HL-T, A New Cell Line Derived From HL-60 Promyelocytic Leukemia Cell Cultures Expressing Terminal Transferase and Secreting Suppressor Activity

By Elisabeth Paietta, Richard J. Stockert, Theresa Calvelli, Peter Papenhausen, Stephanie V. Seremetis, Marilena Fotino, Peter H. Wiernik, Lucy Chang, and Fred J. Bollum

A cell line with immature blast cell morphology was isolated from HL-60 promyelocytic leukemia cell cultures and designated HL-T. This new cell type is biphenotypic, expressing terminal transferase (TdT) together with myelomonocytic immunologic features. TdT enzymatic activity, undetectable in HL-60, was determined to be 140 to 180 units/10⁶ HL-T cells by the dGTP-assay, approximately 20% of the activity found in lymphoblastoid cell lines. HL-T predominantly synthesize the known 58-kDa TdT-protein plus a minor 54/56-kDa doublet. The 58-kDa steady state form is nonglycosylated and is phosphorylated. Precursor antigens S3.13 and MY-10, absent on HL-60, are expressed by HL-T; however, the cells are negative for HLA-DR. Southern blot analysis by hybridization with immunoglobulin heavy chain (JH) and T cell-receptor chain gene (Tβ) probes shows JH to be in the germ-line configuration in both cell lines and the Tβ gene to be in germ-line in HL-60 but to be rearranged in HL-T. Truncation of the gene encoding the granulocyte–macrophage–colony-stimulating factor (GM-CSF), as found in HL-60, is not observed in HL-T. HL-T are resistant to differentiation–induction by retinoic acid and 1,25-dihydroxyvitamin D₃. Cytogenetically HL-T share with HL-60 a deletion of the short arm of chromosome 9 at breakpoint p13, an aberration frequently found in patients with T cell leukemia. In addition, HL-T display t(8;9)(p11;p24) and trisomy 20. Tetraploidy is observed in 80% of HL-T metaphases with aberrations identical to those in the diploid karyotype. Like HL-60, the new line shows some surface-antigenic–T cell characteristics. Despite an antigenic pattern most consistent with that of helper–inducer T cells (T₄⁺, D₄⁺, B₄⁺, 2H₄⁺, TQ1⁺), HL-T cells and their conditioned culture medium suppress antigen, mitogen, and mixed-leukocyte-culture-mediated lymphocyte proliferation.

THE HL-60 CELL LINE has been established from the peripheral blood of a patient with acute promyelocytic leukemia. Morphologically, histochemically, and immunologically, the cell population consists predominantly of promyelocytes with a small number of more mature myeloid elements present, reflecting a low rate of spontaneous maturation. These cells have attracted wide interest because of their potential to respond to various agents by differentiation in vitro along the myeloid (eg, induced by retinoic acid), the monocyctic (eg, induced by 1,25-dihydroxyvitamin D₃), or eosinophilic lineage (eg, in response to butyric acid and alkaline conditions). In one instance, commitment of HL-60 cells toward nonlymphoid cells was suspected from the reactivity with null lymphoblasts of antibodies raised against phorbol ester-induced HL-60 cells. Inhomogeneity of the HL-60 cell population is suggested by the selection of differentiation-resistant subclones through growth in the presence of inducer drugs.

We have observed the spontaneous outgrowth from HL-60 cultures of a cell line with immature blast cell morphology, HL-T. A parental relationship of this new cell type with HL-60 cells is discussed in view of antigenic, cytogenetic, and functional similarities between the lines. The unique features of HL-T as a TdT⁺/myelomonocytic biphenotype with T cell-receptor β chain gene rearrangement and the antigenic profile of T₄⁺ helper-inducer cells despite marked suppressor activity should make this line a valuable tool for various aspects in hematopoietic research.

MATERIALS AND METHODS

Cell cultures. HL-60 cells, as the counterpart for the variant HL-T cells, were provided by Dr R. Gallagher at Montefiore Medical Center, Bronx, NY. The HL-60 culture from which the HL-T line arose was purchased from the American Type Culture Collection, Rockville, MD. Cell lines were maintained in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 20% fetal bovine serum, 0.3% L-glutamine, 100 mg/mL streptomycin, and 100 U/mL penicillin in a humidified atmosphere of 5% CO₂ in air at 5 x 10⁶ and 2 x 10⁵ cells/mL. For experimentation harvested cells were freed of debris and dead cells by Ficoll-Hypaque (FH) density centrifugation.

In vitro induction of differentiation. Late log-phase HL-T or HL-60 cells were washed twice to remove spent medium and cultured at 5 x 10⁶ cells/mL of complete culture medium containing 10⁻⁸ mol/L retinoic acid (Sigma, St. Louis) or 10⁻⁻ mol/L 1,25-dihydroxyvitamin D₃ (generously provided by Hoffmann-La Roche, Nutley, NJ) for four days before phenotypic changes were evaluated. Control cultures contained the appropriate ethanol concentrations.

Electron microscopy studies. Cell pellets were fixed in 2.5% glutaraldehyde in 0.067 mol/L phosphate buffer, pH 7.4, postfixfixed

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Supported in part by Grant No. P30CA13330 awarded by the National Cancer Institute, DHHS; by grants from the Chemotherapy Foundation and from the Henry M. and Lillian Stratton Foundation, Inc; by National Institutes of Health Grants No. AM-32972 and AM-17702; and by the NCI merit award CA42081.


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in Dalton’s chromic osmium, dehydrated in ethanol, and embedded in Epon after two changes in propylene oxide. Thin sections were stained with uranyl and lead salts and viewed in a Siemens 101 electron microscope.

**Cytogenetics.** Cytogenetic analysis was done on unstimulated cells using the methotrexate synchronization technique and trypsin-Giemsa banding. Twenty metaphases were examined for each cell line.

**Immunophenotyping.** A series of mouse monoclonal antibodies (MoAbs; Table 1) was tested by indirect immunofluorescence with fluoresceinated affinity-purified F(ab’)2 fragment–goat antimouse immunoglobulins (Cappel Laboratories, Cooper Biomedical, Inc.).

### Table 1. Immunophenotype

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Source</th>
<th>Cluster Designation (CD)</th>
<th>Percent Fluorescent Cells*</th>
</tr>
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<tbody>
<tr>
<td>Polyclonal rabbit antisera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TdT</td>
<td>(a)</td>
<td>Immature lymphocytes</td>
<td>0</td>
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<tr>
<td>Monoclonal murine antibodies</td>
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<td></td>
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<tr>
<td>Stem cell markers</td>
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<td></td>
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<tr>
<td>S3.13</td>
<td>(b)</td>
<td>CFU-GEMM-myeloblast, mono, T cell</td>
<td>13</td>
</tr>
<tr>
<td>MY-10</td>
<td>(c)</td>
<td>CD34, CFU-GM, monoblast</td>
<td>28</td>
</tr>
<tr>
<td>S8.6</td>
<td>(b)</td>
<td>CFU-GEMM-promyelocyte, mono</td>
<td>5</td>
</tr>
<tr>
<td>S16.144</td>
<td>(b)</td>
<td>CFU-GEMM-metamyelocyte, mono</td>
<td>95</td>
</tr>
<tr>
<td>MY-9</td>
<td>(d)</td>
<td>CD33, CFU-GEMM-metamyelocyte, mono</td>
<td>89</td>
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<tr>
<td>RFD-1</td>
<td>(a)</td>
<td>HLA-DR</td>
<td>0</td>
</tr>
<tr>
<td>VIP-2b</td>
<td>(f)</td>
<td>CD38, T10, committed stem cells</td>
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</tr>
<tr>
<td>Myelocytic monocytic markers</td>
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<td></td>
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<td>S4.7</td>
<td>(b)</td>
<td>CFU-GM-neutrophil, mono</td>
<td>74</td>
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<tr>
<td>VIM-2</td>
<td>(f)</td>
<td>Myeloblast-neutrophil, mono</td>
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<td>VIM-D5</td>
<td>(f)</td>
<td>CD15, promyelocyte to neutrophil</td>
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</tr>
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<td>(f)</td>
<td>Myelocyte to neutrophil, mono</td>
<td>10</td>
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<td>VIM = 13</td>
<td>(f)</td>
<td>CD14, MO2-antigen, mono</td>
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<td>903 = Mo1</td>
<td>(d)</td>
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<td>RFD-9</td>
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<td>Macrophages, B, and erythroid cells</td>
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<td>OKT6</td>
<td>(g)</td>
<td>CD1, thymocyte antigen</td>
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<td>OKT3s*</td>
<td>(g)</td>
<td>CD3, mature T cells</td>
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<td>OKT3c*</td>
<td>(g)</td>
<td>TdT* thymic blasts</td>
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<td>Leu-1</td>
<td>(h)</td>
<td>CD5, pan-T</td>
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<tr>
<td>RFT-12</td>
<td>(e)</td>
<td>CD6, peripheral T cells</td>
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<td>CD7, pan-T</td>
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</tr>
<tr>
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<td>CD4, helper T cells</td>
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<td>OKT4a</td>
<td>(g)</td>
<td>CD4, helper T cells</td>
<td>83</td>
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<tr>
<td>Anti-T4</td>
<td>(i)</td>
<td>CD4, helper T cells</td>
<td>86</td>
</tr>
<tr>
<td>D44</td>
<td>(k)</td>
<td>Corticocytomocyte, helper, NK cells</td>
<td>43</td>
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<tr>
<td>4B4†</td>
<td>(i)</td>
<td>CDw29, helper–inducer cells</td>
<td>96</td>
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<tr>
<td>Leu-2a</td>
<td>(h)</td>
<td>CD8, suppressor cells</td>
<td>2</td>
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<tr>
<td>2H4†</td>
<td>(i)</td>
<td>Suppressor–inducer cells</td>
<td>83</td>
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<td>TQ1†</td>
<td>(i)</td>
<td>Suppressor–inducer cells</td>
<td>74</td>
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<tr>
<td>VIP-1b</td>
<td>(f)</td>
<td>T9 antigen, transferrin-receptor</td>
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<td>B lymphoid markers</td>
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<tr>
<td>VIL-A1</td>
<td>(f)</td>
<td>CD10, common ALL antigen</td>
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<td>(e)</td>
<td>CD20, B1 antigen, pan-B</td>
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<tr>
<td>VIB-C5</td>
<td>(f)</td>
<td>CD24, BA-1 antigen</td>
<td>5</td>
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<tr>
<td>NK markers</td>
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<td></td>
<td></td>
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<tr>
<td>Leu-7</td>
<td>(h)</td>
<td>T cell and NK subset</td>
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<tr>
<td>VEP-13</td>
<td>(f)</td>
<td>CD16, Fc IgG receptor on NK, neutrophil</td>
<td>78</td>
</tr>
</tbody>
</table>

Both cell lines were negative for erythroid and megakaryocytic antigens.

The letters in brackets refer to the sources by whom the reagents are made or distributed: (a) F.J. Bollum, Bethesda, MD; (b) G. Rovera, The Wistar Institute, Philadelphia; (c) R. Tindle, Sera-Lab, Sussex, UK; (d) J.D. Griffin, Dana Farber Cancer Institute, Boston; (e) G. Janossy, Royal Free Hospital, London; (f) W. Knapp, Institute for Immunology, University of Vienna, Austria; (g) Ortho Diagnostics Systems, Raritan, NJ; (h) Beckton-Dickinson, Mountain View, CA; (i) Coulter Immunology, Hialeah, FL; (k) A. Bernard, Institut Gustave-Roussy, Villejuif, France.

Abbreviation: mono, monocytes.

*Fluorescent cells were analyzed with a FACStar flow-cytometry system (except for TdT-staining)

†These antibodies recognize widely distributed structures so that their designation as functional antigens needs to be considered cautiously.
Malvern, PA) as second antibodies. Intracytoplasmic T3 antigen was detected on methanol-fixed (30 minutes at 4°C) cytospin preparations using the VIT-3 antibody (40 minutes at 22°C). Background fluorescence was determined with P3X63NSI myeloma culture supernatant with added mouse IgG at 100 μg/mL (Cappel). Fluorescent cells were assessed either manually, using a Nikon microscope (Nikon Inc, Garden City, NY) with incident illumination and equipped for the dual wavelength method, or by flow cytometry (Beckton-Dickinson FACSTAR, Mountain View, CA), using an argon laser emitting 170 mW at 488 nm. Control samples consistently gave 5% or less of positive cells. Narrow-angle forward light scatter was used as a measure of cell size and for excluding debris. Fluorescence intensity was measured on log scale.

HLA-typing of the cell lines was done by a microcytotoxicity method using complement that had been extensively absorbed with light scatter as a measure of cell size and for excluding using an argon laser emitting I 70 mW at 488 nm. Control samples preparations using the VIT-3 antibody (40 minutes at 22°C). Terminal transferase (TdT). TdT activity was detected microscopically in methanol-fixed cells (30 minutes, 4°C) by indirect immunofluorescence using monospecific rabbit antiserum and fluorescence-conjugated F(ab')2 goat antirabbit IgG as second antibody. For two-color immunofluorescence, myeloid-specific antibodies were counterstained with phycoerythrin-conjugated streptavidin via biotinylated affinity-purified F(ab')2 fragment—goat antimouse Ig (Beckton-Dickinson, Mountain View, CA) and combined with TdT staining. For immunoblotting, polyclonal anti-TdT antiserum was used at 15 μg IgG/mL of incubation mixture; preimmune rabbit serum served as control. A cocktail of four monoclonal anti-TdT antibodies, HTdT-1, -2, -3, and -4,19 (10 μL equal to 0.5 μg of total IgG) or control Cappel supernatant media was employed in immunoprecipitation. The combination of all four monoclonal HTdT antibodies is required to fully reproduce the fluorescence intensity obtained with the rabbit heterologous anti-TdT antiserum.

TdT enzymatic activity was determined as described.24 Cells (4 x 10⁶), washed with RPMI 1640 medium, were lysed in 160 μL of 0.333 mol/L phosphate buffer, pH 7.4, by freeze-thawing. The lysate was cleared at 175,000 g for 45 minutes and aliquots assayed using 5 μmol/L d(pA),, as initiator, 1 mmol/L 3H-dGTP (44 cpm/pmol) as substrate, 8 mmol/L MgCl₂, 0.2 mol/L potassium cacodylate buffer at pH 7.2 and 1 mmol/L 2-mercaptoethanol. Products of the reaction were scored as acid-insoluble radioactivity on glass fiber filters. One unit of enzyme activity is defined as 1 nmol/L dGMP incubated in 60 minutes at 36°C.

DNA analysis. DNA was extracted from lysed HL-60 and HL-T by proteinase K digestion, phenol extraction, and ethanol precipitation according to established procedures.25 For hybridization with cloned TdT complementary DNA (cDNA), cellular DNA (15 μg) was digested with the restriction enzymes Pst I, HindIII, or BamHI (Bethesda Research Laboratories, Gaithersburg, MD), sized in 1% agarose gels, transferred to nitrocellulose sheets, and hybridized according to Southern26 with the nick-translated probes pT711 and pT106, solving the total coding sequence of TdT.27 For studying immunoglobulin and T cell receptor—β chain (Tβ) gene rearrangements, DNA was digested with EcoRI or BamHI restriction endonucleases, electrophoresed in 0.8% agarose gels, transferred, hybridized, and processed as described.28 The Tβ probe used was derived from a human cDNA Tβ clone (YTT-2) isolated from the Jurkat-T2 T lymphoma cell line (a gift from Dr T. Mak, University of Ontario Cancer Institute, University of Toronto, Canada). This clone hybridizes to both alleles (Cβ1 and Cβ2) of the constant (TβC) region and to one or more alleles of the variable (V) regions.29 The probe for the immunoglobulin locus, representative of the J region (JH) of the heavy chain (IgH) locus, was obtained from Dr Stanley Korsmeyer (National Institutes of Health, Bethesda, MD), as were other probes. Studies on the genomic organization of the GM-CSF gene were performed by Dr James Ible, Frederick Cancer Center, Frederick, MD, using the probe pCH5.2, a 5.2-kb HindIII fragment of genomic DNA containing three exons of the GM-CSF gene cloned in pBR322.30

Immunoblotting. Cell aliquots (10⁶ cells/200 μL) and pre-stained mol wt (Bethesda Research Laboratories) were heated at 100°C for ten minutes in SDS-sample buffer containing 1 mmol/L dithiothreitol, proteins resolved on 10% SDS-polyacrylamide gel electrophoresis (PAGE),31 electrophoretically transferred to nitrocellulose paper,32 and processed as described33 using 100μL of polyclonal anti-TdT antiserum for probing and iodinated protein A to detect protein-bound immunoglobulin on the nitrocellulose paper.34 The nitrocellulose sheet was blotted with paper towels, air dried, and exposed to Kodak XAR-5 x-ray film for 18 hours at ~70°C.

L-25's methionine metabolic labeling. Cells were methionine-deprived for 30 minutes at 37°C before 500 μCi of L-35S methionine (specific activity 1.110 Ci/mmol; Amersham, Arlington Heights, IL) were added for three hours at 37°C. Cells were then washed with serum-containing RPMI 1640 medium and lysed in ice-cold lysis buffer (0.1 mmol/L Naphosphate, pH 7.2, 1% NP40, 2.5 mmol/L phenylmethylsulfon Ме, 1 mmol/L EDTA). The cleared supernatant was prepurified with 10 μg of mouse IgG and 50 μL of a 1:20 suspension of protein A Sepharose CL-4B (PharMacia Fine Chemicals, Piscataway, NJ) before 10 μL of monoclonal anti-TdT antibody cocktail was added overnight at 4°C. Antigen—antibody complex were recovered with 50 μL of protein A sepharose CL-4B, released into reducing sample buffer as described and resolved on 10% SDPAGE.36 Gels were fixed, impregnated in Enhance (New England Nuclear, Boston, MA) for 60 minutes, dried, and fluorographed at ~70°C for 1 week using Kodak SB 5 film.

To test for glycosylation of the TdT protein, cells were grown in the presence of 2 μg/mL tunicamycin (Boehringer Mannheim, Indianapolis, IN) for four hours. This antibiotic is classically used to inhibit cotranslational protein N-glycosylation.35

Phosphorylation of the TdT protein. Cells were precipitated in phosphate-free Eagle's minimum essential medium (GIBCO) for 15 minutes at 37°C before [32P]Pi, (carrier-free; Amersham) was added at 1 μCi/mL.37 Cells were washed with ice-cold phosphate-buffered saline (PBS), solubilized, and subjected to immunoprecipitation. Immunoprecipitates were resolved on 10% SD-PAGE followed by fluorography as described above.

Functional studies. The proliferative response to mitogens in vitro of normal peripheral blood lymphocytes isolated by Ficoll-Hypaque density centrifugation was measured by incorporating 3H-thymidine during six hours after stimulation for 72 hours with the mitogens phytohemagglutinin (0.1%), concanavalin A (con A, 10 μg/mL), pokeweed mitogen (PWM, 0.01%), and phytohemagglutinin plus interleukin 2 (10%; Cetus, Emeryville, CA) for or 120 hours with the specific antigens Candida (2 units), PPD (2.5 μg) or tetanus toxoid (340 μg) with the automatic cell harvester Mash 2. For mixed lymphocyte cultures they were treated with mitomycin C (30 μg/mL/10⁶ cells, 20 minutes, 37°C), washed in PBS, and added to lymphocyte cultures at 7.5 x 10⁶ cells per well (200 μL), if not indicated otherwise. Conditioned media from three-day HL-60 or HL-T cultures (inoculated at 10⁶ cells/10 mL) were filtered (0.45 μm) and used at a ratio of 1:1 with 10% fetal bovine serum (FBS) containing RPMI 1640 medium. Viability of lymphocytes in the presence of 50% HL-T-conditioned medium was assayed by measuring the spontaneous release of radioactivity from [35S]labeled lymphocytes cultured for 18, 36, and 72 hours as compared with culture in control medium.

Natural killer (NK) and lymphokine-activated killer (LAK) cell activity were studied in a four-hour chromium release assay using the erythroleukemia cell line K562 as natural killer-sensitive target and the lymphoblastoid cell line Daudi (both provided by Dr K.)
Welte, Memorial Sloan-Kettering Cancer Center, New York) and fresh tumor cells isolated from the ascites fluid of patients with solid tumors as LAK-cell targets. Target cells (2 x 10⁶ cells/0.5 mL) were labeled with 200 μCi Na¹⁵⁶CrO₄ (specific activity 490 to 670 mCi/mg, New England Nuclear) at 37°C for one hour, washed, incubated for an additional 30 minutes, filtered through 110-mesh Nytex filter, and distributed in round-bottom 96-well dishes at 10⁴ cells/well (50 μL). To these, the effector cells (HL-60, HL-T) were added (100 μL) at effector:target cell ratios 40:1, 10:1, and 2.5:1. Spontaneous release was measured in wells containing only target cells and medium. Total release was obtained by adding 2% SDS. After four hours of incubation at 37°C in 5% CO₂ in air, the well supernatants were harvested in a Skatron-Titentek System (Skatron AS, Sterling, VA) and counted in a gamma counter. Lytic activity is expressed as the mean percentage of ¹⁵⁶Cr released in triplicate assays.

To test for induction of LAK-cell activity, HL-T cells were cultured with 1,500 units of recombinant interleukin 2 (Cetus) for four days in RPMI 1640 medium with 20% FBS. Expression of the interleukin 2 receptor was evaluated using the anti-Tac MoAb (provided by Dr T.A. Waldmann, NCI, Bethesda, MD).

RESULTS

Phenotype. HL-T cells express the morphology of immature myeloblasts with a high nuclear-cytoplasmic ratio, loose chromatin texture, occasionally inconspicuous nucleoli, and basophilic, vacuolated cytoplasm without granulation. Electron microscopy, performed to reveal microgranules, as seen in some cases of acute promyelocytic leukemia, detected neither granulation nor viral particles. HL-T are negative for special histochemical stains including myeloperoxidase, α naphthyl butyrate, chloroacetate esterase, and periodic acid-Schiff reaction.

Testing for HLA antigens revealed HLA-A1, A2, B22, B17, Bw4, Bw6, Cw6 plus weak reaction with typing antisera defining B8 and B40 for HL-60, and HLA-A1, A19/31, B8, B40, Bw6 for HL-T, indicating that, with respect to their predominant HLA markers, the lines are only remotely related.

Karyotype. Cytogenetic analysis of HL-T revealed tetraploidy in 80% of the metaphases, 94,XXXX,9p-(p13),t(8;9)(p11;p24),+20,+20, and a diploid karyotype with identical abnormalities in 20% of the cells (Fig 1). Compared with initial passages 10 months ago, the percentage of tetraploid cells has since then increased from 50% to 80%. HL-T share with HL-60 a rare deletion of the short arm of chromosome 9 at breakpoint p13. Cytogenetic analyses of HL-60 at earlier and later passages performed in our laboratory and reported by others show chromosomal instability. In the most recent analysis we find two clones, 45,X0,9p-(p13),t(10;13)(p11;q11),17p+,−5,+18 in 45% of

![Fig 1. Banded karyotype of a diploid metaphase of HL-T cells. The two characteristic abnormalities, del 9 (p13) and t(8;9) are indicated by arrows.](image-url)
the metaphases and in the 10% of hypotetraploid cells; an additional 4q + is present in the rest of the hypodiploid cells.

As expected from the differential karyotypes of HL-60 and HL-T, both alleles of the gene encoding GM-CSF are unarranged in HL-T cells, which have two intact chromosomes no. 5, while the well-described 30 partial deletion of the GM-CSF gene was found in HL-60 cells.

**Terminal transferase (TdT).** By indirect immunofluorescence, 60% to 70% of HL-T cells express the lymphoid-specific enzyme TdT. Enzymatic activity in HL-T is 140 to 180 units/10⁸ cells using dGTP as substrate or 20 to 25 units/10⁸ cells using dATP, consistent with a higher sensitivity of the dGTP assay. In comparison, the lymphoblastoid cell line RPMI 8402 (100% of TdT + cells by immunofluorescence) contains 160 to 200 dATP units/10⁸ cells. This suggests a lower level of enzymatic TdT activity per HL-T cell compared with lymphoblastoid cells. HL-60 cells are negative for TdT by both assays.

In metabolically labeled HL-T, anti-TdT antibodies recognize a major 58-kDa protein plus a minor 54/56-kDa doublet. The 58-kDa protein corresponds to the high mol wt form of TdT found in various lymphoid tissues. Lower mol wt peptides of TdT of 32 kDa and below have been attributed to proteolytic degradation. As seen in Fig. 2, such TdT-specific bands did not appear during the three-hour synthesis period in HL-T cells investigated. Whether the 54/56 kDa pair represents beginning degradation or a new product remains unclear at present. By immunoblotting, the 58-kDa protein was confirmed as the steady state form of the enzyme; it is nonglycosylated and possesses a phosphorylation site (data not shown), both characteristic features of the enzyme (F.J. Bollum, unpublished findings). We were unable to demonstrate the presence of TdT proteins in HL-60 cells either by metabolic labeling or by immunoblotting.

Blot hybridization of HL-T and HL-60 DNA with the cloned TdT-cDNA inserts pT711 and pT106 revealed no difference in the restriction enzyme-fragment patterns for genomic TdT sequences in the two cell types (Fig 3).

**Immunophenotype.** The reactivity patterns of HL-T and HL-60 cells with a series of MoAbs are compared in Table I. HL-T coexpress TdT and myelomonocytic antigens recognized by S16.144 and S4.7, neither of which has ever been demonstrated on lymphoid cells but are unreactive with MY-9, an antibody with overlapping specificity. HL-T express the precursor cell antigens S3.13 and MY-10, both lacked by HL-60. The presence of S3.13 antigen on HL-T may reflect either immaturity, monocytic nature, or close

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### Fig 2

Fluorograph of 10% SDS-PAGE of TdT proteins in HL-T. Immunoprecipitation from extracts of ³⁵S-methionine labeled HL-T cells with a cocktail of monoclonal anti-TdT antibodies (Lane TdT) or with control mouse immunoglobulin demonstrates a TdT-specific 58-kDa band plus a 54/56-kDa doublet. All additional proteins were precipitated nonspecifically. MW, mol wt standards.

### Fig 3

Hybridization of the human TdT cDNA probes pT711 and pT106 to DNA from HL-60 (lane A) and HL-T cells (lane B) cleaved with restriction enzymes Pst, Hind III, or Bam HI.
affiliation with the T cell lineage.\textsuperscript{10} In favor of the latter, HL-T lack another very early hematopoietic antigen not expressed by T lymphocytes,\textsuperscript{10} the S8.6 antigen. The distribution of MY-10 on normal and leukemic cells parallels the expression of HLA-DR, except that monocytes and B cells are MY-10 negative.\textsuperscript{11} Hence the finding of MY-10 on the expressed by T lymphocytes,' the S8.6 antigen. The distniciation with the T cell lineage.'\textsuperscript{10} In favor of the latter,

expression of HLA-DR, except that monocytes and B cells

distribution of MY-bO on normal and leukemic cells parallels the

antigens, including the X-hapten first appearing at the

ehematopoietic antigen not

HL-T lack another very early hematopoietic antigen not

related antigens, HL-T are negative for NK-specific antici

expression of surface antigens.

HL-60 cells additionally express the thymocyte antigen CD1 (T6)

HL-60 cells have germ-line configurations of both T\( \beta \)

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HL-60 cells with TdT activity, on expression of surface antigens.

HL-60 cells non-culture supernatant demonstrate a comparari-

HL-T, EcoRI on BamHI DNA digests\textsuperscript{28} were tested in

HL-T for responsiveness to retinoic acid and 1,25-
dihydroxyvitamin D\( _3 \), two effective inducers of HL-60 differ-

tion.'\textsuperscript{13} Exposure of the cells to either agent did not alter

HL-T with respect to karyotype and immunophenotype, is

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DISCUSSION

We describe the isolation of a new cell type from cultures of the human promyelocytic leukemia cell line, HL-60. Emergence of the line followed a period of growth cessation and spontaneous maturation of the HL-60 culture. Although this new line, designated HL-T, shares with HL-60 some antigenic, cytogenetic, and functional features, its origin remains to be established. With respect to the HLA-

phenotype, the two lines differ at the A and B loci. However,

weak reactivity of HL-60 cells with antisera defining the

major B antigens of HL-T may reflect the presence of a

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HL-T with respect to T\( \beta \) probe. Genomic DNA was digested with EcoRI and

probed with T\( \beta \). Lane C represents an identical Southern analysis

germ-line DNA obtained from human fibroblasts. The sizes of

the restriction fragments detected by the T\( \beta \) probe in the control,

that is, the germ-line bands at 11 to 12 and 4.2 kb pairs, are
denoted by the solid lines on the left side of the figure. The arrows

on the right side mark the location of rearranged bands in HL-T.

tested HL-T for responsiveness to retinoic acid and 1,25-
dihydroxyvitamin D\( _3 \), two effective inducers of HL-60 differ-

entiation.'\textsuperscript{12} Exposure of the cells to either agent did not alter

morphology, TdT activity, or expression of surface antigens.

4. DISCUSSION

We describe the isolation of a new cell type from cultures of the human promyelocytic leukemia cell line, HL-60. Emergence of the line followed a period of growth cessation and spontaneous maturation of the HL-60 culture. Although this new line, designated HL-T, shares with HL-60 some antigenic, cytogenetic, and functional features, its origin remains to be established. With respect to the HLA-

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FIG. 4. Southern blot analysis of HL-60 (lane 60) and HL-T (lane T) DNA with T\( \beta \) probe. Genomic DNA was digested with EcoRI and

probed with T\( \beta \). Lane C represents an identical Southern analysis

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denoted by the solid lines on the left side of the figure. The arrows

on the right side mark the location of rearranged bands in HL-T.
Fig 5. T cell function of HL-T cells. HL-T and HL-60 cells as well as their conditioned culture media suppressed (A) mitogen- or (B) antigen-induced lymphocyte proliferation (5B). These effects are illustrated as % suppression of the mitotic responses measured in control cultures. See "Methods" for details.

parental line is the most plausible explanation for the presence of both the BI1 and the HL-T subline. By cytogenetic analysis, both HL-60 and HL-T possess a rare deletion on the short arm of chromosome 9 at break point p13, an abnormality frequently present in acute lymphoblastic leukemia (ALL) with T cell features and poor prognosis.29-31 Such blast cells often lack the receptor for sheep RBCs despite the expression of other T-associated antigens;30,31 as is true for HL-T, but lack HLA-DR and TdT, consistent with a more mature T cell phenotype.29,31 However, 9p- together with trisomy 20, another cytogenetic aberration in HL-T, has been detected in the T lymphoblastoid, TdT+ cell line CCRF-CEM.32 Although 9p- is generally affiliated with a lymphoid phenotype, one case with biphenotypic features has been reported.33 Chilcote et al33 found 9p- to be associated with an inactivation of the enzyme methylthioadenosine phosphorylase, resulting in an increased sensitivity of such blast cells to agents that inhibit de novo purine synthesis.

Like others,1,40-42 we have found marked chromosomal instability of HL-60. Most consistently, the cells are predominantly hypodiploid (modal chromosome number 45) with −X, del 9 (p13), −5, +18, a rearrangement between chromosome 17 and some unidentified material, and t(10;13)(p11;q11). Since the gene for human TdT is localized on chromosome 10,34 the translocation involving chromosome 10 present in HL-60 but absent in HL-T prompted us to compare their genomic organization for TdT. Although no gross differences were detected, point mutations cannot be excluded.

Expression of TdT in acute nonlymphoid leukemias is frequently associated with clonal immunoglobulin or T cell receptor–gene rearrangements.5-35 Our finding in the lineage of promiscuous, TdT+, HL-T cells of a rearranged Tβ gene further supports the suggested putative role of TdT in lymphocyte ontogenesis.56 Since during intrathymic differentiation T cell receptor genes are rearranged and expressed synchronously with certain surface antigens,57 the expression by HL-T of CD5 but not of CD2 on CD3 localizes an arrest of the T lymphoid differentiation program in these cells after the Tβ gene rearrangement.

Inhomogeneity of the HL-60 cell population is further suggested by the possible selection of sublines for their ability to continue growing in the presence of either differentiation-inducing agents58 or of cytostatic drugs.59 Resistance has been in part related to alterations in cellular drug metabolism58,59 or protein glycosylation60 and in part to the gain or loss of chromosomes.58,61 The high level of tetraploidy in

Table 2. Effect of HL-T and HL-60 on Three-Way Mixed Lymphocyte Culture Proliferation

<table>
<thead>
<tr>
<th>Proliferative Responses (100%)</th>
<th>HL-T</th>
<th>HL-60</th>
<th>HL-T</th>
<th>HL-60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Decrease</td>
<td>% Decrease</td>
<td>% Decrease</td>
<td>% Decrease</td>
</tr>
<tr>
<td>Donor A + donor B</td>
<td>37.2 ± 8*</td>
<td>0†</td>
<td>78.0</td>
<td>64.8</td>
</tr>
<tr>
<td>Donor A + donor B</td>
<td>46.0 ± 17*</td>
<td>19.6</td>
<td>90.0</td>
<td>56.8</td>
</tr>
<tr>
<td>Donor A + donor B</td>
<td>55</td>
<td>0‡</td>
<td>87.2</td>
<td>72.3</td>
</tr>
</tbody>
</table>

Values are derived from single experiments or are given as the mean ± SEM from two experiments.

Abbreviations: M, mitomycin C-treated cells.

*Not-mitomycin C-treated HL-T cells suppressed the proliferative responses by 81% and 86.2% in the case of donor B and donor A, respectively.

†,‡Mitomycin-treated HL-60 cells increased the proliferative response by 52% and 28.9%, respectively.
HL-T cells may be related to their lack of responsiveness to differentiation induction by retinoic acid and 1,25-dihydroxyvitamin D₃.

As apparent from Table 1, the immunophenotype of HL-T is unique. The cells express TdT, reflecting immature lymphoid features, but lack HLA-DR, CD1, or common acute lymphoblastic leukemia antigen (CALLA). Positivity for D44, an antigen present at high density on cortical thymocytes (and peripheral T cells), for S3.13 and MY-10 antigen, although indicative of a precursor differentiation state, is aberrant in view of the other antigenic characteristics. That the recognition of TdT in HL-T cells by indirect immunofluorescence is not attributable to nonspecific interaction of the heteroantisenum with proteins other than TdT, as found in phytohemagglutinin-stimulated lymphocytes, is confirmed by measurable enzymatic activity, immunoprecipitation from metabolically labeled cells, and immunoblotting.

HL-T cells coexpress lymphoid and myeloid antigens and are therefore to be considered biphenotypic. Only two other such cell lines have been described to date: RS4;11 with B lineage and monocytic features and the t(4;11) typically associated with this cell type in a subgroup of pediatric acute lymphoblastic leukemia and RED-3, positive for TdT, T4 antigen, the myeloid-associated X-hapten and myeloperoxidase, which upon induction with retinoic acid differentiate along the myeloid cell lineage. RED-3 and HL-T clearly differ with respect to the expression of the X-hapten, an antigen not expressed before the stage of promyelocytes during normal hematopoiesis. The finding of T antigens, especially T4, on otherwise myeloid cells is not uncommon. In the HL-60 cells, however, the antigenic profile appears to be more complex in that both the thymocytic antigen CD2 and the mature T antigen CD3 are present (Table 1). We have described a patient with TdT⁺ acute promyelocytic leukemia with blast cells positive for CD1 and developing CD3 upon differentiation induction in vitro. Since then we have observed a minor TdT⁺ component in six of eight patients with acute promyelocytic leukemia. These data support a closely related differentiation potential of promyeloblasts and T lymphoblasts.

The TdT positivity of HL-T, suggesting an immature lymphoid differentiation state, should preclude cell-mediated lymphocyte function. Instead, strong suppression of both T-dependent (mitogen-induced) and T-independent lymphocyte proliferation (antigen-stimulated, three-way mixed leukocyte reaction) was observed with HL-T; with HL-60 cells, only the latter activity was seen (Figs 5A and 5B). These suppressor activities were not predicted by the antigenic phenotype, which is most consistent with that of helper-inducer cells in the case of HL-T and with that of suppressor-inducer cells in the case of HL-60, even though the antibodies 4B4, 2H4, and TQ1 recognize broadly distributed structures. The simultaneous expression of CD16 and CD3 antigens by HL-60 cells is consistent with their being effector cells in antibody-dependent cellular cytotoxicity without significant NK cell activity. This antigenic and functional profile is characteristic of a unique, small, subset of peripheral blood T lymphocytes.

The potent suppressive activity present in the conditioned medium of HL-T cells, and to a lesser extent in HL-60 culture medium, suggests the production and secretion of an inhibitory factor reminiscent of soluble suppressor lymphokines recently described in T cell hybridoma supernatants. Whether the suppression of lymphocyte proliferation resides in a lymphokine unique to HL-T cells has to await its isolation and characterization.

ACKNOWLEDGMENT

We gratefully acknowledge Drs P. Lalezari and M. Khoshidi for valuable critical discussions and their help in the flow cytometric analyses of fluorescent cells; N. Becker for performing the histochemical staining of HL-T cells; H. Dembitzer for electronmicroscopy studies; Drs T. Mak and S. Korsmeyer for the gift of Tß and Ig probes; and Dr J. Ihle for performing the analysis of the genomic organization of GM-CSF gene. We are indebted to D. Thompson and M. McManus for excellent technical assistance.

REFERENCES


53. Chilcote RR, Carrera C, Le Beau MM, Morgan R, Matthay...


59. Marsh W, Sicheri D, Center MS: Isolation and characterization of adriamycin-resistant HL-60 cells which are not defective in the initial intracellular accumulation of drug. Cancer Res 46:4053, 1986


70. Rigby WFC, Shen L, Ball ED, Fanger MW: 1,25-dihydroxyvitamin D₃ induces a myelomonocytic phenotype with enhanced effector cell function in the HL-60 promyelocytic leukemia cell line. Mol Immunol 22:567, 1985


HL-T, a new cell line derived from HL-60 promyelocytic leukemia cell cultures expressing terminal transferase and secreting suppressor activity

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