Establishment of Two New Myeloma Cell Lines From Bilateral Pleural Effusions: Evidence for Sequential In Vivo Clonal Change

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Two new human myeloma cell lines have been established from a 36-year-old woman with refractory IgGx multiple myeloma in whom bilateral malignant pleural effusions developed. The malignant plasma cells from each effusion were set up in a liquid culture using an L-15 medium containing catalase, glutathione, selenious acid, ascorbic acid, insulin, transferrin, additional glutamine hydrocortisone, and 2-mercaptoethanol and designated as M-3 medium. Two IgGx cell lines, LB-831 and LB-832, were established and proved to be Epstein-Barr virus negative using the internal repeat sequence DNA probe. Characteristic plasma cell morphology was evident by light and electron microscopy. Immunotyping revealed an IgG, B+, D2-, La (HLA-DR)+, CALLA+ phenotype for each cell line as well as for the original pleural fluid and bone marrow.

CASE REPORT

A 36-year-old woman presented in June 1983 with a short history of lower back pain and clinical findings suggestive of spinal cord compression at the lumbar 4–5 level. A plasmacytoma was found at laminectomy and subsequent evaluation confirmed a diagnosis of multiple myeloma of the IgGx type. The serum contained 4.6 g/dL IgGx monoclonal protein, and the bone marrow contained 60% plasma cells, many immature and with atypical morphology. Skeletal x-rays revealed multiple lytic lesions. Serum creatinine and calcium levels were set up in a liquid culture using an L-15 medium containing catalase, glutathione, selenious acid, ascorbic acid, insulin, transferrin, additional glutamine hydrocortisone, and 2-mercaptoethanol and designated as M-3 medium. Two IgGx cell lines, LB-831 and LB-832, were established and proved to be Epstein-Barr virus negative using the internal repeat sequence DNA probe. Characteristic plasma cell morphology was evident by light and electron microscopy. Immunotyping revealed an IgG, B+, D2-, La (HLA-DR)+, CALLA+ phenotype for each cell line as well as for the original pleural fluid and bone marrow.
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then frozen in aliquots for future baseline evaluation such as viral studies and cytogenetics, as needed. The rest of the cells were used for studies of thymidine incorporation, cytochemistry, immunologic phenotype, and colony culture, as well as being set up in routine tissue culture conditions in Falcon 50-mL tissue culture flasks (Becton Dickinson Labware, Oxnard, Calif). The initial cell concentration in the Falcon culture flasks was $5 \times 10^5$ cells per milliliter of medium. The standard medium used was RPMI 1640 medium supplemented with 15% fetal calf serum, 100 IU/mL of penicillin, and 50 µg/mL of streptomycin. The medium was changed approximately twice weekly. During early growth, an autologous adherent feeder layer became established, which was essential for initial passaging of both LB-831 and LB-832 until well established. Initial growth was poor, but improved significantly in parallel culture flasks using a specially prepared M-3 medium. This medium incorporates glutamine, and 2-mercaptoethanol, as outlined in Table I. M-3 medium was based upon prior experience as well as previously published results. After approximately three months, both LB-831 and LB-832 were well established and could be maintained using M-3 medium supplemented with fetal calf serum. Aliquots from each passage were frozen for future study.

Cytogenetic Studies

Cells from the original pleural fluids and the LB cell lines were suspended in 5 mL of fresh RPMI 1640 medium (GIBCO, Grand Island, NY) containing 15% fetal calf serum, l-glutamine, penicillin, and streptomycin for 24 hours. Colcemid (GIBCO, Lawrence, Mass) (10 µg/mL) was added for the last one hour of incubation, with cells harvested and banded as previously described. Based upon previously published confidence limits, a minimum of 75 metaphases from each cell line were examined for this study. Comparisons were made between (1) initial direct preparations, (2) short-term (four- to seven-day) cultures, and (3) cells from the established LB-831 and LB-832 cell lines. To illustrate the major stem line karyotype of both the direct and culture material, a G-banded karyotype from passage 10 of the LB-831 cell line was used.

Transmission Electron Microscopy

The myeloma cells from LB-831 and LB-832 were initially fixed in suspension for one hour at room temperature with 1.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) and then spun in conical plastic tubes at 2,500 rpm for ten minutes in a Sorvall (Dupont, Wilmington, Del) GLC-1 swinging-bucket table-top centrifuge. The resulting pellets were fixed with 3% glutaraldehyde in 0.1 mol/L phosphate for one hour at 4 °C and then postfixed for 1/2 hours in 1% OsO4 in a 0.1 mol/L phosphate buffer. The pellets were dehydrated through a graded series of ethanol and embedded in Spurr's (Electron Microscopy Sciences, Ft Washington, Pa) low-viscosity epoxy resin. One-micron sections were cut and stained with toluidine blue and examined with a light microscope. Thin sections were cut with a DuPont diamond knife using a Dupont/Sorvall MT-5000 ultramicrotome and mounted on uncoated 200-mesh copper grids. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Hitachi (Hitachi Scientific Instruments, Mt View, Calif) HU-12 electron microscope.

Cytochemistry

The myeloma cells were evaluated for acid phosphatase and β-glucuronidase (β-gluc) using routine methods as previously published.

Immunologic Marker Studies

Cyto centrifuge (Shandon Southern Instruments, Sewickley, Pa) slide preparations of the myeloma cell material were used for immunologic evaluation. Biotin-avidin conjugation and horseradish peroxidase labeling with diaminobenzidine tetrahydrochloride (DAB) as the detection agent were used, as previously reported. Briefly, cyto centrifuge slides were fixed in aceton at 4 °C for ten minutes, air-dried at room temperature, and then hydrated in

Fig 1. Diagrammatic plot of the viable cell number $v$ time in months for the myeloma cells from the right pleural effusion, which were used to establish the myeloma cell line LB-831. It can be seen that after the first six weeks the cell number in the RPMI culture medium drops off substantially. In contrast, the cell number in the M-3 medium was retained and started to increase subsequently to the three-month point.
phosphate-buffered saline (PBS) at pH 7.4 for ten minutes. Following this wash, mouse monoclonal antibody was applied to human cell surface (and/or cytoplasmic) antigen. All monoclonal antibodies were obtained from Becton Dickinson.

After a second PBS wash, a second-stage reagent, biotin-conjugated F(ab')2 goat anti-mouse IgG (Tago, Burlingame, Calif) was added, followed by a 15-minute incubation and a three-minute PBS wash. The cytocentrifuge slides were next incubated with avidin D conjugated with horseradish peroxidase (Vector Laboratories, Norwalk, Conn). After another 15-minute incubation period, slides were again washed twice with PBS. Next, each slide was incubated with a DAB solution consisting of 3 mg of DAB per milliliter of PBS, along with 0.010 mL of 30% H2O2. After the five-minute incubation in DAB solution, slides were washed in PBS for three minutes and rinsed in distilled water. Slides were incubated in a copper sulfate solution for five minutes, rinsed with PBS, dipped in distilled water, and counterstained with methylene blue for five minutes, followed by a brief 100% ethanol dip, two changes of xylene, and coverslipping with Permount (Fisher Scientific, Fair Lawn, NJ). The specific antigens evaluated were: B1, B2, Ia (HLA-DR [L243]), CALLA, terminal deoxynucleotidyl transferase (TdT), heavy chains, \( \gamma \) heavy chain, \( \mu \) heavy chain, \( \kappa \) light chain (163–42), and \( \lambda \) light chain (1-155-2).

RESULTS

Cell Culture, Morphology, and Cytochemistry

Cells from the first pleural effusion (LB-831) proliferated well in liquid culture from the outset. Within the first three weeks, however, a nonproliferating adherent layer of mesothelial cells and monocyte-macrophages attached to the plastic surface. This adherent layer was essential for early passaging; however, by approximately passage 10, the LB-831 myeloma cells were established and proliferated without any feeder in M-3 medium and subsequently had to be split twice per week to prevent overcrowding in the flasks. The plasma cell morphology was retained and the cells were positively staining for both acid phosphatase and \( \beta \)-gluc. A characteristic nuclear morphology with a clover-leaf shape present in both prior bone marrows and the original pleural fluid was retained (Fig 2). There was heterogeneity in size and shape of cells. Some cells were multinucleated, although this feature was more prominent in LB-832. With serial passaging of LB-831, there was an increasing trend to multinucleation, reaching \( \approx 20\% \) of cells in later passages.

In contrast, cells from the second pleural effusion (LB-832) did not proliferate well in culture initially. However, with establishment of the autologous adherent feeder layer and use of the M-3 medium described above, this cell line also became established, although continuing to proliferate much more slowly than LB-831. After multiple passages without the feeder layer, the more indolent nature of LB-832 was retained. The one-hour "flash labeling" (labeling index [LI\%]) with \([\text{H}]\)-thymidine was 55\% for LB-831 and 34\% for LB-832. Detailed cell cycle analysis of LB-831 compared to LB-832 using a BrDu/sister chromatid exchange technique is the basis for a separate publication.16,17 The generation time (Te) for LB-831 was 38.37 hours. The generation time for LB-832 has been more difficult to measure accurately, but was approximately ten hours longer. In contrast, using the same BrDu technique, the generation time for the 8226 myeloma cell line (ABJ type) was 26.4 hours. Both LB-831 and LB-832 grew in plate assays with good cloning efficiency, although colonies were hard to count because of diffuse growth. The plasma cell morphology of LB-832 remained obvious, many cells being bi- or multinucleated. Acid phosphatase and \( \beta \)-gluc were both found in LB-832, but the \( \beta \)-gluc was much weaker than for LB-831.

Karyotype Analysis

Both fresh and cultured myeloma cells from LB-831 and LB-832 were used for detailed karyotypic analysis. Although there was a tendency toward greater polyploidy with serial passaging, the patterns of chromosome changes for LB-831 and LB-832 were retained from fresh to cultured material. The stem line karyotype of passage 10 of the LB-831 cell line is illustrated in Fig 3. The chromosome abnormalities found

![Fig 2. The heterogenous myeloma cell morphology in the direct bone marrow aspirate (A), pleural fluid (B), and the LB-831 cell line (C and D). Cells with more typical plasma cell morphology are identified by the arrow heads (p), whereas the atypical multinucleate plasma cells (these are not megakaryocytes) are indicated by the full arrows (→). The LB-831 myeloma cells are immunotyped by the avidin-biotin/immunoperoxidase technique with \( x \) antisera (C), which demonstrates dense cytoplasmic staining and is strongly positive, in marked contrast with the \( \lambda \) antisera (D), which completely lacks cytoplasmic staining and is negative. The positivity with IgG, CALLA, HLA-DR, and B were of similar magnitude to the \( x \) positivity.](image-url)
Fig 3. Giemsa-banded karyotype from passage 10 of LB-831. The major cytogenetic abnormalities found in both LB-831 and LB-832 are illustrated in this karyotype. Partially identifiable structural marker chromosomes include: two translocations involving chromosome 1[t(1;15) and t(1;?)], an interstitial deletion of chromosome 3[p21;p25], an unidentified segment translocated to the long arm of chromosome 8(q35), a putative HSR at band p22 of chromosome 7, and a t(13;13) marker chromosome. A number of unidentifiable markers which were detected in the karyotypes of LB-831 and/or LB-832 are shown at the bottom. Examples of unidentifiable markers that were common to both cell lines and other unidentifiable markers that were unique to either LB-831 or LB-832 are presented.

to be clonal in both LB-831 and LB-832 are summarized in Table 2, whereas those clonal alterations differing between these lines are described in Table 3. Although most of the clonal abnormalities (structural, markers, and numeric changes) present in LB-831 were also seen in LB-832, there were changes in marker frequencies, with three markers (M7, M8, and M9 [rings]) found exclusively in LB-832, as listed in Table 3. As stated previously, these clonal markers were observed in both the direct material and the established cell lines. Particularly striking was a large putative homogeneously staining region (HSR) on chromosome 7p present in both LB-831 and LB-832. Of further interest, no structural changes were noted in chromosomes 2, 14, or 22—those involved with immunoglobulin synthesis—although there was trisomy of chromosome 14.

Immunotyping Studies

Immunotyping of direct patient specimens as well as cells from culture was carried out. The myeloma cells from all the direct and cultured preparations had the same phenotype: IgG, κ, B1, + Ia (HLA-DR)+, and CALLA + (Fig 2). All cells were negative for λ, IgM, IgA, B2, TdT, and the whole range of T cell antigens. Specimens tested included fresh

Table 2. Clonal Structural and Numeric Chromosome Abnormalities Present in Both LB-831 and LB-832

<table>
<thead>
<tr>
<th>Structural Alterations</th>
<th>Numeric Alterations</th>
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<tbody>
<tr>
<td>t(1;7)(q32;?)</td>
<td>+2</td>
</tr>
<tr>
<td>t(1;15)(p11;p11)</td>
<td>+3</td>
</tr>
<tr>
<td>del(3)(p21;p26)</td>
<td>+4</td>
</tr>
<tr>
<td>del(5;7)(q35;?)</td>
<td>+5</td>
</tr>
<tr>
<td>t(7;7)(p22;?)</td>
<td>+6</td>
</tr>
<tr>
<td>del(7;7)(p15;.)</td>
<td>+8</td>
</tr>
<tr>
<td>t(7;7)(p22;7)</td>
<td>-9</td>
</tr>
<tr>
<td>?HSR 7(p22)</td>
<td>+10</td>
</tr>
<tr>
<td>?HSR 7(p22;7)</td>
<td>+11</td>
</tr>
<tr>
<td>t(7;7)(p22;7)</td>
<td>+14</td>
</tr>
<tr>
<td>del(7;7)(p15;?)</td>
<td>+16</td>
</tr>
<tr>
<td>del(7;7)(p15;?)</td>
<td>+17</td>
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<tr>
<td>del(7;7)(p15;7)</td>
<td>+18</td>
</tr>
<tr>
<td>del(7;7)(p15;7)</td>
<td>+19</td>
</tr>
<tr>
<td>del(7;7)(p15;7)</td>
<td>+20</td>
</tr>
<tr>
<td>inv(11)(p11;q13)</td>
<td>-22</td>
</tr>
</tbody>
</table>

Table 3. Clonal Chromosomal Abnormalities Differing Between LB-831 and LB-832

<table>
<thead>
<tr>
<th>Structural alteration</th>
<th>Percentage of Cells With Change</th>
</tr>
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<tbody>
<tr>
<td>t(7;7)(p22;7)</td>
<td>LB-831 72 27</td>
</tr>
<tr>
<td>inv(11)(p11;q13)</td>
<td>LB-832 54 9</td>
</tr>
<tr>
<td>M4</td>
<td>LB-831 45 9</td>
</tr>
<tr>
<td>M5</td>
<td>LB-831 27 0</td>
</tr>
<tr>
<td>M6</td>
<td>LB-831 35 9</td>
</tr>
<tr>
<td>M7</td>
<td>LB-831 0 45</td>
</tr>
<tr>
<td>M8</td>
<td>LB-831 0 54</td>
</tr>
<tr>
<td>M9 (rings)</td>
<td>LB-831 0 45</td>
</tr>
</tbody>
</table>
bone marrow cells, myeloma cells in the cerebrospinal fluid (CSF), plus both the right and left pleural effusion cells taken directly from the patient. The culture material (LB-831 and LB-832) has retained the same phenotype after serial passaging. A minor exception was with respect to CALLA positivity. Cell line LB-831 was tested five times for CALLA with serial passaging and was positive four times. On the remaining occasion (passage 7) LB-831 was CALLA –, although the rest of the phenotype was unchanged. In subsequent passages CALLA positivity was again evident.

**Secreted Products and Other Testing**

The cell line material LB-831 and LB-832 has been tested for secretion of immunoglobulin, β2 microglobulin, and bone-resorbing activity. The monoclonal IgGκ secretory rates for LB-831 and LB-832 were measured on several occasions. Quantitative radioimmunoassay determinations of the cumulative amounts of IgGκ released into the supernatant medium by 72-hour cultures yielded an average of $6.9 \times 10^4$ ng per $10^6$ viable myeloma cells. The range of IgGκ content was 2.4 to $7.15 \times 10^4$ ng per $10^6$ myeloma cells. β2 microglobulin was also readily detected in the supernatants with approximate secretory rates of 5 to 10 pg per cell per day. The cells from LB-831 were also tested for epidermal growth factor (EGF) receptors and were completely negative. Initial testing for mycoplasma was also negative. The assay for bone-resorbing activity (osteoclast activating factor [OAF]) was carried out by Dr Gregory Mundy (University of Texas, San Antonio). Very high levels of OAF were detected in the supernatant fluids tested using the standard bioassay system.18

**Electron Microscopy Results**

**LB-831 culture.** The neoplastic cells had the typical appearance of myeloma cells at various stages of development. Many cells appeared plasmacytoid, with abundant rough endoplasmic reticulum arranged in parallel stacks (Fig 4A). Many cells displayed broad bands of microfila-

![Fig 4. Electron micrographs of myeloma cells from culture. (A) LB-831. The nucleus is bilobed and contains sparse heterochromatin and a prominent nucleolus (Nu). The cytoplasm has a typical plasmacytoid appearance with the rough endoplasmic reticulum arranged in parallel stacks (arrow). Filiform mitochondria and lysosomal-like bodies are present (uranyl acetate, lead citrate; original magnification ×9,800, current magnification ×9,880). (B) LB-832. The nucleus has a polylobated appearance. Five distinct lobes can be observed in this cellular profile. The heterochromatin is more abundant than in A (uranyl acetate, lead citrate; original magnification ×6,100, current magnification ×3,660).](https://www.bloodjournal.org/content/101/3/552.full)

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ements that were predominantly found in a perinuclear location. The mitochondria were unremarkable and displayed both orthodox and condensed configurations. The nuclei had sparse heterochromatin and contained large prominent nucleoli with a characteristic nucleolomere. The nuclear profiles were quite variable in appearance.

**LB-832 culture.** This myeloma culture line was morphologically similar to LB-831 and had a distinct plasmacytoid appearance. Although microfilaments were present, their arrangement into broad bands was not as evident as in LB-831. The nuclear profiles were also quite variable. Nuclei with a distinct polylobated experience (Fig 4B) were, however, much more frequently observed in LB-832 compared with LB-831.

**Epstein-Barr Virus (EBV) Testing**

Both the initial pleural fluid material and the cell lines LB-831 and LB-832 were either negative or very weakly positive (<5% cells) when tested with the fluorescent Epstein-Barr nuclear antigen (EBNA) method. When tested with the specific EBV DNA probe (internal repeat sequence, BamHI enzyme restricted [Dr. J. Pagano, Duke University, Durham, NC]), all of the material was consistently negative.

**DISCUSSION**

The data presented confirm the establishment of two new myeloma cell lines. Routine light microscopy, cytochemistry, and electron microscopy indicated a plasma cell morphology that was retained in long-term culture. Although the presence of abundant stacks of rough endoplasmic reticulum in the cytoplasm of the tissue culture cells is consistent with both reactive and neoplastic plasma cells, the sparse heterochromatin pattern and prominent nucleoli are indicative of a young nucleus. Bernier and Graham believe that this nuclear cytoplastic asynchrony is specific for the malignant immunoproliferative disorders. The striking nuclear polylobation observed in the myeloma cell line LB-832 is most probably a reflection of the polyplid nature of many of these cells. Polylobation or hypersegmentation of the nucleus has previously been reported in myeloma cells and in the neoplastic plasmacytoid cells present in malignant lymphomas of follicle center cells. The broad bands of cytoplastic microfilaments that were present in culture LB-831 are a characteristic feature of normal monocytes and macrophages and are not commonly observed in normal plasma cells. Their presence is probably a reflection of the neoplastic nature of the myeloma cells and has previously been reported in a solitary myeloma. Abundant microfilaments may be related to increased cell surface activity inasmuch as we have recently described these broad bands as a prominent feature of phagocytic myeloma cells.

The in vitro synthesis of IgGx immunoglobulin, β, microglobulin, and OAF also indicated a myeloma cell phenotype. The negative results with the specific DNA probe for EBV were important to exclude the development of transformed lymphoblastoid cell lines. These two new EB negative cell lines increase the number currently available for biological studies from nine to 11. However, of the nine previously available, only two or three have retained a stable plasma cell phenotype in vitro and good potential as hybridoma fusion partners. Although LB-831 and LB-832 seem likely to result in 8-azaguanine-resistant sublines, their true value as fusion partners remains to be established.

The use of M-3 medium was clearly important in the early establishment of LB-831 and LB-832. In comparison with routine RPMI 1640 medium (Fig 1), after the initial one to two months, the growth in M-3 was significantly better. For LB-831 a low level of growth continued in RPMI 1640; however, for LB-832 the establishment of maintained growth without a feeder layer was possible only with M-3. The exact combination of constituents in M-3 was based upon extensive prior experience (Leibovitz2) and published data indicating enhanced growth of B cells or plasma cells in the presence of catalase, ascorbic acid, insulin, selenium, transferrin, and 2-mercaptoethanol. Continued use of the M-3 medium has resulted in the establishment of several additional myeloma cell lines (in less than one year), the full characterization of which is currently in progress. This degree of success is clearly better than in any prior experience.

The complete immunologic phenotype of the two cell lines, LB-831 and LB-832—IgGx, B+, HLA-DR+, CALLA+—is of considerable interest. Myeloma cells are generally considered to be mature B cells although some data have suggested that plasma cells and/or myeloma stem cells may be pre-B cells or even more immature cells in the B cell series. LB-831 and LB-832 were not pre-B cells (c IgM−, S Ig−); however, the HLA-DR positivity and CALLA positivity (normally associated with acute lymphoblastic leukemia) were consistent features. Additionally, the direct bone marrow, pleural fluid, and colony-stimulating factor (CSF) cells all showed this same immature phenotype. Thus, the malignant plasma cells in this patient with clinically aggressive myeloma demonstrated in vitro colony growth and cell line growth (self-renewal) properties and had a combined mature plasma cell and immature B cell phenotype. This range of phenotypic expression is similar to that recently found in other B cell malignancies such as non-Hodgkin's lymphoma. In 1982, three patients have been found to have this same CALLA+/HLA-DR+ plasma cell phenotype associated with very aggressive disease (survival ≤6 months) and form the basis for a separate report.

The clinical phenotype includes both extramedullary and CNS disease.

The malignant nature of the cell lines, LB-831 and LB-832, is strongly supported by the very abnormal karyotypes. The specific details have several interesting aspects. First, in marked contrast to many B cell neoplasms, no structural abnormalities were noted involving the regions of chromosomes 2, 14, or 22—encoding genes important in immunoglobulin synthesis. However, the involvement of chromosomes 2, 14, or 22 as a portion of the unidentified markers could not be absolutely excluded. Of possible interest is that trisomy for chromosome 14 was noted in both LB-831 and LB-832. Those chromosomes most frequently involved in structural abnormalities in both cell lines included 1, 3, 5, 7, 13, and 15 (Table 2). Alterations, particularly of chromosomes 1, 3, and 7, have frequently
been observed in multiple myeloma as well as in several other hematologic malignancies.  

The presence of the putative homogeneously staining region (HSR) on chromosome 7p is of particular interest because several specific DNA sequences have been localized to this area, including the c-erb-B oncogene, and T cell antigen receptor (which has some homology with immunoglobulin), and a recently recognized immortalizing or oncogenic sequence. Preliminary studies in our laboratory have been performed on the LB-831 and LB-832 cell lines using the blot hybridization technique of Southern to evaluate whether amplified oncogene sequences were detectable. Oncogenes tested that are not amplified in these lines include c-myc, B-lym, N-ras, K-ras, H-ras, c-abl, and c-sis. Further study of the cell lines for analysis by the Roninson gel assay technique also failed to demonstrate the presence of significant amplification of DNA domains (above 20 copies). Further testing is currently underway to determine whether the putative HSR represents the site of an amplified domain.

The additional chromosome changes noted in LB-832 not present in LB-831 are also worthy of comment. The additional presence of ring and marker chromosomes as well as the numeric differences and polyploidy in LB-832 were confirmed to be statistically significant by a review of >75 metaphases from both LB-831 and LB-832. Therefore, there was strong evidence for in vivo sequential clonal change with disease progression in this patient who retained the same IgGx, B1+, CALLA+, HLA-DR+ phenotype throughout. The additional changes in LB-832 occurred after the course of cytosine arabinoside therapy. Since this agent has not been reported to cause direct DNA damage, the new cytogenetic abnormalities were not felt to be directly attributable to that therapy. In addition, the chromosome changes were observed in both direct and culture material; this finding represents, as far as we know, the best documentation of this type of sequential clonal evolution in human myeloma, thus strongly supporting the recently proposed concept of clonal heterogeneity in myeloma. Similar cytogenetic heterogeneity with tumor progression has been reported in other types of malignancy such as, for example, ovarian carcinoma.

In conclusion, the data presented document the establishment of two new human myeloma cell lines that have retained a whole range of myeloma cell properties. Interesting and important aspects of these cell lines include the use of the new M-3 medium, the documentation of an unusual immunotype (HLA-DR and CALLA positivity), cytogenetic abnormalities including the presence of an HSR on chromosome 7p+, and evidence of sequential clonal change. These results provide new insights into the biology of myeloma and also make two new cell lines available for future studies.

ACKNOWLEDGMENT

We gratefully acknowledge the help of Paul Finley, MD, and David Lucas, PhD, in qualitative and quantitative evaluation of the monoclonal protein.

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