SUP-HD1: A New Hodgkin's Disease-Derived Cell Line With Lymphoid Features Produces Interferon-γ

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A new cell line, SUP-HD1, was established from the pleural effusion of a patient with nodular sclerosing Hodgkin's disease (NSHD). The SUP-HD1 cells had the characteristic morphology of Reed-Sternberg cells and contained acid phosphatase and nonspecific esterase. The cells lacked the Epstein-Barr virus (EBV) genome and reacted with monoclonal antibodies (MoAbs) against CD15 (Leu-M1), CD25 (Tac), CD71 (OKT9), Ki67, and HLA-Dr. However, the SUP-HD1 cells were nonreactive with MoAbs that specifically identify T lymphocytes, B lymphocytes, and macrophage/myeloid cells. Karyotype analysis of the cell line showed clonal abnormalities involving 1p13, 7p15, 8q22, and 11q23, chromosomal locations, at which breakpoints have been reported in HD. Southern blot analysis demonstrated rearrangement of the immunoglobulin heavy chain and κ light chain genes as well as the gene for the β chain of the T-cell receptor (TCR). Transcriptional analysis showed expression of RNAs for κ light chain, interferon-γ (IFN-γ), and interleukin-2 receptor (IL-2R) but not IL-2. The SUP-HD1 cells lacked cytoplasmic and surface immunoglobulin heavy chain, but a small amount of cytoplasmic κ light chain was detected. The presence of nuclear factor κB (NFκB), a B-lymphocyte–associated transcription factor, was demonstrated in stimulated and unstimulated cells. In addition, the SUP-HD1 cell line produced IFN-γ, a T-lymphocyte–associated lymphokine. Based on these data, the SUP-HD1 cells appear to be aberrant lymphocytes with characteristics of both activated B and T lymphocytes. Elaboration of lymphokines such as IFN-γ by the malignant cells may represent one explanation for the unique clinical and pathologic features of HD.

HODGKIN'S DISEASE (HD) is a malignancy characterized by the presence of classical Reed-Sternberg (RS) cells and Hodgkin's (H) cell variants in a stromal background consisting of lymphocytes, plasma cells, histiocytes, and eosinophils.[1] The lineage of RS and H cells is unclear, and these cells have been variously postulated to be derived from lymphoid, myeloid, monocyte/macrophages, or interdigitating reticulum cells.[2-7] The RS and H cells generally constitute a small percentage of the cells in tumor-involved tissues, and the difficulty in classifying this malignancy accurately may result in part from the presence of mixed populations of cells or from heterogeneity within HD.

Recent studies aimed at determining the cell of origin of HD have focused on molecular genetic studies of (a) gene rearrangements[8-11] and (b) the presence of Epstein-Barr virus (EBV) DNA in RS cells.[12,13] Molecular analysis of patient tumor tissue showed clonal rearrangements of immunoglobulin genes that were more often detected in samples with high RS cell content, but the clonal bands were generally faint and have not been shown directly to be derived from the RS and H cells.[8-11] Weiss et al did not detect immunoglobulin gene rearrangements in 15 cases of HD that contained low numbers of RS cells; however, clonal immunoglobulin gene rearrangements were detected in 7 of 8 HD samples containing large numbers of RS cells.[8]

The presence of EBV DNA has been demonstrated in approximately 20% of HD tumor samples.[12,13] Furthermore, EBV DNA was specifically localized to the RS cells, which represent a clonal proliferation of cells. This evidence strongly suggests that at least a subset of cases of HD are derived from cells capable of being infected with EBV.

At least nine established cell lines have been derived from patients with HD.[14-21] Recent genotypic, immunophenotypic, cytogenetic, and enzymatic studies have suggested a lymphoid lineage for these cell lines.[22-25] Characterization of further cell lines derived from HD would be important in assessing the lineage of origin and increasing our understanding of the pathogenesis of HD. We describe the establishment and characterization of a cell line (SUP-HD1) with morphologic properties of the RS and H cells. Immunophenotyping, immunogenotyping, and expression of lymphocyte-associated proteins suggest a lymphoid cell origin for this HD cell line. The SUP-HD1 cell line produces IFN-γ, a lymphokine which may in part explain the unique histopathology of HD.

MATERIALS AND METHODS

Case report. A 33-year-old man with a history of left upper quadrant pain lasting several months was examined at Stanford University Hospital in April 1983. The patient had splenomegaly and a mediastinal mass. Pathologic review of a lymph node biopsy revealed nodular sclerosing HD (NSHD) (Fig 1A). After a full clinical evaluation and staging laparotomy, the patient was treated with procarbazine, melphalan, velban/adriamycin, bleomycin, velban, and decarbazine chemotherapy for pathologic stage III SA NSHD. However, 15 months after diagnosis, the patient relapsed (with abdominal periaortic lymphadenopathy) and subsequently was treated with aggressive chemotherapy and irradiation. One month before his death, pleural effusions developed (from which the

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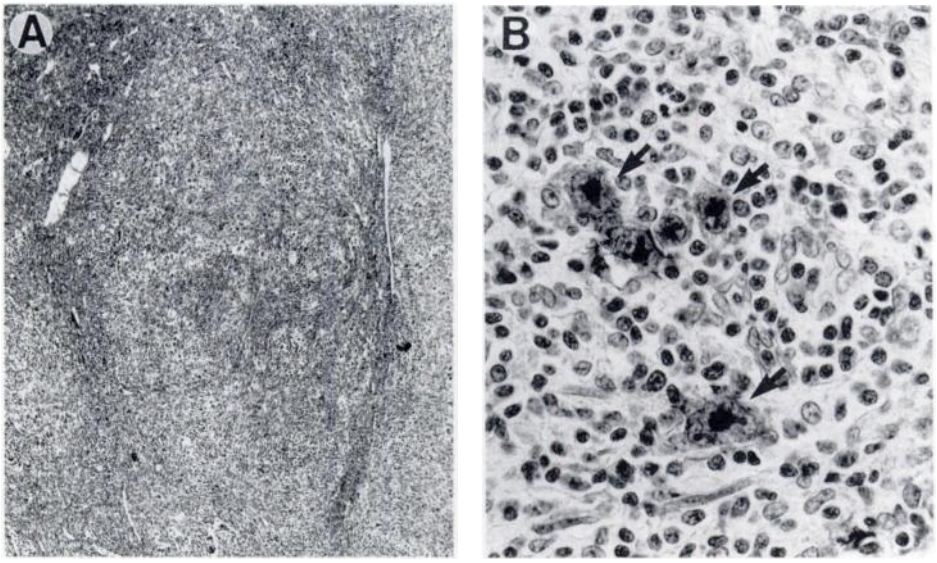

Fig 1. Section of abdominal lymph node removed at the time of diagnostic laparotomy. (A) Nodule containing many lacunar cells surrounded by sclerotic bands of collagen (hematoxylin and eosin, original magnification ×32). (B) Reaction with anti-CD15 (Leu-M1) showed large globules of paranuclear reaction product in abnormal cells (arrows) (immunoperoxidase original magnification ×400).

cell line was derived). Pathologic review of the pleural effusion did not show classical RS or H cells but did show numerous mesothelial cells, lymphocytes, and macrophages. The patient subsequently died of respiratory failure 46 months after diagnosis. At autopsy, large bilateral pleural effusions secondary to extensive tumor involvement of the pleural surfaces were found, as was diffuse lymph node involvement. Microscopic review of lymph nodes showed a malignant lymphoma consistent with HD of lymphocyte-depleted subtype.

Source of malignant cells. Cells were collected from the pleural effusions and separated into aliquots for cell culture experiments, immunophenotyping, and immunogenotyping. The protocol procedures were approved by the Medical Committee for the Use of Human Subjects in Research at Stanford University, and informed consent was obtained from the patient.

In vitro culture of pleural effusion. The technique for cell culture was a modification of our previously reported method.[26] Pleural effusion cells were centrifuged, resuspended in modified McCoy 5A media with 10% fetal calf serum (FCS) and supplemental insulin-like growth factor I (10 ng/mL), mixed with agar (0.3%), plated on Petri dishes and cultured at 38.5°C in an incubator gassed with 5% O_2, 6% CO_2, and 89% N_2. Each dish contained a feeder layer consisting of a mixture of media, agar (0.5%), and human serum (10%).

Morphology of cell line. The morphology of the SUP-HD1 cell line was evaluated by light microscopy, standard cytochemical stains, and electron microscopy. For ultrastructural studies, cells from a pellet preparation were embedded in LX-112 epoxy and thin sections were examined on a Hitachi H-300 electron microscope (Hitachi, Tokyo, Japan).[27]

Characterization of cellular antigens. The fresh HD tumor tissue was snap-frozen, processed, and stained as previously described.[28] Cytospin preparations from the pleural effusion and the SUP-HD1 cell line were stained in an identical manner. The staining consisted of a first stage-incubation with one of the antibodies shown in Table 1. After washing, biotinylated horse anti-mouse immunoglobulin (Vector Laboratories, Burlingame, CA) was applied, followed by a third stage of horseradish peroxidase-conjugated avidin. The specimens were then incubated in diaminobenzidine followed by copper sulfate, fixed in absolute methanol, and counterstained with methylene blue.

Enzyme analysis. Adenosine deaminase (ADA) and nucleoside phosphorylase (NP) were determined as previously described.[26] Terminal deoxynucleotidyl transferase (TdT) activity was assayed by a testing system from Supertechs (Bethesda, MD).

Chromosome preparation and analysis. Chromosomes were prepared for analysis from the SUP-HD1 cell line during the first 6 months in culture. For cell synchronization and high-resolution chromosome banding, cells were exposed to methotrexate (10^{-7} mol/L) for 18 hours and released with thymidine (10^{-5} mol/L) for 6 hours. After incubation at 37°C in 5% CO_2 in air for 24 hours, cells were arrested in the presence of colcemid for 30 minutes. The arrested cells were pelleted, suspended in hypotonic KCl solution, and fixed as described previously.[29] Chromosome spreads were trypsin-Giemsa banded, and 10 karyotypes were analyzed.

Heterologous transplantation of SUP-HD1 in nude mice. Ten to one hundred million viable SUP-HD1 cells in 0.1 to 1.0 mL were injected into the subcutaneous tissue or into the peritoneum of 6- to 8-week-old nude mice.

Gene rearrangement studies. The patient's tissues available for Southern blot analysis were his spleen and the pleural effusion from which the cell line was established. Although the spleen originally had foci of involvement with HD, pathologic review of the frozen specimens available for Southern analysis showed only normal tissue. High molecular weight (mol wt) DNA was isolated from the SUP-HD1 cell line and from cells in the pleural effusion and spleen, and Southern analysis was performed. DNA probes were used to detect possible rearrangement of immunoglobulin heavy chain genes (*Bgl*II restricted genomic DNA-J_H probe), immunoglobulin light chain genes (*Eco*RI restricted genomic DNA-C λ probe and *Bam*HI restricted genomic DNA-C κ probe) and T-cell receptor β (TCR β) genes (*Hind*III restricted genomic DNA-T_β probe).[8] An HLA-B probe was also used in Southern blot analysis of *Bam*HI-digested DNA.[30] DNA from the cell line and positive and negative controls were analyzed by a dot blot for the presence of the EBV genome using two different DNA probes.[12]

Assay of RNA transcripts. Approximately 500 mL SUP-HD1 cells at 5×10^5 cells/mL were divided into five treatment groups: unstimulated, stimulated with 1 μmol/L ionomycin (Calbiochem, San Diego, CA) and 50 ng/mL phorbol myristate acetate (PMA, Sigma, St Louis, MO) (for either 3 or 6 hours); or stimulated with 2 μmol/L ionomycin and 50 ng/mL PMA (for either 3 or 6 hours). Total RNA was isolated, and mRNA transcripts were detected with a ribonuclease protection assay.[31] Uniformly labeled RNA probes were prepared for hybridization to the mRNA's encoding interferon-γ (IFN-γ), interleukin-2 (IL-2), and the α chain of the IL-2 receptor

Table 1. Immunophenotyping of the Patient's Tumor and Corresponding Cell Line

Antibody	Cluster Designation	Patient Tumor*	SUP-HD1 Cell Line
Panleukocyte			
PD7	45	–	–
HD associated			
Leu-M1	15	+	+
Ki-1/BerH2	30	/–	–/–
T Lineage			
Leu-1	5	–	–
Leu-2	8		–
Leu-3	4		–
Leu-4	3		–
Leu-5	2		–
Leu-9	7		–
BF1			–
B Lineage			
Leu-12	19		–
Leu-14	22		–
Leu-16	20		–
MB1	37		–
B2	21		–
L26		–	–
Ig			–
α			–
γ			–
μ			–
κ†			–
κ‡			+
Macrophage/Myeloid			
Leu-M3	14		–
Leu-M5	11C		+§
Y182	68		–
NK Associated			
Leu-7	57		–
Leu-11b	16		–
Leu-19	56		–
Other			
TAC	25		+
OKT9	71		+
Ki-67			+
LN2	74		+
HLA-Dr			+

*Tumor sample from diagnosis.

†Peroxidase-conjugated polyclonal anti-human κ (TAGO, Immunologic, Burlingame, CA).

‡Mouse anti-human κ (Becton Dickinson, Sunnyvale, CA).

§Only rare cells were stained.

(IL-2R). Probes were hybridized to 10 μg total cell RNA in 50% formamide and digested with 4 μg/mL RNase AIII (Sigma), yielding protected fragments of approximately 430, 280, and 205 base pairs (bp), respectively for IFN-γ, IL-2, and IL2R. Controls included 10 μg transfer RNA and 10 μg RNA from the human T-cell line Jurkat which, when stimulated for 3 hours with 2 μmol/L ionomycin and 50 ng/mL PMA, produced detectable levels of all three transcripts. A Northern blot using κ DNA as a probe was performed with RNA prepared from the SUP-HD1 cell line and a mature $\mu+$ $\kappa+$ B-cell line as a control.

Assay of IFN-γ protein levels. The Jurkat and SUP-HD1 cell lines (5×10^5 cells/mL) were incubated with ionomycin (2 μmol/L) and PMA, 50 ng/mL while in log-phase growth. The supernatant was removed after 0, 1, 3, 6, and 24 hours of incubation. Samples were stored at 0°C, and part of the supernatant was concentrated 10-fold with Centriprep-10 concentrators (Amicon, Danvers, MA). The presence of IFN-γ was determined using the human IFN-γ enzyme-linked immunosorbent assay (ELISA) test kit for cell culture media (Amgen Biologics, Thousand Oaks, CA). Test samples were assayed in duplicate, and a standard curve was prepared with 0, 6.25, 12.5, 25, and 50 U/mL IFN-γ.

Assay of lymphocyte-associated transcription factors. Nuclear extracts were prepared from 5×10^7 cells by a small-scale technique modified from that of Ohlsson and Edlund.[32] Protein concentrations were measured using the Bradford assay and 5 μg protein for nuclear factor κB (NFκB)[33] and 10 μg protein was used for nuclear factor of activated T cells (NFAT1).[34] Gel mobility shifts were performed using 10,000 cpm of the appropriate kinased, double-stranded oligonucleotide, 150 mmol/L NaCl, 200 ng salmon sperm DNA, and 10 ng specific inhibitor when present. The components were incubated for 1 hour at room temperature before electrophoresis and subsequent autoradiography.

RESULTS

Establishment of the SUP-HD1 cell line. The pleural effusion cells were initially plated in agar as a single cell suspension. Microscopic clusters of proliferating cells representing 0.001% of the cells that were initially plated were observed after the first week of culture. Cells were fed fresh media and transferred onto new plates (containing a feeder layer) after 2 weeks of culture and into a flask after 3 weeks of culture. There was no appreciable lag period from the time of initial plating of the cells to establishment of the SUP-HD1 cell line as has been noted for other HD-derived cell lines.[22] SUP-HD1 cultured cells have been growing for more than 2 years in suspension culture in a hypoxic environment, without a feeder layer and without supplemental cytokines. The SUP-HD1 line grows as single, nonadherent cells (up to 1×10^6 cells/mL) doubling every 3 to 4 days, with a cloning efficiency in agar of 0.5% to 0.7%.

Morphologic findings. The SUP-HD1 cells demonstrated a marked heterogeneity of size and structure with mono-, bi-, and multinucleated cells ranging in size from 15 to 100 μm. The predominant cell was a large (15 to 20 μm), round, mononuclear cell that lacked villi in phase-contrast microscopy. Approximately 15% of the cells were binucleate cells with conspicuous nuclei (Fig 2A), and 1% of the cells were multinucleated giant cells.

Evaluation by electron microscopy showed that the cells had large nuclei, either multiple or hyperlobate, with each lobe containing a prominent nucleolus (Fig 2). The chromatin was generally fine, with some peripheral clumping. The cytoplasm contained many mitochondria, dilated endoplasmic reticulum, and a few lysosomes but was without a significant collection of filaments. No cell junctions of any type were present.

Cytochemical stains. Staining revealed a strong positive reaction for acid phosphatase and a mild positive reaction for periodic acid-Schiff (PAS) and nonspecific esterase. SUP-HD1 cells were negative when stained for peroxidase and with Sudan black.

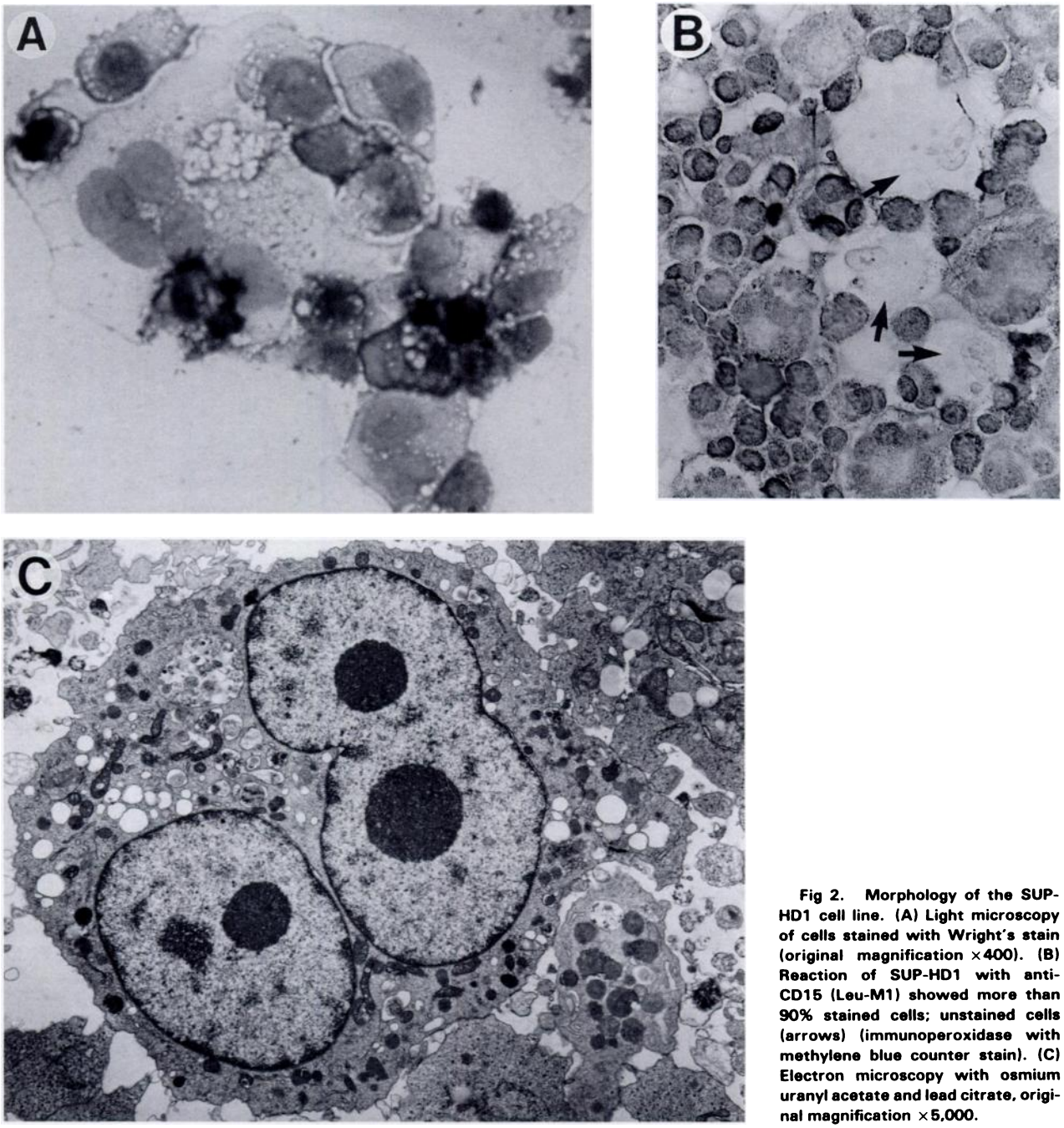

Fig 2. Morphology of the SUP-HD1 cell line. (A) Light microscopy of cells stained with Wright's stain (original magnification ×400). (B) Reaction of SUP-HD1 with anti-CD15 (Leu-M1) showed more than 90% stained cells; unstained cells (arrows) (immunoperoxidase with methylene blue counter stain). (C) Electron microscopy with osmium uranyl acetate and lead citrate, original magnification ×5,000.

Cellular antigens. Various cellular antigens were analyzed by immunohistochemistry and immunocytochemistry. Both the cell line and the patient's tumor (paraffin sections of lymph nodes at initial examination and lymph nodes and spleen at relapse) were CD15 (Leu-M1) positive and nonreactive with CD45 (PD7), CD30 (Ki-1/BerH2), and L26. The SUP-HD1 cells reacted strongly, though variably from cell to cell, with antibodies to CD15 (Leu-M1) (Fig 2), CD25 (Tac), CD71 (OKT9), CD74 (LN2), Ki-67, and HLA-Dr (Table 1). Nearly all cells expressed CD71 and CD74 with little variability in staining intensity. Approximately 75% of the mononuclear, binucleated, and multinucleated cells reacted with Tac and Ki-67. A similar percentage of cells stained for HLA-Dr, but a relatively greater percentage of binucleated and multinucleated cells stained and the staining of these cells was of greater intensity than that observed in mononuclear cells. A small portion of the SUP-HD1 cells (<5%) reacted with CD11C (Leu-M5) but did not react with monoclonal antibodies (MoAbs) to heavy chains. Although the SUP-HD1 cells failed to react with a peroxidase conjugated polyclonal anti-human κ (TAGO Immunologic, Burlingame, CA), there was weak to moder-

ate cytoplasmic staining with a mouse monoclonal anti-human κ (Becton Dickinson, Sunnyvale, CA). In addition, cytospin preparations on thawed pleural effusion revealed a rare cell that stained positive for CD15 (Leu-M1) and CD25 (Tac).

Enzyme levels. SUP-HD1 cells were negative for TdT activity. The ADA activity was 0.5 U/mg protein, and the NP activity was 0.23 U/mg protein.

Karyotype analysis. Cytogenetic analysis of the cell line showed: 44, X, −Y, −1, −2, −4, −7, −9, −13, dup(1)(p13q32), +der(1)t(1;1;6)(q44→q25::p34→q32::p25), del(2)(p23p25), +der(2)t(2;7)(p25;p15), del(5)(p13p15.3), +der(7), dic(4;7)(q31;p15), del(8)(p21p23), t(8;22)(q22;q13), del(11)(q23q25), t(11;?,11;?)(p15;?;q23;?), t(14;?)(p11.2-q11.2;?), del(21)(q21q22.3), +2mar (Fig 3). Seven metaphases with GTG banding showed the near-diploid abnormal karyotype, and three showed tetraploidy of this karyotype.

Heterotransplantation in nude mice. Subcutaneous or intraperitoneal inoculation of the SUP-HD1 cell line into nude mice failed to induce tumors in five attempts.

Molecular genetics. An immunoglobulin heavy-chain J-region probe was used in a Southern analysis of DNA from the cell line, the patient's pleural effusion, and normal germline which were digested with *Bgl*II. Germline bands were observed for the germline DNA control and the pleural effusion DNA, whereas the cell line showed a unique band corresponding to both a rearranged and deleted heavy chain gene (Fig 4A). An immunoglobulin κ probe used on a Southern blot of cell line and germline DNA restricted with *Bam*HI also showed that both κ light chain genes were rearranged in the cell line (Fig 4B). A J_β probe used on *Hind*III restricted genomic and cell line DNA showed a single rearrangement of cell line DNA (data not shown). Analysis of the cell line and germline DNAs with an immunoglobulin λ probe showed a germline configuration for both (data not shown).

In addition, molecular probing showed that the cell line was not infected with EBV (data not shown). With the HLA-B probe used on DNA restricted with *Bam*HI, there was exact comigration of six bands from the patient's spleen with six bands from the SUP-HD1 cell line (data not shown).

The cell line produces IFN-γ messenger RNA and protein. The cell line was tested for the production of IFN-γ, IL-2, and IL-2R mRNAs that are coordinately expressed in T cells using an RNA protection assay with IFN-γ, IL-2R, and IL-2 probes. The SUP-HD1 cells produced a small amount of IFN-γ RNA (Fig 5A, lane 1) constitutively, which increased approximately five- to 10-fold on stimulation for 3 hours (Fig 5A, lanes 2 and 3) but then declined on further stimulation for 6 hours (Fig 5A, lanes 4 and 5). The cells constitutively produced the IL-2R RNA (Fig 5B, lane 1) which increased slightly on stimulation at both 3 and 6 hours (Fig 5B, lanes 2 through 5). No IL-2 RNA was detected in SUP-HD1 cells even on stimulation (Fig 5C). With each probe, Jurkat cells made the expected RNA.

The cell line was tested for production of IFN-γ protein that was detected by an ELISA assay. Concentrated supernatants from the Jurkat cells after incubation with PMA and

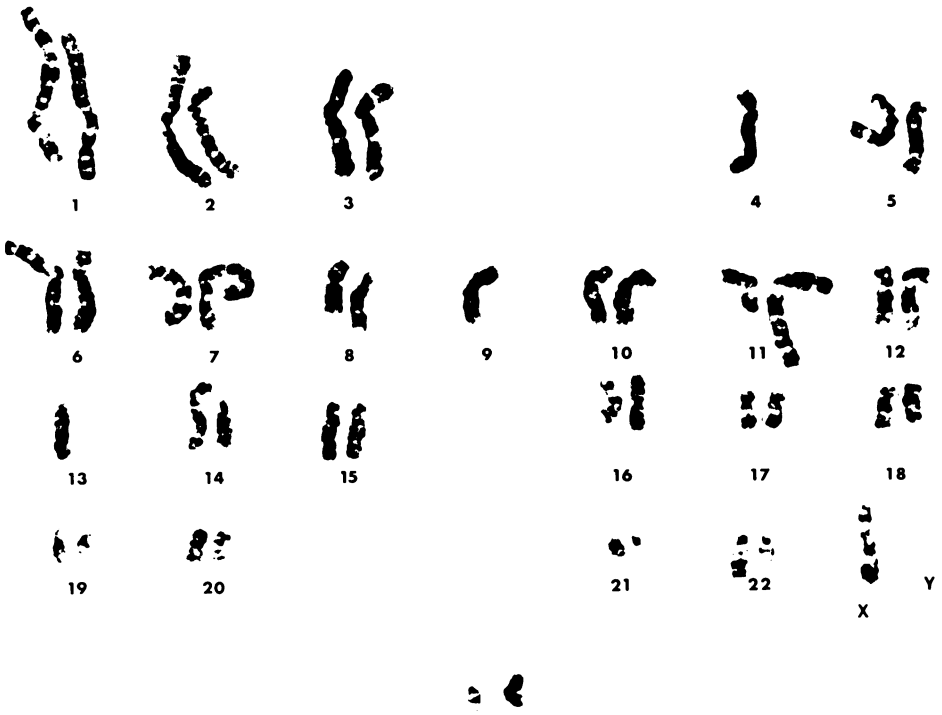

Fig 3. Karyotype of the HD cell line. Full karyotype analysis is given in the Results section.

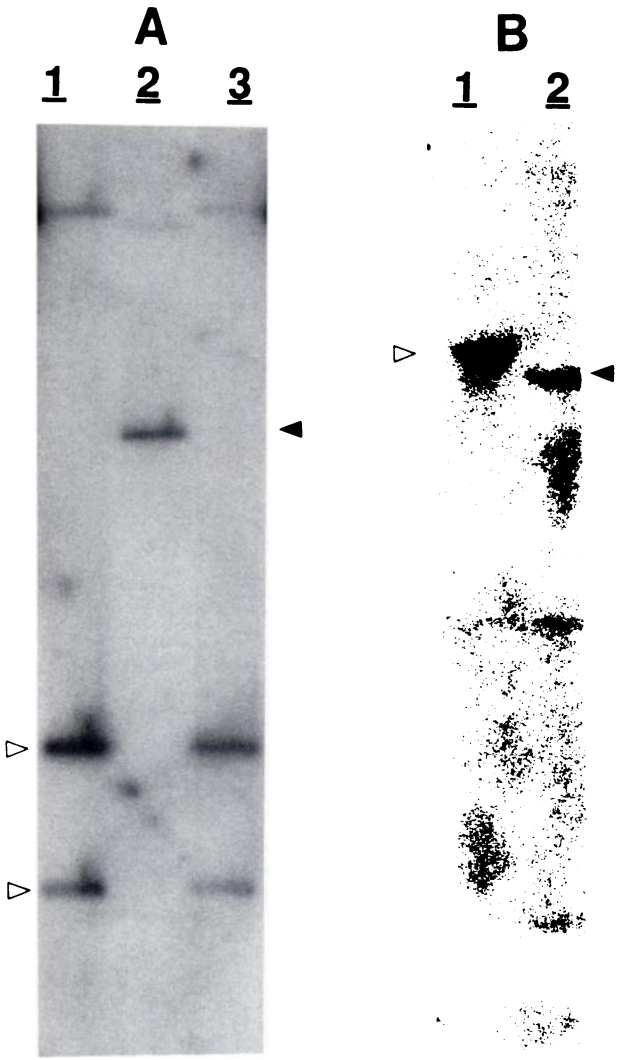

Fig 4. Southern blot to show rearrangements of (A) immunoglobulin heavy chain: lane 1, patient's spleen DNA; lane 2, SUP-HD1 cell line DNA; lane 3, pleural effusion DNA, all restricted with *Bgl*II and probed with J_H (B) immunoglobulin κ light chain. Lane 1, germline DNA; lane 2, SUP-HD1 cell line DNA restricted with *Bam*H1 and probed with C_κ. Rearranged bands (arrowheads); positions of germline DNA bands (open arrowheads).

ionomycin for 0, 1, 3, 6, and 24 hours yielded IFN-γ levels of 0, 0, 6, 12, and 15 U/mL, respectively. Concentrated supernatants from the SUP-HD1 cells incubated under the same conditions for 0, 1, 3, 6 and 24 hours showed IFN-γ levels of 0, 0, 3, 6, and 3 U/mL, respectively.

Assay of lymphocyte-associated transcription factors. Extracts of Jurkat, SUP-HD1, and liver cells were analyzed for expression of the lymphocyte-associated transcription factors NFκB[33] and NFAT1[34] using a gel mobility shift assay. Jurkat cells produced a protein that bound an oligonucleotide carrying an NFκB binding site only when stimulated (Fig 6, lane 3). SUP-HD1 cells produced an activity that bound the oligonucleotide both when they were unstimulated (Fig 6, lane 7) and when they were stimulated (data not shown). For both cell lines, binding was competed by an unlabeled oligonucleotide carrying the NFκB binding site (Fig 6, lanes 4 and 8) but not with an oligonucleotide containing a mutated binding site (Fig 6, lanes 5 and 9) or with an oligonucleotide containing a binding site for hepatocyte nuclear factor 1, HNF1 (Fig 6, lanes 6 and 10). Jurkat cells produced NFAT1, but SUP-HD1 cells even with stimulation did not (data not shown). Control experiments showed that neither cell line produced a liver-associated binding protein, HNF1,[35] and liver cells did not produce NFκB (data not shown).

DISCUSSION

Although historically it has been difficult to grow HD cell in vitro,[36-39] recently several cell lines have been established as continuous suspension cultures.[24] Each of these HD-derived cell lines has been EBV negative and, although different, each possesses many characteristics of the HD cell.[24] Most HD-derived cell lines were cultured from the pleural effusions of patients with treatment-resistant recurrent NSHD.[24] The SUP-HD1 cell line appears to resemble three HD-derived cell lines closely: L428, HDLM-2, and KM-H2.[14,19,20] These four cell lines have morphologic heterogeneity manifested by mononucleate, binucleate, and multinucleate giant cells. These pleiotropic features represent spontaneous differentiation in culture that has not been observed in cell lines from patients with acute leukemia and lymphoma.[26,29,40,41] Each of the four cell lines is acid phosphatase and esterase positive and each reacts with the same MoAbs as the H cell: CD15 (Leu-M1), CD25 (Tac), CD71 (OKT9), Ki-67, and HLA-Dr.[24,25] In contrast to other HD-derived cell lines, SUP-HD1 cells lacked the CD30 (Ki-1) antigen; however, the patient's tumor also lacked this antigen at diagnosis and relapse.

To establish concordance between the patient's tumor and the SUP-HD1 cell line, a direct comparison of the morphology, cytochemical stain profile, immunophenotype, and immunogenotype was performed. The SUP-HD1 cells had the same general morphology as the patient's tumor, and the presence of both acid phosphatase and nonspecific esterase is characteristic of HD.[42] In addition, the MoAb profile was concordant with the patient's tumor tissue with the four antibodies tested. Comigration of the HLA-B bands on Southern analysis supports the origin of the SUP-HD1 cell line from the patient. Although rearrangements of the immunoglobulin heavy chain gene were detected in the SUP-HD1 cell line, the pleural effusion and spleen tissue from the patient showed only germline bands. These discordant results are most likely due to minimal tumor involvement in the spleen and pleural effusion that was below the level of detection by Southern blotting (1% to 5% tumor cells) because the spleen tissue (which was available for DNA analysis) and pleural effusion (from which the cell line was derived) were pathologically normal. Thus, the SUP-HD1 cells have many features concordant with the patient's tumor, as have other cell lines previously established in our laboratory.[26,29,40]

Karyotype analysis of HD has been limited by a low yield of dividing cells and by complex chromosomal changes.[43] In contrast to other lymphoid malignancies, the ploidy of HD is

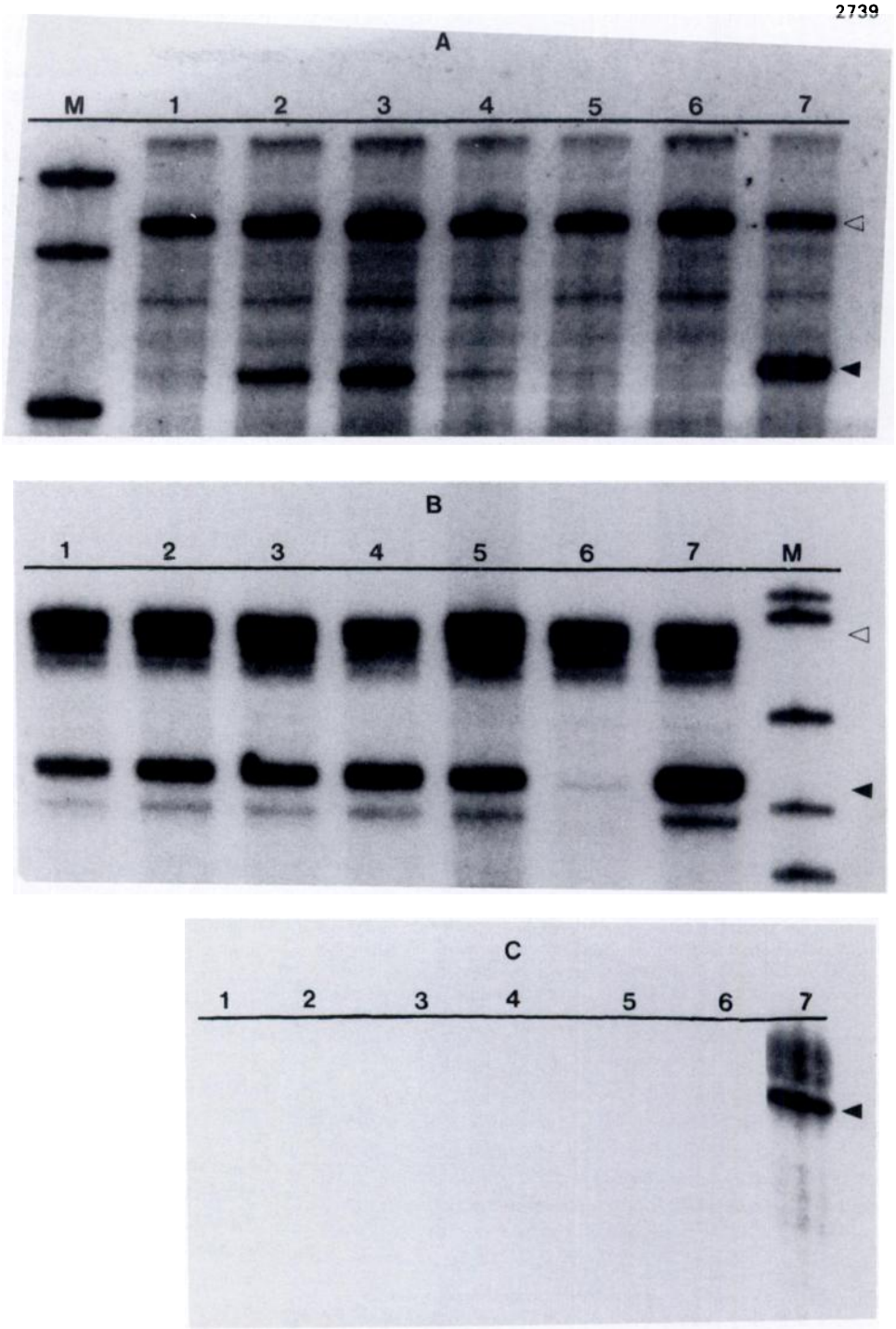

Fig 5. Transcription analysis of IFN-γ (A), IL-2R (B), and IL-2 RNAs (C). Lanes 1 through 5 are RNA from SUP-HD1 cells: lane 1, unstimulated; lane 2, treated with PMA and 1 μmol/L ionomycin (I) for 3 hours; lane 3, treated with PMA and 2 μmol/L I for 3 hours; lane 4, treated with PMA and 1 μM I for 6 hours; and lane 5, treated with PMA and 2 μmol/L I for 6 hours. Lane 6 contained t-RNA as a control and lane 7 was RNA from Jurkat cells stimulated with PMA and 1 μmol/L I for 3 hours. Lanes labeled M were mol wt markers and *Msp*I digests of pBR322; the markers shown in A had a mol wt of 600, 527, and 404; the markers shown in B had a mol wt of 242, 238, 217, 201, and 190. Undigested probe (open arrowheads); digested probe (solid arrowheads), reflecting the amount of specific RNA protected.

often in the triploid to tetraploid range, but near-diploid karyotypes have been reported.[44,45] Recent karyotypes from cases of HD showed common breakpoints at 11q23, 14q32, 6q11-21, 8q22-24, and within the short arm of chromosome 1 and 7.[46] The SUP-HD1 cells share many of these chromosomal abnormalities as well as chromosomal changes observed in cell lines derived from patients with HD (Table 2).[20,47] The SUP-HD1 cells have both a translocation and a deletion at 11q23 and, as suggested by Cabanillas et al, HD with breakpoints at 11q23 may share some of the unusual and biphenotypic features of acute leukemia with a t(4;11)(q21;q23).[46] Similar to HD, acute leukemias with a t(4;11)(q21;q23) often stain positive for acid phosphatase and nonspecific esterase, react with anti–Leu-M1 and anti–HLA-Dr antibodies, have variable expression of other B-lymphocyte antigens, and have immunoglobulin heavy-chain gene rearrangements.[48-50]

Studies of the SUP-HD1 cells suggest a lymphoid cell lineage and do not support a monocyte/macrophage or interdigitating reticulum cell origin for this cell line. The SUP-HD1 cells possess several features characteristic of B lymphocytes but also possess some features associated with T lymphocytes (Table 3). The B-lymphocyte-associated features include low cellular ADA,[51] absence of TdT, rearrange-

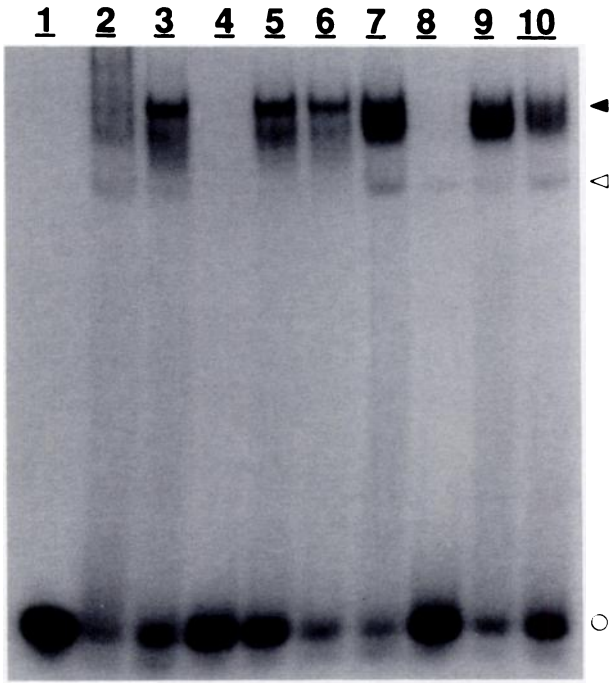

Fig 6. Gel mobility shift of NFκB in Jurkat and SUP-HD1 cells: NFκB complex is specifically competed with unlabeled NFκB binding site but not other unrelated oligonucleotide binding sites: lane 1, free NFκB labeled oligonucleotide probe; lane 2, nonstimulated Jurkat cells; lane 3, stimulated Jurkat cells; lane 4, stimulated Jurkat cells with unlabeled NFκB oligonucleotide; lane 5, stimulated Jurkat cells with NFκB mutant oligonucleotide; lane 6, stimulated Jurkat cells with HNF-1 oligonucleotide; lane 7, SUP-HD1 unstimulated cells; lane 8, SUP-HD1 unstimulated cells with unlabeled NFκB oligonucleotide; lane 9, SUP-HD1 unstimulated cells with NFκB mutant oligonucleotide; lane 10, SUP-HD1 unstimulated cells with HNF-1 oligonucleotide. Specific NFκB-oligonucleotide complex (solid arrowhead); position of free oligonucleotide probe (open circle); nonspecific lower band (open arrowhead).

ment of immunoglobulin heavy-chain and κ light-chain genes, and transcription of a κ light-chain RNA. A small amount of κ light-chain protein was detected, but immunoglobulin heavy-chain protein was not detected, indicating that a block to mature immunoglobulin production exists.

The SUP-HD1 cells also produce a B-lymphocyte–

Table 2. Comparison of the Chromosomal Abnormalities in SUP-HD1 Cell Line, HD, and HD-Derived Cell Lines

Chromosomal Changes Observed in SUP-HD1	Chromosomal Changes Observed in Tumors From Patients With HD[43-46]	Chromosomal Changes Observed in HD-Derived Cell Lines[20,47]
del(11)(q23q25)	del(11)(q23qter)	del(11)(q21qter)
t(11;?11;?)(p15;?;q23;?)	t(11;_)(q23;_)	
t(8;22)(q22;q13)	t(8;_)(q22-24;_)	
der(2)t(2;7)(p25;p15)	Breakpoints on 7p	
dic(4;7)(q31;p15)		
dup(1)(p13q32)	Breakpoints on 1p	dup(1)(p22p32)
del(21)(q21q22.3)		del(21)(q21qter)

Table 3. Comparison of Characteristics of SUP-HD1 Cell Line With Features of Normal B and T Lymphocytes

Characteristics of SUP-HD1	Features of Normal Lymphocytes: B Lymphocytes	T Lymphocytes
Enzymes		
ADA (low)	+	
TdT	+	
Gene rearrangements		
Immunoglobulin heavy chain	+	
Immunoglobulin κ light chain	+	
β Chain of T-cell receptor		+*
Transcription factors		
NF κ B constitutively	+	
Transcripts		
IL-2R		+*
IFN-γ		+
κ Light chain	+	
Protein		
κ Light chain	+	
IFN-γ		+

*Predominantly associated with but not restricted to T lymphocytes.

associated transcription factor, NFκB, which binds to functionally important sequences in the regulatory region of the κ light chain and the IL-2R.[33] NFκB is constitutively present in B lymphocytes and plasma cells and can be induced in pre-B and T lymphocytes by stimulation with PMA. The SUP-HD1 cells constitutively express this factor, as demonstrated by gel mobility shift assays. As would be expected for cells producing an active NFκB, the SUP-HD1 cells also constitutively express κ light chain and IL-2R RNA. A second factor, NFAT1 binds to a conserved sequence in the IL-2 gene enhancer that is required for maximal tissue-specific gene expression.[34] Although only a few tissues have been studied (ie, lymphocytes, liver cells, and cervical carcinoma cells), this protein appears to be present only in nuclear extracts derived from activated T lymphocytes. Neither stimulated nor unstimulated SUP-HD1 cells make this factor, as determined by gel mobility shift assays, consistent with a complete absence of IL-2 RNA in these cells. The constitutive expression of NFκB and the lack of NFAT1 activity strongly imply that the SUP-HD1 cell line has differentiated predominantly along a B-lymphocyte lineage rather than a T-lymphocyte lineage.

The SUP-HD1 cells also have two features associated with but not restricted to T lymphocytes. The SUP-HD1 have rearranged the gene for the β chain of the TCR, which appears to be nonfunctional because neither cytoplasmic nor cell membrane antigen was detected (with BF-1, an antibody against the nonpolymorphic portion of TCR-β). The SUP-HD1 cells also produce IL-2R RNA and express the IL-2R (Tac antigen) on the cell surface. The SUP-HD1 cells constitutively produce IFN-γ RNA, normally a product of activated T lymphocytes, which is inducible five- to 10-fold on stimulation of the cells to levels approximately half of those of the stimulated Jurkat cell line. Regulation of IFN-γ is aberrant since after 6 hours of stimulation IFN-γ RNA in

the SUP-HD1 cells decreases while the level of IFN-γ RNA in Jurkat cells continues to increase. In comparison, both the MO cell line (established from a patient with hairy cell leukemia) and the KT-3 cell line (established from a patient with Lennert's lymphoma) produce IFN-γ for 5 days after stimulation.[52,53] However, normal T lymphocytes coordinately express the genes encoding IFN-γ, IL-2, and IL-2R.[54] In the SUP-HD1 cell line, the three genes are no longer coordinately regulated since the SUP-HD1 cells do not make detectable IL-2 transcript even when stimulated under conditions that elicit transcription in the Jurkat cell line. Several other HD-derived cell lines also do not produce IL2.[55]

This is the first report of IFN-γ produced by an HD-derived cell line; the L428 HD-derived cell line secretes a transforming growth factor[56] along with a variety of uncharacterized substances that affect the immune response and granulopoiesis.[57] The L428 cell line also secretes an "IL-1-like" activity[55] but apparently does not produce IL-1 mRNA.[58] IFN-γ has pleiotropic effects, including enhanced expression of class I and II major histocompatibility antigens and IL-2R.[59] Furthermore, IFN-γ causes lymphocyte proliferation, activation of mononuclear phagocytic cells, and formation of polykaryons.[59] Production of IFN-γ by malignant cells in HD may be related to the unique clinical and histopathological manifestations of this disease. For example, the numerous T lymphocytes in tissues involved by HD may result in part from local IFN-γ production since intradermal injections of IFN-γ cause a local influx of CD4-positive T cells and monocytes. Furthermore, injections of purified recombinant IFN-γ can cause fever and chills, clinical findings similar to "B" symptoms in HD. IFN-γ also causes increased expression of the monocyte Fc receptor, as do HD biopsy supernatants.[21] The supernatant from one of the HD cell lines (HDLM-2) induced morphologic changes in myelomonocytic cell lines, leading to cells with monocyte-macrophage characteristics,[19] an observation consistent with the monocyte-activating property of IFN-γ.

Clinical heterogeneity is apparent in HD, and HD cell lines will probably show heterogeneity. The cell line we describe may represent a malignant cell differentiated along both B- and T-lymphocyte lineages. Production of IFN-γ and possibly other lymphokines may be related to the clinical and pathologic findings in HD. Further characterization of this cell line may be helpful in providing more information on the nature of HD.

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