The Human Myeloma Cell Line LP-1: A Versatile Model in Which to Study Early Plasma-Cell Differentiation and c-myc Activation

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The characteristics of a human cell line (LP-1) derived from the peripheral blood of a patient with IgG-lambda myeloma in leukemic transformation are described. The cells resemble immature plasma cells in that they exhibit a membrane phenotype that is intermediate between late B lymphocytes and plasma cells, even though they secrete IgG-lambda chains. Treatment of LP-1 cells with 12-O-tetradecanoxyphorbol-13-acetate (TPA) or pokeweed mitogen (PWM) induces the appearance of surface markers and ultrastructural features typical of mature plasma cells but does not affect their proliferative activity. Molecular analysis of the cell line showed an increased expression of the c-myc protooncogene and the presence of abnormally sized transcripts. Conventional cytogenetics and pulsed-field gel electrophoresis showed no structural rearrangements of the c-myc gene, suggesting that the abnormal c-myc expression may be due to point mutations or small deletions within the gene. The LP-1 cell line is a useful model in which to study the process of B-cell maturation; such study may lead to the uncovering of unusual mechanisms of c-myc activation. Furthermore, the LP-1 cell is a potential partner in the generation of human hybridomas.

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CASE REPORT

A 56-year-old woman complaining of lower back pain was admitted to the hospital in January 1985 with a diagnosis of stage III A IgG-lambda multiple myeloma. She had multiple lytic bone lesions, and a bone-marrow tap revealed that she had 30% immature plasma cells. The patient reached a complete remission after six courses of chemotherapy with vincristine, melphalan, Cytoxan, (cyclophosphamide; Mead Johnson & Co, Evansville, IN), and prednisone alternating with vincristine, bleomycin, Adriamycin (doxorubicin; Adria Laboratories Inc, Dublin, OH), and prednisone. In November 1985 the patient presented with pain in the right shoulder. Computerized axial tomography (CAT) showed a tumor mass infiltrating the mediastinum and the shoulder. A biopsy indicated an extensive muscular infiltration of IgG-lambda immunoblastic and plasmablastic cells, with no evidence of bone-marrow relapse. In spite of intensive salvage chemotherapy, the disease progression was marked by the appearance of immature plasma cells in the peripheral blood (March 1986). One month later the patient died of overt plasma-cell leukemia.

MATERIALS AND METHODS

Cell culture. The leukemic cells were collected from samples of the patient’s peripheral blood in the terminal phase of the disease and subjected to separation on a Lymphoprep density gradient (Nyegaard Co, Oslo, Norway) for the removal of erythrocytes and granulocytes. The cells recovered at the interface (>90% plasma cells) were maintained in culture at a concentration of 1 x 10⁶/mL in Iscove-modified Dulbecco’s medium (IMDM) containing 20% fetal calf serum (Gibco Laboratories, Grand Island, NY), to which 5 µg/mL transferrin and 5 µg/mL porcine insulin (Sigma Chemical Co, St Louis) were added.

The cell line was determined to be free of mycoplasma contamination after colony formation in an agar-broth selective medium.
(MycoTrim-TC, Hana Media, Inc, Berkeley, CA), microscopic study with fluorescent Hoechst 33258 stain, and incorporation of 3H-thymidine by culture supernatant. The cultures were maintained at 37°C in 5% CO₂.

**Morphologic and cytochemical studies.** Cytospin preparations were used for May-Grünwald Giemsa, periodic acid-Schiff (PAS) reaction, and acid-phosphatase stainings, which were performed by standard methods.

**Assay for Epstein-Barr virus nuclear antigen (EBNA).** The assay for EBNA was performed according to the method of Reedman and Klein.

**Electron microscopy.** Cells were fixed in 1.7% glutaraldehyde in 0.1 mol/L phosphate-buffered saline (pH 7.4) for 90 minutes and processed as previously described.

**Cytogenetic studies.** Karyotypic analysis was performed on freshly obtained cells from the patient and on cells that had been in culture for 9 consecutive months. After 20 minutes of incubation with 0.05 µg/mL colchicine, the cells were subjected to hypotonic treatment and processed for G banding as described by Seabright.

**Immunologic studies.** Analysis for cytoplasmic immunoglobulins (clg) in fixed cytocentrifuged cells was performed by direct immunofluorescence with fluorescein isothiocyanate (FITC)-labeled F(ab)₂ goat antihuman Ig (kappa and lambda light chains) (Cappel, Westchester, PA). To test for surface immunoglobulins (slg), 5 x 10⁶ cells were incubated with the aforementioned antisera for 30 minutes at 4°C, washed twice, and analyzed with a fluorescence microscope. Ig secretion in the medium was evaluated by an indirect immunofluorescent technique with FITC-labeled goat antimouse Ig (goat and mouse) used as a second antibody (Cappel).

**Molecular analysis.** DNA from LP-1 cells and normal donor lymphocytes was extracted with phenol chloroform, digested with several restriction enzymes, and fractionated on 0.8% agarose gel as described previously. Hybridization was carried out in a solution containing 50% Kd (vol/vol) formamide at 42°C. Filters were washed three times at 51°C for 30 minutes in 0.1% sodium dodecyl sulfate (SDS), 15 mmol/L NaCl, and 1.5 mmol/L trisodium citrate, and exposed to x-ray films for various periods.

Total RNA samples were obtained from LP-1 cells and a lymphoblastoid cell line by the guanidine-HCl extraction method, and 10 µg was subsequently run on a denaturing containing gel 7% formaldehyde and 1% agarose, blotted to nitrocellulose filters, and hybridized by the method of Thomas.

The c-myc-specific probes used in this study were a 3-kb PstI fragment derived from the plasmid pMC415PP, which corresponds to the 5' portion of the c-myc gene (probe A in Fig 2), and the plasmid pMC413RC, which corresponds to the c-myc exon 3 (probe B in Fig 2). The c-myc probe used was the human cDNA, containing pGEM3 plasmid.

**Ribonuclease (RNase) protection assay.** This assay was performed by hybridizing total cellular RNA to a uniformly labeled antisense RNA transcript generated by the SP6 promoter in pGEM vectors (Promega Biotec, Madison, WI) as described. Two probes were used: (a) a 72-bp ScrFI fragment spanning from 34 bp 5' to 38 bp 3', and (b) a 1,41-bp probe spanning a region from the XbaI site in intron 1 and the 5' PstI site in c-myc exon 2. The latter probe protects a 133-bp fragment in the case of a normally spliced exon 2.

After hybridization with the two probes, samples were digested with RNase A and T1 at 37°C for two hours and then loaded on a 10% polyacrylamide 8 mol/L urea gel as described elsewhere.

**Pulsed-field gel electrophoresis.** The analysis was performed according to the methods described by Kenwrick et al. Briefly, LP-1 and HUT-78 cells and human lymphocytes from healthy donors were suspended in 0.5% low-melting agarose blocks at a concentration of 5 x 10⁶ cells per block. The cells were lysed and the DNA was released by incubating the blocks in 0.25 mol/L EDTA (pH 8.0), 2 mg/mL protease K, and 1% w/vol N-lauroylsarcosine at 50°C for 48 hours. NotI and SfiI enzymes were from Boehringer (Mannheim, FRG), and digestions were performed according to the manufacturer's recommendations. DNA was fractionated on an LKB pulsaphor (LKB, Bromma, Sweden) with a pulse time of 65 seconds at 330 V for 40 hours at 13.5°C, transferred to a nylon membrane, and hybridized as described above.

**Induction of cell differentiation.** Cells were seeded in 1-mL wells at 5 x 10⁶ per mL and treated with PWM only (1:100 final concentration), or in combination, at a 1:1 ratio, with normal T lymphocytes irradiated with 5,000 rad. Control and treated cells were harvested after six days, assessed for viability by staining with trypan blue, and analyzed for immunologic markers and Ig secretion. TPA treatment (1 x 10⁻⁶ mol/L) was performed for four days.

**RESULTS**

**Establishment of the LP-1 cell line.** After one week in the stationary phase, the cells spontaneously began to prolif-erate in suspensions, with a doubling time of 50 hours. Tests for mycoplasma contamination were negative on all occasions.

**Morphologic studies.** The morphology of the LP-1 cells after 9 months of culture was similar to that of the cells obtained from the patient, with the exception of giant cylindrical cells (± 10%) with two or three nuclei that were present in the line population. The average cells showed an intense basophilic cytoplasm, a clear perinuclear area, and a round nucleus with fine chromatin and one to three nucleoli. The acid-phosphatase reaction (non-tartrate-resistant) was intensely positive on almost all the cells, whereas the PAS reaction was negative.

**Karyotypic analysis.** Karyotypic analysis was performed on cells from the patient and from the cell line on several occasions. Those from the patients showed a pseudo-
Fig 1. Representative karyotype of LP-1 cells after 9 months of continuous passage, showing 73. XX, dup(1)(p13;p23), +3, +3. inv(4) (p12;p16), inv(4)(p12;p16), −4, der(5) t(5;7)(q31;7), +5, del(6)(q23), +7, +7, +8, +8, der(9)t(9;12)(q34;q22), +9, +10, +10, +11, der(12) t(12;13)(p13;q11), + der(12), −13, −13, +15, +15, +16, +17, +17, +18, +18, +19, +20, +21, +21, +22, +22, +3mar.

Fig 2. Southern blot analysis of the c-myc gene of LP-1 cells (t) and of normal donor lymphocytes (c). (I) No abnormal bands are detectable with probe A in the HindIII or EcoRII or in other digestions not shown (see text). (II) With probe A, BgIII and PstI digestions generate abnormal c-myc bands of 12.5 and 4.6 kb, respectively. (III) With probe B, only the 12.5-kb fragment generated by BgIII is still visible. These findings suggest the loss of the BgIII and PstI restriction sites marked by asterisks in the bottom panel. E, EcoRII; S, SstI; X, XbaI; B, BglII; H, HindIII; P, PstI; C, ClaI; R, RsaI; PvuII; c, control DNA; t, LP-1 DNA.
diploid modal number with several chromosomal rearrangements. Throughout 9 months of culture and 57 continuous passages, the chromosomal model number progressively increased to 73, with a wide distribution ranging from 60 to 79 chromosomes, while still maintaining the complex translocations observed in the patient's cells. The following abnormalities were consistently observed in the 50 mitoses scores: dup(1)(p13p23), inv(4)(p12p16), der(5), t(5;?)(q31;?), del(6)(q23), der(9), t(9;12)(q34;q22), der(12), t(12;13) (p13;q11), +3 mar. The representative karyotype of LP-1 cells is shown in Figure 1.

Molecular analysis. We studied by Southern blot analysis the structure of the c-myc locus of the LP-1 cells, as amplification of this oncogene has been previously reported to occur frequently in plasma-cell leukemia.\textsuperscript{19,20}  
EcoRI and HindIII restriction endonucleases and a probe corresponding to the c-myc exon 1 and intron 1 (probe A) did not demonstrate an abnormal structure or amplification of the gene (Fig 2I). However, Northern analysis of the c-myc transcript present in the cell line showed high expression of the c-myc oncogene as compared to a human lymphoblastoid cell line (Fig 3). In addition, a longer-than-normal (3.2 kb) c-myc RNA transcript was detected. This led us to perform additional Southern blots. After digestion of the LP-1 DNA with XbaI, PvuII, SstI, and BamHI and use of the c-myc probe A corresponding to c-myc exon 1 and intron 1 (see Fig 2), only the normal fragments were seen (data not shown). By contrast, in the BglII and PstI digestions, c-myc probe A detected, besides the normal 6.2- and 3-kb fragments, faint abnormal bands of 12.5 and 4.6 kb, respectively (Fig 2II). Rehybridization of the same filters with the c-myc probe B, which corresponds to the c-myc exon III, showed only normal fragments with XbaI, PvuII, SstI, and BamHI (data not shown). In contrast, with c-myc probe B, besides the normal 6.2-kb band, an abnormal band of 12.5 kb was again visible in the BglII digestion, whereas the PstI digest was normal (Fig 2III). These findings exclude the occurrence of a major rearrangement of the c-myc locus and instead suggest the presence of point mutations or small deletions affecting the restriction sites for BglII andpstI marked with asterisks in Fig 2.

Indeed, the sizes of the BglII and PstI abnormal bands detected with c-myc probes A and B are compatible with this
interpretation. Point mutations around the PvuII site located within c-myc exon 1 are frequent in Burkitt’s lymphoma and have been associated with altered c-myc transcription.\textsuperscript{15} We were therefore interested in knowing whether in addition to the point mutation detected in noncoding regions of the c-myc gene, point mutations of the first exon, similar to those observed in Burkitt’s lymphoma, were also present in the LP-1 cells. An RNase protection analysis—performed on total cellular RNA obtained from LP-1 cells with use of two probes that allow detection of the presence of point mutations within relevant regions of c-myc exons 1 and 2 (Fig 4B)—failed to reveal abnormalities.

Even in the absence of detectable cytogenetic abnormalities involving chromosome 8, we wanted to determine if the minor modifications observed in the c-myc structure were due to rearrangements taking place at some distance from the gene and undetectable with the common Southern blotting procedure. Analysis with the pulsed-field gel electrophoresis technique showed normal patterns with both NotI (Fig 5) and SfiI endonucleases, which generate c-myc–containing fragments of 600(\text{ca}) and 50(\text{ca}) kb, respectively. In contrast, in agreement with our previous finding,\textsuperscript{12} an abnormal c-myc band was obtained from HUT-78 cells.

Finally, as the 6q- cytogenetic abnormality has been associated with c-myb overexpression in different kinds of human malignancies, we evaluated the level of expression of this protooncogene in the LP-1 cell line. The amount of c-myb transcript, however, was not particularly elevated compared with that of a control lymphoblastoid cell line, and no significant variation was found after mitogen stimulation (data not shown).

Immunotyping studies. Table 2 summarizes the results of the phenotypic analysis performed on fresh cells and after 9 months of continuous passage. Only a small fraction of the cells expressed surface IgA and IgD-lambda, whereas 20% tested positive for cytoplasmic IgG-lambda. The markers of
Table 2. Phenotypic Features of Primary Cells and the LP-1 Cell Line

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</table>

*µg/10⁶ LP-1 cells every 24 hours.
†Percent of positive cells.

Table 3. Phenotypic Changes of LP-1 Cells After TPA and PWM Treatment

<table>
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<tr>
<td>Ig secretion</td>
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*Percent of positive cells.
†µg/10⁶ LP-1 cells every 24 hours.

The early and intermediate stages of B-cell differentiation, such as B1, B2, B4, and J5, are not detectable; neither are Fc and complement receptors or EBNA and T-cell lineage (eg, CD2 and CD3) markers. The unique markers (PC1 and PCA1), considered to be typical of differentiated plasma cells, were never detected on untreated cells. The fraction of HLA class II-positive cells increased from 15% to 30% during the 9-month period. Quantitative determination of the cumulative amounts of IgG-lambda released into the culture medium yielded an average of 50 µg/10⁶ LP-1 cells every 24 hours.

Induction of plasma-cell differentiation. After six days of culture in the presence of TPA or PWM alone or in combination with T lymphocytes, LP-1 cells underwent several phenotypic and functional changes. As shown in Table 3, HLA class II-positive cells decreased, whereas PC1 and PCA1 antigens appeared on 80% and 60% of the cells, respectively, and clg-positive cells increased dramatically. The amount of IgG-lambda secreted in culture medium did not change significantly.

The effects of TPA and PWM on the proliferation of LP-1 cells were also evaluated. The results (not shown) demonstrated that none of the inducers affected the doubling time of the cells over a period of two weeks.

Electron microscopy studies. The ultrastructural features of LP-1 cells are shown in Fig 5. Untreated cells (Fig 6A) show large nuclei with dispersed chromatin and prominent nucleoli and a high nuclear/cytoplasmic ratio. Following six days of PWM treatment (Fig 6B), the cells exhibit plasma cell–like features, with abundant cytoplasm containing many strands of irregularly dispersed endoplasmic reticulum. The nucleus is peripheric, with clumped chromatin and without evident nucleoli.

DISCUSSION

This report deals with the functional and molecular characterization of a new human myeloma cell line spontaneously...
established in vitro from the cells of a patient with IgG-lambda myeloma in the leukemic terminal phase. In agreement with the morphologic features, the immunologic characterization of the LP-1 cells showed a differentiative block intermediate between the late B-lymphocyte and plasma-cell step as they spontaneously secreted small amounts of IgG-lambda.

Several human cell lines with plasmacytoid features have been described, but only a few of them were proven not to be EBV-derived or able to preserve their phenotypic and functional features for a long time in culture. Among these, LP-1 seems to belong to the category with the more immature phenotype. Both RPMI-822626 and HCl-H92926 cell lines do not express HLA class II antigens, whereas they are PCA1-positive, a marker of mature plasma cells. In addition, the latter line does secrete relatively high amounts of Ig. The U-266 line,27 which still retains the HLA class II antigen, is also positive for the PCA-1 antigen. The cell lines U-1957, U-1958, and U-1996 recently described by Jemberg et al28 correspond to different levels of plasma-cell maturation and all express the PCA-1 antigen. The incomplete phenotypic characterization of LB-83124 and KMM-5625 lines prevented a detailed comparison with the LP-1 line.

Cyto genetic analysis demonstrated that the LP1 cells share several chromosome abnormalities with pre-B leukemias,26-28 whereas they do not carry rearrangements involving chromosomes 1, 3, 11, 14, and 17, commonly occurring in plasma-cell disorders.29

As already reported in other plasma-cell leukemias,19,20 LP-1 cells show an increased expression of the c-myc protooncop gene and the presence of transcripts of abnormal size. However, at variance with the reported cases, it was not possible to demonstrate amplifications or genomic rearrangements involving the c-myc gene, but only minor changes, probably due to single-point mutations. However, the pathogenetic relevance of these abnormalities has still to be assessed, and only functional studies on the cloned abnormal c-myc gene may illuminate this point. One of the most relevant findings on the LP-1 cells is represented by their ability to overcome the maturation block after treatment with PWM and TPA. As reported in common lymphocytic leukemia antigen (CALLA)-positive lymphoblasts from fetal bone marrow30 and from myeloma patients,31 the LP-1 cells can be induced by TPA and PWM, either alone or in combination with normal T cells, to express late markers of B-cell maturation, such as the PCA1 antigen. It must also be stressed that this provides unique evidence that PWM, whose ability to trigger B-cell differentiation in the presence of normal T lymphocytes is well known, may transduce the same signal on leukemic cells. In addition, the plasma-cell features of LP-1 cells treated with PWM are further confirmed by the ultrastructural findings.

Unlike most human tumor systems, in which the induction of differentiation is associated with the arrest or the reduction of cell proliferation, that of the LP-1 cells was not decreased by either TPA or PWM. This finding is in agreement with the limited degree of maturation obtained, as shown by the unchanged rate of Ig secretion and the failure to reach the stage of terminal differentiation.

The new cell line described here exhibits some unusual characteristics: it can be induced to differentiate by PWM, which is believed to act only on normal lymphocytes, and it can be maintained in the presence of TPA and PWM at a plasma cell–like differentiation stage without affecting its proliferating capacity. Moreover, LP-1 cells exhibit an altered expression and an abnormal transcript of the c-myc oncogene. The LP-1 cell line may therefore represent a valuable tool with which to dissect the B-cell maturation process and to clarify the role of c-myc derangement in B-cell neoplasias. It may also provide an additional potential partner in the generation of human–human hybridomas.

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