Primary Myeloma Cells Growing in SCID-hu Mice: A Model for Studying the Biology and Treatment of Myeloma and Its Manifestations

By Shmuel Yaccoby, Bart Barlogie, and Joshua Epstein

Progress in unraveling the biology of myeloma has suffered from lack of an in vitro or in vivo system for reproducible growth of myeloma cells and development of disease manifestations. The SCID-hu mouse harbors a human microenvironment in the form of human fetal bone. Myeloma cells from the bone marrow of 80% of patients readily grew in the human environment of SCID-hu mice. Engraftment of myeloma cells was followed by detectable human Ig levels in the murine blood. Myeloma-bearing mice had high levels of monoclonal human IgS, high blood calcium levels, increased osteoclast activity, and severe resorption of the human bones. The human microenvironment was infiltrated with Epstein-Barr virus-negative monoclonal myeloma cells of the same clonality as the original myeloma cells. Active angiogenesis was apparent in areas of myeloma cell infiltration; the new endothelial cells were of human origin. We conclude that the SCID-hu mouse is a favorable host for studying the biology and therapy of myeloma and that a normal bone marrow environment can support the growth of myeloma cells.

MATERIALS AND METHODS

Myeloma cells. Heparinized bone marrow aspirates were obtained from patients with active myeloma during scheduled clinic visits. Signed informed consents were obtained and are kept on record. Relevant patient information is provided in Table 1. The samples were separated using ficoll hypaque centrifugation (Histopaque; Pharmacia, Uppsala, Sweden). The proportion of myeloma cells in the light-density cell preparations (specific gravity ≤1.077 g/mL) was determined using CD38/CD45 flow cytometry. When indicated, myeloma cells were sorted on the basis of CD38/CD45 expression. The myeloma clone in each sample was characterized by CDRIII polymerase chain reaction (PCR).

SCID-hu system. CB.17/Ic/SCID mice were obtained from Harlan Sprague Dawley and were housed and monitored in our animal facility. All experimental procedures and protocols had been approved by the Institutional Animal Care and Use Committee. SCID-hu mice were generated as reported. Before inoculation of myeloma cells, the mice were exposed to 150 rad X-irradiation, using a model 143-45 Irradiator. Cesium source, at a rate of 125 rads/min. Light-density bone marrow cells (1.5 to 10 × 10⁶) were injected directly into the human bone in the SCID-hu mice in a final volume of 25 to 50 µL of phosphate-buffered saline (PBS). An increase in the levels of circulating monoclonal human Ig (hlg) of the M protein isotype was used as an indicator of myeloma cell growth.

Determination of hlg levels. Levels of human IgG, IgA, κ, and λ light chains were determined by enzyme-linked immunosorbent assay (ELISA). Antibodies were purchased from The Binding Site (San Diego, CA). Plates were coated with 50 µL/well of primary antihuman κ and λ (5 µg/mL) and antihuman IgA and IgG (10 µg/mL) and incubated overnight at 4°C. The plates were washed three times in PBS containing 0.5% (vol/vol) of Tween 20 and washed one more time with blocking buffer containing 4% bovine serum albumin (BSA) in PBS. Serial dilution of samples in PBS-containing 1% BSA (50 µL/well) were incubated at room temperature for 2 hours. Standards consisting of each purified Ig were added to the appropriate plates at concentrations ranging from 0.4 to 300 ng/mL. After washing three times with PBS/Tween, plates were incubated with 50 µL/well of biotinylated Ab (affinity-purified antihuman κ and light chains at 0.5 µg/mL) and antihuman IgA and IgG at 0.2 µg/mL) for 1 hour. Fifty microliters per well of streptavidin-horseradish peroxidase were added to each well after washing and allowed to bind for 1 hour. After a final washing, 50 µL/well of OPD solution (DAKO, Carpinteria, CA) containing 3% H₂O₂ was added. Absorbance at 450 nm was determined on a Auto-Reader II ELISA reader (Ortho Diagnostic Systems, Raritan, NJ).

Methods of analysis. Tissues and organs recovered from SCID-hu mice were processed as reported. Myeloma cells were identified morphologically by immunohistochemical staining for cytoplasmic Ig (cIg; DAKO immuno peroxidase kit) and their clonality was determined.

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by in situ hybridization with patient-specific probes (ASO-ISH). Changes in bone remodeling were identified by x-radiography and by increased osteoclast activity as demonstrated by immunohistochemical staining for tartarate-resistant acid phosphatase (TRAP; Sigma, St Louis, MO). Immunohistochemical staining with a monoclonal antibody to CD34 (Cell Marque, Austin, TX) was used to demonstrate neo-vascularization as an indicator for the presence of a human microenvironment in areas of myeloma cell growth.

**Determination of calcium.** Calcium levels were determined using a calcium determination kit (Sigma Diagnostics) according to the manufacturer’s recommendations.

**In situ hybridization.** Clonality of the tumor cells grown in SCID mice was demonstrated by in situ hybridization using an adaptation of published methods.25,26 Antisense oligonucleotide sequences (24–32 bp) complementary to CDR III regions of the myeloma clone of each patient were biotinylated during synthesis (Life Technologies, Rockville, MD). Tissue sections were dewaxed in xylene, defatted in chloroform, and then rehydrated. The sections were treated with proteinase K (10 µg/mL in Tris-HCl buffer at 37°C for 1 hour). Hybridization mixture containing 50% formamide, 10% dextran, 4× SSC (SSC is 150 mmol/L NaCl, 15 mmol/L trisodium citrate, pH 7), 25 mg/mL herring sperm DNA, and biotinylated probe (1 to 2 µg/mL) was applied to each section. The sections were covered with Parafilm and incubated in a humidified chamber overnight at 37°C. After hybridization, the slides were washed twice with 1× SSC at room temperature and twice with 1× SSC at 37°C to 42°C (depending on the size of the probe). Signals were visualized using an in situ hybridization kit (DAKO). Specificity was determined by using irrelevant patient probes and sections from different patients.

**Screening for Epstein-Barr virus.** Myeloma cells recovered from SCID-hu mice were analyzed for the presence of EBV sequences by PCR.27 All samples were negative.

### RESULTS

Bone marrow cells from 15 patients were studied in the SCID-hu system. Mice inoculated with cells from 12 patients had detectable circulating hIg 2 to 19 weeks after inoculation. Two mice had no detectable hIg even at 23 and 29 weeks, and one mouse, inoculated with cells from a patient with nonsecreting myeloma, also had no detectable circulating hIg (Table 1). In these 3 cases, myeloma growth could not be detected by histological and immunohistochemical examinations. With two

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Stage*</th>
<th>Prior Therapy</th>
<th>Isotype</th>
<th>Cells (&gt;10^6) inoculated†</th>
<th>Plasma Cells (%)‡</th>
<th>Weeks to hIg Detection§</th>
<th>Weeks to End of Experiment</th>
<th>Final hIg Levels (µg/mL)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>III A</td>
<td>21 mo</td>
<td>IgG k</td>
<td>3</td>
<td>78</td>
<td>2</td>
<td>17</td>
<td>IgG = 657, k = 458</td>
</tr>
<tr>
<td>2</td>
<td>III A</td>
<td>2 mo</td>
<td>IgA k</td>
<td>3</td>
<td>40</td>
<td>19</td>
<td>25</td>
<td>IgA = 504, k = 341</td>
</tr>
<tr>
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<td>III A</td>
<td>None#</td>
<td>IgG k</td>
<td>3</td>
<td>26</td>
<td>9</td>
<td>36</td>
<td>IgG = 130, k = 330</td>
</tr>
<tr>
<td>4</td>
<td>III A</td>
<td>12 mo</td>
<td>IgA k</td>
<td>2</td>
<td>35</td>
<td>6</td>
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<td>IgA = 365, k = 1415</td>
</tr>
<tr>
<td>5</td>
<td>II A</td>
<td>26 mo</td>
<td>Nonsecretor</td>
<td>4</td>
<td>20</td>
<td>29</td>
<td>None detected</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>III A</td>
<td>None</td>
<td>k light chain</td>
<td>5</td>
<td>75</td>
<td>9</td>
<td>29</td>
<td>Ig = 560, k = 230</td>
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<tr>
<td>7</td>
<td>I A</td>
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<td>IgG k</td>
<td>10</td>
<td>11</td>
<td>2</td>
<td>15</td>
<td>IgG = 16, k = 200</td>
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<tr>
<td>8</td>
<td>III B</td>
<td>None</td>
<td>k light chain</td>
<td>1.5</td>
<td>42</td>
<td>36</td>
<td>None detected</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>III A</td>
<td>None</td>
<td>IgA k</td>
<td>3</td>
<td>35</td>
<td>&gt;23**</td>
<td>None detected</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>II A</td>
<td>4 mo</td>
<td>IgA λ</td>
<td>5</td>
<td>25</td>
<td>4</td>
<td>&gt;15</td>
<td>IgG = 88, IgA = 173</td>
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<tr>
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<td>IgG λ</td>
<td>5</td>
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<td>4</td>
<td>&gt;15</td>
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<tr>
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<td>4 mo</td>
<td>IgG k</td>
<td>6</td>
<td>20</td>
<td>2</td>
<td>12</td>
<td>IgG = 2100, k = 3600</td>
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<tr>
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<td>None</td>
<td>IgG k</td>
<td>5</td>
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<td>3</td>
<td>&gt;12</td>
<td>IgG = 2100, k = 1700</td>
</tr>
<tr>
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<td>IgG k</td>
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<td>23</td>
<td>2</td>
<td>&gt;7</td>
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<tr>
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<td>Nonsecretor</td>
<td>5</td>
<td>89</td>
<td>3</td>
<td>&gt;3</td>
<td>IgG = 156, k = 334</td>
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</tbody>
</table>

| *Stage according to the Durie Salmon staging. †Total number of bone marrow cells inoculated. ‡Percentage of plasma cells in inoculates as determined by CD38/CD45 flow cytometry. §Time from myeloma bone marrow cells inoculation, measured in weeks. ¶Experiments were ended when monotypic hIg levels were $\geq 200$ µg/mL. ‡Human Ig levels in the mouse serum at the end of experiment as measured by ELISA. #Newly diagnosed patients. **Experiment still underway. |
exceptions, the circulating hIg was identical to the M protein isotype in terms of both the heavy and light Ig chains; for 1 patient with IgA λ myeloma, all 4 isotypes were expressed at 15 weeks (patient no. 10), and in 1 patient with nonsecretory myeloma, IgG κ was found at 3 weeks (patient no. 15). In this latter patient, the original myeloma cells contained IgG κ cytoplasmic Ig (data not shown). The kinetics of the increase of hIg levels varied among patients; examples are presented for 3 of these patients in Fig 1. Although the number of patients studied is too small to draw firm conclusions, there was no apparent correlation between the time to detection of hIg and the number of myeloma cells inoculated, marrow plasmacytosis, or any other patient characteristic, whether analyzed for the whole group or only for the 6 patients with IgG κ myeloma (Table 1).

Further analysis is available only for the 8 patients for whom the experiments have been completed. Histological examination of decalcified sections of the human bone showed extensive infiltration of plasma cells (Fig 2). The cells uniformly contained monotypic clg (Fig 3), coexpressed Ig heavy and light chains, and reacted homogeneously and exclusively with the patient’s specific ASO (Fig 4), indicating their clonal identity with the original patient’s myeloma cells. In all cases, myeloma plasma cells were found only in the human bone. Neither myeloma cells nor any other human cells were detected in any of the murine organs, bone marrow, or blood, as determined histologically, and by CD38/CD45 and HLA-ABC flow cytometry.

Growth of the myeloma cells was associated with increased blood Ca levels (summarized in Fig 5), suggesting anomalies in bone remodeling. Immunohistochemical examination of the kidneys showed varying amounts of light chain deposits (not shown). Decalcified bone sections stained for TRAP showed markedly increased osteoclast activity (Fig 6). Severe loss in human bone density was readily visible upon x-ray examination in all cases. Although density loss was severe in all mice, the degree of human bone resorption varied. An example depicting a moderate level of density loss is presented in Fig 7.

Expression of the endothelial cell antigen CD34 in a decalcified section of the human bone is demonstrated in Fig 8. An active vascularization process is readily visible. In all cases, neo-vascularization was restricted to areas infiltrated with myeloma cells and was not visible in the human bones of control SCID-hu mice, suggesting that vascularization occurred in response to the presence of myeloma cells.

**DISCUSSION**

Primary myeloma cells, hitherto considered not capable of sustained proliferation when removed from the patient, readily grew in the human bone of SCID-hu mice. Interestingly, no
other human hematopoietic cells were detectable in the murine bone marrow and blood. Growth of myeloma cells was restricted to the human environment, similar to reports with primary leukemic cells and very different from myeloma cell lines that readily grow in SCID mice and disseminate out of the human bone microenvironment in the SCID-hu system.

Myeloma growth was accompanied by increased osteoclast activity and resorption of the human bone, a typical manifesta-
tion of myeloma. Increases in osteoclasts were also observed in the murine bones (femurs and vertebrae; data not shown). However, loss in density of murine bones was not discernible on x-radiograms.

As in other malignancies, vascularization is an important part of myeloma growth. In the SCID-hu system, newly formed blood vessels were made of human cells and were visible only in areas infiltrated with myeloma cells. These endothelial cells could originate from dormant stem cells in the implanted human bone or, alternatively, from the myeloma patients’ bone marrow. Because primary myeloma cells cannot grow in SCID mice (23 failed attempts, including some samples that grew in the SCID-hu system; data not shown), it appears that a normal human bone environment can support the growth of primary myeloma cells from bone marrow aspirates of patients with myeloma. Studies are underway to determine the source of the endothelial and other cells that constitute the human microenvironment in the SCID-hu mice and whether purified myeloma plasma cells will also engraft in this model.

The SCID-hu mouse provides a favorable host environment for reproducible growth of primary myeloma cells. This system is a suitable model for studying the biology of myeloma, its treatment, and manifestations.

**REFERENCES**


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