6 months. If the doubling time is constant, this would imply that in such patients the disease began with a single cell undergoing malignant transformation approximately 20 yr before diagnosis. Indeed, in studies of the growth rate of myeloma inferred from changes of serum paraprotein concentrations, Hobbs (28) has calculated an average doubling time of 6.3 months for IgA paraproteins, and has estimated the average duration of IgA myeloma as 21 yr. His estimate for IgG myeloma was somewhat longer, but he did not correct the IgG calculation for the concentration-dependent changes in serum IgG catabolism. It would, therefore, appear likely that a similar growth rate can be inferred for both IgG and IgA myeloma, although quantitative cell mass studies remain to be done in the latter variant.

Although the assumption of a constant growth rate provides a means for approximating disease duration in myeloma, it may not be the optimal model. In certain solid tumors, growth slows when the tumor becomes large (24). This form of tumor growth is described better with a Gompertzian growth curve (24, 29) than with a first-order kinetic model. The Gompertzian model would yield shorter estimates of total duration of myeloma if growth slows significantly before diagnosis or early in the period of clinical observation. Serial tumor mass measurements in selected patients may indicate which model of tumor growth is applicable to this disorder.

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