IgE Myeloma: Total Body Tumor Cell Number and Synthesis of IgE and DNA

By Sydney E. Salmon, O. Ross McIntyre, and Makio Ogawa

Studies of myeloma cell DNA and immunoglobulin synthesis and total body tumor cell number were performed on a patient with IgE multiple myeloma and plasma cell leukemia; results were contrasted to similar observations in patients with IgG myeloma. Autoradiographic studies demonstrated significantly greater DNA labeling in the IgE patient's bone marrow plasma cells than in cells from IgG myeloma patients who did not have leukemic manifestations; an additional patient with plasma cell leukemia also had a high labeling index of the myeloma cells in the blood. The IgE myeloma patient's molecular synthesis rate for immunoglobulin averaged 26,000 molecules of IgE per minute per myeloma cell, a synthetic rate similar to that found in IgG myeloma. These observations have relevance to the normal immune response, as they can be used to approximate the number of normal IgE- and IgG-producing cells in the body. In the patient with IgE myeloma, the total body myeloma cell number was $2.7 \times 10^{12}$ cells at the time of study and was of the same order of magnitude as in IgG myeloma; however, the distribution of tumor cells was strikingly different. Serial estimates of tumor cell number were made; such estimates may prove useful in evaluation and treatment of patients with multiple myeloma.

Multiple myeloma is a malignant disorder of antibody-producing cells in which a monoclonal immunoglobulin (M-component) is secreted as a tumor product. The incidence of myeloma of different immunoglobulin classes approximates a ratio to the normal serum concentrations of IgG, IgA, IgD, and IgE. IgE, which carries reaginic antibody specificity, is the most recently discovered immunoglobulin; it is present in the lowest concentration in normal serum. M-components of the IgE class accompany the rarest type of myeloma recognized thus far; only two cases involving IgE clones have been described. Interestingly, both patients had plasma cell leukemia, although this generally is a rare manifestation of myeloma.

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New techniques, recently applied in IgG myeloma, allow an estimate of the body's total burden of tumor cells on the basis of measurement of cellular and total body synthetic rates of the M-component. In the present study, synthesis of DNA and immunoglobulin by the IgE myeloma cell was assessed and related to the total body tumor cell number and the clinical course of the neoplasm. These studies may have relevance to the function of normal IgE-producing clones, which proliferate and synthesize reaginic antibody after allergic sensitization.

**Materials and Methods**

**Patient Studies**

The IgE myeloma patient (P.S.) was a 60-yr-old man, whose case history has been reported elsewhere. Studies of IgE synthesis were performed 7 months after diagnosis, at a time when his myeloma was in relapse after a brief drug-induced remission. Two in vivo metabolic studies of the turnover of radioiodinated IgE were performed, as well as a third study employing \(^{14}C\)-guanido-L-arginine, to determine the total body synthetic rate for the M-component of IgE; the studies were analyzed with techniques described previously.

Studies of immunoglobulin synthesis in vitro were carried out one month after the first metabolic study was begun. Ten milliliters of bone marrow were aspirated into a syringe containing heparin and prepared with a series of washing steps. The aspirate contained \(1 \times 10^9\) myeloma cells, which varied in size and differentiation and ranged from small lymphocytes to large plasma cells. Cells in this entire range could be recognized as myeloma cells when observed by fluorescence microscopy, as they all reacted with a specific fluorescein-conjugated antibody to the IgE of patient P.S. Myeloma cells comprised 93% of the nucleated cells in the marrow sample. Studies of immunoglobulin synthesis in vitro were performed with the materials and methods of Salmon and Smith; myeloma cells were used at a concentration of \(6 \times 10^5\) cells/ml of medium. Initial viability was 97% (trypan blue). At the end of 19 hr in culture, \(6 \times 10^5\) cells/ml of tissue culture medium remained, and viability was 93%.

Culture tubes were prepared with the above cell concentration and with the same tissue culture medium and incubated for 4 hr in the presence of \(1 \mu\)Ci/ml of tritiated thymidine (18 Ci/mM, New England Nuclear Corp., Boston, Mass.). Slides of the cultured cells were fixed in methanol and prepared for autoradiography by dipping in NTB 3 emulsion (Eastman Kodak Co., Rochester, N.Y.). The same autoradiographic study was performed subsequently on marrow cells from five patients with IgG myeloma and one patient with macroglobulinemia of Waldenström, and on peripheral blood myeloma cells from one patient with plasma cell leukemia and Bence Jones proteinuria. Autoradiographs were exposed for 2 wk, developed, and stained with acid Giemsa. Grains over myeloma cells were counted; cells were assigned to one of four classes (0-10, 11-25, 26-50, and more than 50 grains). The percentage of myeloma cells that had significant nuclear labeling (more than 10 grains) was taken as the labeling index.

**Radioimmunoassay of IgE**

A sandwich solid-phase radioimmunoassay, similar to those previously standardized for measurement of IgG, IgA, and IgM, was developed to measure specifically the heavy chains of the monoclonal IgE of patient P.S. (PS protein). The patient's serum was dialyzed against 0.005 M phosphate buffer (pH 8.0) and applied to a diethylaminoethyl (DEAE)-cellulose column equilibrated with the same buffer. Elution of an IgG peak occurred with the starting buffer. PS protein was eluted when the pH 8.0 buffer was increased in molarity to 0.025 M phosphate. The PS protein peak was further purified by

*In collaboration with Dr. Thomas Waldmann.*
passage through a calibrated, upward flow, Sephadex G-200 column (Pharmacia Fine Chemicals, Piscataway, N.J.). To obtain an antiserum to the patient's IgE, 5 mg of the purified PS protein were dissolved in saline, emulsified with complete Freund's adjuvant, and injected subcutaneously into rabbits. The rabbit antiserum was absorbed with appropriate immunoglobulins and light chains to render it specific for the heavy chains of the patient's IgE. When tested by radioimmunoassay, the absorbed antiserum (anti-PS) had less than 0.02% cross-reactivity, and had a binding affinity of 55% for radioiodinated PS protein.

The radioiodinated PS protein was also used for the inhibition immunoassay described below. Sandwich radioimmunoassay tubes were prepared as described previously, with an initial adsorption of purified antigen (PS protein, 0.1 mg/ml) in phosphate-buffered saline, and a final immunochemically bound coat of a suitable dilution of anti-PS. Tubes prepared in this fashion were used both for the direct assay of PS protein labeled biosynthetically with 3H-L-leucine during the tissue culture period and also for an inhibition immunoassay for quantitation of PS protein. A highly purified preparation of PS protein of known nitrogen content (graciously provided by Dr. Kimishige Ishizaka) served as the quantitative reference standard for the inhibition radioimmunoassay of the supernatants and cell sonicates from the tissue culture samples. The range of sensitivity of the inhibition assay for PS protein was from 5 to 500 ng/ml, with a standard error of ±5%.

Calculation of IgE Myeloma Cell Number

Total body myeloma cell number was calculated from the rates of IgE synthesis in vivo and in vitro, corrected for the cell concentration of the in vitro culture:

\[
\text{Total body myeloma cell number} / \text{Number of myeloma cells in vitro} = \frac{\text{Rate of total body IgE synthesis in vivo}}{\text{Rate of total myeloma IgE synthesis in vitro}}
\]

or

\[
MC_{\text{TR}} = \left( \frac{R_{\text{TR}}}{R_{\text{M}}} \right) \times MC_{\text{in vitro}}
\]

where \(MC_{\text{TR}}\) = total body myeloma cell number, \(R_{\text{TR}}\) = rate of total body IgE synthesis (g/24 hr) in vivo, \(R_{\text{M}}\) = rate of total myeloma IgE synthesis (g/24 hr) in vitro, and \(MC_{\text{in vitro}}\) = number of myeloma cells in vitro.

This calculation depends on a series of assumptions which have been discussed previously; most importantly: (1) the myeloma cells sampled are representative of the total body population of myeloma cells, and (2) the average rate of IgE synthesis measured in vitro is a valid reflection of the cellular IgE synthesis rate that occurs in vivo.

Estimations of myeloma cell number at times other than during the period of the metabolic studies were based on serial electrophoretic measurements of the concentration of M-component in serum. Such approximations assume that the patient's plasma volume and the fractional catabolic rate for IgE did not vary substantially during the patient's clinical course. Furthermore, the IgE synthetic rate per myeloma cell is assumed to remain constant, as was the case in serial studies of IgG synthesis in IgG myeloma.

RESULTS

Incorporation of ³H-Thymidine by IgE Myeloma Cells In Vitro

Autoradiographs prepared after a 4-hr incorporation of ³H-thymidine by the patient's bone marrow cells showed heavy labeling over the nuclei of 13% of the IgE myeloma cells. Identical labeling indices were obtained whether exposure to thymidine occurred during the first 4 hr in vitro or after the marrow cells had been cultured for 18 hr. In contrast, the labeling indices of immunoglobulin-producing cells obtained from bone marrow of five patients with IgG myeloma and one patient with macroglobulinemia of Walden-
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Fig. 1.—Four-hour nuclear labeling indices of immunoglobulin-producing cells from the bone marrow of five patients with IgG myeloma (IgG), one with macroglobulinemia of Waldenström (IgM), and the IgE myeloma patient (IgE). Not illustrated is the labeling index of 40% observed in peripheral blood myeloma cells from a patient with plasma cell leukemia and Bence Jones proteinuria.

ström (none of whom had plasma cell leukemia) were significantly lower; the highest index in this latter group of patients was 3.8% (Fig. 1).

Peripheral blood leukocytes from a patient who had a light-chain multiple myeloma of type kappa and plasma cell leukemia were studied with the same in vitro techniques. Only the myeloma cells incorporated tritiated thymidine; the labeling index was 40%. Bone marrow was not available for study from the latter patient.

Fig. 2.—Synthesis and secretion of $^3$H-IgE by myeloma cells in vitro as demonstrated by direct solid phase radioimmunoassay. After a lag of 30 min, the intracellular pool of labeled IgE rose (white circle), and reached a plateau within 3–5 hr. Labeled IgE was also detectable in the extracellular medium (black circle) by 30 min and increased in linear fashion after 2 hr. Secretion of labeled IgE occurred before uniform labeling of the intracellular pool was achieved. Inhibition immunoassay of the same tissue culture samples (not illustrated) showed a stable total intracellular pool size and secretion rate from the time of initiation of the in vitro cultures.
Fig. 3.—Radioimmunoassay of \(^{3}H\text{-IgE}\) and related protein fractions after elution from a calibrated Sephadex G-200 column. The intrinsically labeled IgE secreted in vitro was eluted at the same position as the peak position of tubes of the purified PS protein isolated from the patient's serum (IgE). Small amounts of \(^{3}H\)-lambda light-chain dimers were present, and traces of IgE-related labeled protein also were detected at elution positions subsequent to that of the intact immunoglobulin.

**Synthesis of IgE In Vitro and In Vivo**

In immunosynthetic experiments in which the IgE myeloma cells were exposed continuously to \(^{3}H\)-L-leucine, labeled IgE was detectable intracellularly within 30 min. The intracellular pool of labeled IgE rose steadily for approximately 3–5 hr and then remained constant (Fig. 2). Immunoassay of Sephadex G-200 fractions of the labeled intracellular IgE demonstrated that intact IgE was predominant, but smaller amounts of IgE-related protein were found that eluted from the gel at the positions of lower weight precursors of the intact immunoglobulin.

Studies of secretion of labeled IgE showed that, after an initial equilibration period, the secretion rate of the labeled IgE remained constant during the initial 18 hr of culture (Fig. 2). In contrast to the heterogeneity of the intracellular pool, more than 95% of the labeled IgE synthesized and secreted into the in vitro medium was eluted from Sephadex at the same position as IgE from the patient's serum, indicating that they were of the same molecular weight (Fig. 3). Small amounts of lambda light-chain dimers were also secreted, and they could also be detected clinically as Bence Jones proteinuria.

Quantitation of the intracellular and secreted IgE was done with the inhibition radioimmunoassay. The total intracellular pool of IgE averaged \(2.5 \times 10^{12}\) g/myeloma cell and, after reaching a plateau, remained at a constant level throughout the culture period. The IgE secretion rate was linear for the first 18 hr of culture, and averaged \(12.9 \times 10^{12}\) g/myeloma cell/day. Expressed in molecular terms, each of the patient's myeloma cells contained an average of \(7.5 \times 10^{6}\) molecules of IgE, and secreted 26,000 molecules of IgE/min. The rate of cellular secretion of IgE was similar to the rates previously determined for IgG secretion in patients with IgG myeloma (Table 1).\(^{10}\)

As determined by metabolic turnover study, the patient's total body immunoglobulin synthetic rate in vivo was 26.3 g of IgE per day. Data used to calculate the synthetic rate are summarized in Table 1. Radioimmunoassay of the patient's urine showed that the equivalent of 0.2 g of IgE-related protein was excreted in 24 hr.
Table 1.—Calculations of Immunoglobulin Synthesis and Tumor Cell Mass in IgE and IgG Myeloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight (kg)</th>
<th>Serum M-Component (g/100 ml)</th>
<th>Plasma Volume (liters)</th>
<th>Intravascular M-Component (g)</th>
<th>Fractional Catabolic Rate (per cent)</th>
<th>Total Body M-Component Synthesis (g/24 hr)</th>
<th>Cellular M-Component Synthesis (g/Myeloma Cell/24 hr)</th>
<th>Total Number of Myeloma Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE myeloma</td>
<td>P.S.</td>
<td>91</td>
<td>5.8</td>
<td>3.8*</td>
<td>220</td>
<td>16*</td>
<td>35.3</td>
<td>1.3 × 10^{-11}</td>
</tr>
<tr>
<td>IgG myeloma</td>
<td>L.M. †</td>
<td>52</td>
<td>2.8</td>
<td>2.15</td>
<td>60</td>
<td>9.3</td>
<td>5.6</td>
<td>1.2 × 10^{-11}</td>
</tr>
<tr>
<td></td>
<td>D.R. †</td>
<td>62.5</td>
<td>6.3</td>
<td>3.19</td>
<td>201</td>
<td>12.2</td>
<td>24.5</td>
<td>0.8 × 10^{-11}</td>
</tr>
</tbody>
</table>

* Data kindly provided by Dr. T. A. Waldmann.
† Previously reported cases.\(^{10}\)
Fig. 4.—Approximations of tumor cell numbers during the IgE myeloma patient's entire clinical course. Synthetic measurements used to determine tumor cell numbers were made 7 months after diagnosis; estimates of tumor cell numbers at other times were based on changes in serum concentrations of M-component. Chemotherapy is shown by bars below the graph: M, melphalan; P, prednisone; C, cyclophosphamide; and B, 1,3-bis-(2 chloroethyl)-1-nitrosourea (BCNU).

Tumor Cell Number and Clinical Course

The patient's calculated total body myeloma cell number at the time of the synthetic studies was $2.7 \times 10^{12}$ myeloma cells (Table 1). Serum electrophoretic determinations were used to estimate the tumor cell number at other times during the patient's course; these estimates were based on the assumptions described above. The myeloma cell number present at the time of initial presentation with plasma cell leukemia was calculated to be $3.6 \times 10^{12}$ myeloma cells (Fig. 4). At that time the patient had clinical evidence of an enlarged liver, spleen, and lymph nodes. The change in concentration of the M-component after administration of melphalan and prednisone suggests that more than $2.0 \times 10^{12}$ cells had been killed with treatment, and a brief partial remission of symptomatology occurred. Despite continued therapy with alkylating agents, regrowth was soon in evidence. During the period of early relapse, the apparent tumor doubling time was less than 4 months (Fig. 4). Although the rate of growth apparently decreased during the later part of the patient's clinical course, many plasmaphereses were performed in order to collect IgE, and any estimation of tumor cells based on electrophoretic data may be an underestimate. Death from bacteremia occurred 1 yr after diagnosis. At autopsy, several small lytic bone lesions were noted in the vertebral column, and extensive myeloma cell infiltrates were present in the liver, spleen, lymph nodes, and bone marrow, and in other organs.\footnote{Ogawa, M., Altschule, J., and McIntyre, O. R.: In preparation.}
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DISCUSSION

Although plasma cell neoplasms appear to have a number of common morphological and clinical features, the two known patients with IgE myeloma did differ from the usual pattern of myeloma, both in the absence of extensive skeletal involvement and in their clinical presentation with plasma cell leukemia. Unlike IgG myeloma, tumor cell mass was not closely correlated with the degree of skeletal involvement in these patients. Although the tumor cell number was large in our patient and the bone marrow was heavily involved, the skeleton itself was spared, and extensive deposits were present in various organs in a fashion more akin to the chronic leukemias and lymphomas.

Our studies of DNA and IgE synthesis were undertaken to characterize the malignant IgE clone further. Similar clinical observations in both patients with IgE myeloma suggest to us that properties common to IgE-producing clones might underlie the leukemic manifestations, rapid development of drug resistance, and short survival time. These observations may have relevance to normal production of IgE (and IgG), as myeloma clones appear to have immunosynthetic rates similar to normal clones. The normal total body immunoglobulin synthetic rate for IgE in vivo is but a fraction of that for IgG (0.02 mg/kg/day versus 33 mg/kg/day), but the average number of molecules produced per plasma cell is the same for IgE and IgG myeloma clones when measured in vitro. Therefore, if our determinations of immunoglobulin synthetic rates do apply to normal clones, we can then calculate that a normal adult has approximately 10^11 IgG-producing cells but only 10^8 IgE-producing cells.

Earlier in vivo studies of the incorporation of ^3^H-thymidine by myeloma cells (presumably IgG) showed a labeling index of 3.5% or less and a calculated generation time for myeloma cells of the order of 2 to 6 days. The labeling in vivo agrees with our observed in vitro labeling indices in IgG myeloma. In contrast, the studies of DNA synthesis in vitro in IgE myeloma, combined with our subsequent studies of an additional patient with plasma cell leukemia, indicate that more of the tumor cells may be in DNA synthesis in these variant situations. Although studies of ^3^H-thymidine incorporation were not performed on fresh bone marrow from the initial patient with IgE myeloma (N.D.), his cells have proliferated in tissue culture for approximately 3 yr, study of these cells also showed a high thymidine labeling index. The higher labeling indices in these three instances mean that there is either a longer S phase, a shorter cell cycle time, or fewer tumor cells not in the cell cycle. Since it is unlikely that lengthening of the S phase would result in this large an increase in the labeling index, the results are probably due to other factors (that is, the proliferative rate might be higher). Further clarification of these alternatives would require in vivo thymidine studies and analysis of generation time.

^*Current investigations of cells producing monoclonal IgA and IgM by one of us (S.E.S.) suggest that these cells have similar immunosynthetic rates for their respective globulins.*
In any case, increased thymidine labeling does indicate that more cells are in DNA synthesis, and this may have considerable clinical significance for patients with plasma cell leukemia. Evidence for an increased fraction of plasma cells in DNA synthesis provides a rationale for the use of antimetabolites that damage cells during the mitotic cycle, even though these agents appear to be of limited usefulness as initial treatment in more typical myeloma. Indeed, recent studies of experimental plasmacytoma in the hamster support this contention.16

Serial estimates of tumor cell number may provide insights on a series of questions: (1) How large is a disseminated tumor at the time of its detection? (2) What is its mode of growth? (3) What is the relationship between extent of disease and symptomatology? (4) What is the quantitative effect of a given form of treatment? Two of these questions have now been at least partially answered.

The tumor cell number in the IgE patient was in the same range as that found in the advanced IgG cases. The general concordance of tumor cell estimations in these two myeloma variants may provide evidence for the general applicability of determinations of tumor cell number in myeloma.

An attempt was made to assess the patient's total body tumor cell number serially by means of repeated serum electrophoretic measurements. Although such results are only approximate, and in this case are limited in interpretation by the plasmaphereses, these explorations may nonetheless serve as a model for a useful form of evaluation for patients with the more common monoclonal malignancies. For example, in IgG myeloma, it would appear that sufficient knowledge of the immunoglobulin's catabolism exists7 so that a "synthetic index" could be calculated routinely from the serum concentration of the M-component, the plasma volume, and the concentration-dependent fractional catabolic rate. An index of this type should provide significantly more accurate serial assessments of changes in amount of tumor than does the uncorrected estimate derived from serum electrophoresis. For example, it might be possible to derive the mode of growth of the tumor, and to assess more accurately the magnitude of reduction in tumor size with induction of a clinical remission. Should serial measurements of synthetic index and tumor cell number prove to be more useful than existing techniques for predicting remission, relapse, or survival, they would then likely be brought into routine clinical use.

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15. —: Personal communication.

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