Characterization of a Novel Hodgkin Cell Line, HD-MyZ, with Myelomonocytic Features Mimicking Hodgkin's Disease in Severe Combined Immunodeficient Mice

By Ralf C. Bargou,* Markus Y. Mapara,*‡ Christian Zugck,§ Peter T. Daniel,¶ Michael Pawlita,‖ Hartmut Döhner,§ and Bernd Dörken*‡

*From the *Max Delbrück Center for Molecular Medicine; and ‡Free University of Berlin, Universitätsklinikum Rudolf Virchow, Robert-Rössle-Klinik; O-1115 Berlin-Buch; the §University of Heidelberg, Medizinische Klinik V; and the ‖Applied Tumorvirology Programme INF 242 and ¶Tumorimmunology Programme INF 280, German Cancer Research Center, 6900 Heidelberg, Germany*

Summary

A novel Hodgkin cell line, designated HD-MyZ, was established from the pleural effusion of a 29-yr-old patient with Hodgkin's disease (HD) of nodular sclerosing type. The majority of cells grow adherently and display typical morphological characteristics of Reed-Sternberg (RS) and Hodgkin (H) cells, i.e., large multi- and mononucleated cells with prominent nucleoli. Immunofluorescence analysis revealed a myelomonocytoid immunophenotype (expression of CD13 and CD68, and lack of lymphoid markers). HD-MyZ cells strongly expressed restin, a recently described intermediate filament-associated protein, the expression of which is restricted to H cells, RS cells, and in vitro cultivated peripheral blood monocytes. In addition mRNA expression of c-*fms* (colony-stimulating factor 1 receptor) could be induced in HD-MyZ cells by phorbol myristate acetate (PMA) stimulation. Southern blot analysis did not detect rearrangement of T cell receptor β and immunoglobulin H loci, thus demonstrating the lack of lymphoid commitment. HD-MyZ cells were also devoid of Epstein-Barr virus genomes. HD-MyZ cells constitutively express mRNAs for interleukin 1α (IL-1α), IL-1β, IL-5, IL-6, IL-7, IL-8, IL-10, IL-1 receptor (type I), and IL-6 receptor. Stimulation of cells with PMA increased mRNA expression as well as the secretion of IL-1β, IL-6, and IL-8, and induced the de novo expression of IL-8 receptors. Xenotransplantation into severe combined immunodeficient (SCID) mice by intravenous or subcutaneous inoculation led to development of disseminated tumors with infiltrative and destructive growth. In addition lymphadenopathy, pleural effusion, and infiltration of spleen were observed. Morphological and immunological analysis of tumor cells revealed the same features as HD-MyZ cells. This cell line might be an important tool for understanding the pathogenesis and biology of HD. In addition the SCID mice model might prove helpful in developing new therapeutic strategies.

Hodgkin's disease (HD)[1] is characterized by the presence of typical malignant cells (multinucleated Reed-Sternberg [RS] and mononucleated Hodgkin [H] cells) amid a background of reactive lymphocytes, plasma cells, histiocytes, neutrophils, eosinophils, and stromal cells (1). The cell of origin of HD still remains controversial (2). The establishment of several HD-derived cell lines recently (3) enabled a more detailed analysis of the cellular origin as well as the biology of these tumor cells. These efforts, however, have been hampered by the fact that H or RS cells usually account only for a minority of cells within the cellular background in HD tissue. Most of the cell lines were derived from pleural effusions containing RS or H cells. Various hematopoietic cells, including lymphocytes, monocytes, and interdigitating reticulum cells, have been speculated to be the normal counterpart of RS and H cells (2, 4–10). The majority of tumor cell lines, however, derived from HD that have been described so far were found to be related to lymphocytes (2, 4–7).

[1] *Abbreviations used in this paper:* HD, Hodgkin's disease; RS, Reed-Sternberg; RT, reverse transcription.

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The histopathological as well as a number of clinical features of HD might reflect the unbalanced production of cytokines, e.g., B symptoms (night sweats, fever, weight loss), eosinophilia, acute phase reaction proteins, and thrombocytosis. Subsequently the expression of various cytokines has been studied in HD-derived tumor cell lines (11).

In this report we describe the morphologic, immunophenotypic, molecular genetic, and functional characterization of a novel HD-derived cell line (HD-MyZ) consisting of typical RS and H cells, which display myelomonocytoid characteristics. An in vivo model of HD could be established by xenotransplantation of HD-MyZ cells into SCID mice.

Materials and Methods

Case History. H and RS cells were isolated from a pleural effusion of a 29-yr-old patient with HD refractory to multiple therapy regimen. In 1980 HD of nodular sclerosing type (stage IIIB) was diagnosed in patient MZ. Initial treatment consisted of combined chemo- and radiotherapy. The patient relapsed several times with disseminated manifestations, including abdominal, cervical, and axillary lymph nodes, right proximal humerus, and right iliac crest. In September 1991 tumor progression could be observed with evolution of pleural effusion, infiltration of psoas muscle, and multifocal bone marrow infiltration despite intensive chemotherapy. In addition the patient complained of fevers unresponsive to antibiotics and antifungal therapy. The patient's general condition deteriorated rapidly and the patient died in November 1991. Due to the fact that a high number of H and RS cells could be seen within the smear cytology of the pleural fluid, this was the first patient with HD from whom we made an attempt to derive a cell line.

Culture Conditions and Reagents. Cell population of the pleural fluid consisted predominantly of large mono- and multinuclear cells with morphological features of H and RS cells. After Ficoll-Hypaque separation cells were transferred into culture and maintained in RPMI 1640 (Seromed-Biochrom, Hamburg, Germany), 20% heat-inactivated FCS, 2 mM L-glutamine (Gibco, Karlsruhe, Germany), and penicillin-streptomycin (Seromed-Biochrom). Where indicated, cells were cultured in the presence of PMA (10 ng/ml; Sigma Chemical Co., München, Germany) or LPS (Sigma Chemical Co.). Human PBL were cultured in RPMI plus 5% FCS in the presence of 5 ng/ml PMA plus 0.1% PHA for 24 h. Thereafter, cells were harvested for RNA extraction. The Burkitt lymphoma cell lines BJAB, Raji, and BL60 and the T cell lymphoma cell line Jurkat were used as controls in various experiments.

Analysis of Antigen Expression by Immunofluorescence. Cells were analyzed after staining with various mAbs. The antibodies were obtained from the panel of the Fourth International Workshop on Leucocyte Differentiation Antigens or purchased from Becton Dickinson & Co. (Heidelberg, Germany) and Coulter (Krefeld, Germany). TNF receptor mAbs utr-1 (type II receptor) and htr-9 (type I receptor) were kindly provided by Dr. Manfred Brockhaus (Hoffmann-LaRoche, Basel, Switzerland). mAb anti-APO-1 (IgG3) was a generous gift from Dr. Peter Krammer (German Cancer Research Center, Heidelberg, Germany). Cells (10^6) were incubated with 5 μg/ml mAb in PBS (Containing 0.1% NaN_3 and 10% human IgG; Venimmun Behring, Marburg, Germany). After washing, cells were incubated with Biotin $(Fab)_2$ goat anti-mouse IgG (Dianova, Hamburg, Germany) for 30 min at 4°C. Thereafter cells were incubated with Streptavidin-FITC (Dianova), washed, and fixed with 1% formalin. Indirect immunofluorescence was measured using a FACScan® flow cytometer (Becton Dickinson & Co.). Human tumor cells were detected in SCID mice by staining with an anti-human HLA-DR mAb (B8.12.2; Dianova), which does not crossreact with mouse cells. Double staining of human tumor cells from SCID mice was performed using a PE-labeled anti-HLA-DR mAb (Dianova) and FITC-labeled CD13, CD19, CD71, and CD25 mAbs (Becton Dickinson & Co.).

Cytoplasmic antigens were detected as described using cytocentrifuge preparations (12). Cells were fixed with paraformaldehyde and treated with Triton X-100 (0.5%). After incubation with mAb, cells were stained with FITC-labeled goat anti-mouse IgG (Dianova).

Xenotransplantation of Tumor Cells into SCID Mice. HD-MyZ cells (10^6) were injected either subcutaneously or intravenously into C.B.-17 *scid/scid* mice (13). The mice were obtained from our own breeding colony and were kept in isolators under stringent conditions in the Central Animal Laboratory, German Cancer Research Center. Microbiological controls were performed regularly by addition of sterile sentinel animals to the colony.

Detection of Cytokines by ELISA. Secretion of IL-1β, IL-6, and IL-8 into the supernatant of HD-MyZ was measured by Quantikine assays (Biermann, Bad Nauheim, Germany) according to the manufacturer's manual.

Detection of mRNA by PCR. Total RNA was extracted from viable cells as described by Gough (14). Purification of poly(A^+) RNA from 10 μg of total RNA was performed using the Dynabead mRNA Purification Kit (Dynal, Oslo, Norway). Detection of mRNA by PCR was performed as described by Wang et al. (15) using a Geneamp RNA PCR Kit (Perkin Elmer-Cetus, Überlingen, Germany) and a thermocycler (Bachhofer, Reutlingen, Germany) according to the PCR kit protocol. In detail, 1 μl of the eluted poly(A^+) mRNA (total volume, 20 μl) was used for reverse transcription (RT) with a sequence-specific downstream primer directly followed by a PCR amplification step using an upstream primer. To rule out false-positive results due to DNA contamination, we always ran negative controls without RT. One-third of the PCR volume was run on an agarose gel (1%). After transfer to a nylon membrane and UV crosslinking, filters were prehybridized in hybridization buffer (50%, vol/vol, formaldehyde, 6× SSC, 0.1% SDS) for 4 h at 55°C. cDNA was hybridized with a ^{32}P-labeled internal oligonucleotide complementary to a sequence between the upstream and downstream primers. Probes were labeled using a 3'-oligonucleotide end-labeling kit (British Biotechnology, Oxford, UK). Filters were hybridized for 16 h at 55°C. Filters were then washed twice for 30 min with 6× SSC, 0.1% SDS at 70°C. Table 1 shows oligonucleotide sequences used for RT-PCR and hybridization as well as length of the expected PCR products (16–47).

Southern Blotting and Hybridization Probes. Total cellular DNA was extracted and digested with restriction endonucleases using standard protocols (48). 10 μg of digested DNA was electrophoretically separated in 1% agarose gels and transferred to nylon membranes (Genescreen Plus; DuPont Co., Wilmington, DE). Hybridization probes labeled with ^{32}P using the oligo-primed labeling method (49) were used in 50% formamide, 2× SSC at 42°C (T_m, −20°C). Control DNA was extracted from placenta, the human Burkitt lymphoma lines BJAB, Namalwa, and BL60, as well as the T lymphoma cell line Jurkat.

To analyze the human Ig heavy chain gene and the TCR-β gene for rearrangements, a genomic 2.2-kb Sau3a fragment of the joining region (IgH_J region) (50) and an 800-bp cDNA sequence (JUR-b2) (51) were used, respectively. For the detection of EBV DNA the Bgl2 U fragment (nucleotides 13944–17016) (52) specific for the EBV internal repeat 1 (IR 1) was used.

Table 1. *Oligonucleotide Sequences*

mRNA	bp	5′-3′ Sequence	3′-5′ Sequence	Internal oligonucleotide
β-actin(16)	246	GAGCTGCGTGTGGCTCCCGAGG	CGCAGGATGGCATGGGGGAGGGCATACCCC	GCCAGTGGTAGCGCCAGAGGCGTACAGGG
IL-1β(17)	610	CCTCCAGGGACAGGATATGGAGC	GGCTCAATAAAAGGGCTGGGG	GGAGAGAGCTGACTGTCCTGGCTGATGGAC
IL-2(18)	458	ATGTACAGGATGCAACTCCTGTCTT	GTCAGTGTTGAGATGATGCTTTGAC	CTTCTTCTAGACACTGAAGATGTTTCAGTTC
IL-2R(Tac)(19)	502	CCCTTGGCTCAATCCTCTAAG	CTGGGGAGCTGGGGTTATAGGC	CTCTTAAAGAGGCCAATTAGTAACGCACAG
IL-3(20)	449	ATGAGCCGCCTGCCCGTCCTG	GCGAGGCTCAAAGTCGTCTGTTG	GCCTCCAGGTTTGGCCTTCGAAGGTTATTTTC
IL-4(21)	408	CCTCCCAACTGCTTCCCCC	GCCTTTCCAAGAAGTTTTTCC	CCGTTTCAGGAATCGGATCAGCTGCTTGTG
IL-4R(22)	472	TCTTCCACCTTCGGGAAGTACG	GGCCTGGGTGCCACCCTGCTCC	GGACATTTCGGCGGAGGATCTGTCCCCAGG
IL-5(23)	368	CCCCACAGAAATTCCCACAAG	GGCCTGACTCTTTCTTGGCCC	CCCCCTTGCACAGTTTGACTCTCCAGTGTG
IL-6(24)	595	GGATTCAATGAGGAGACTTGCC	CAAACTGCATAGCCACTTTCC	GTGTCCTAACGCTCATACTTTTAGTTCTCC
IL-6R(p80)(25)	251	CATTGCCATTGTTCTGAGGTTC	AGTAGTCTGTATTGCTGATGTC	CATCTGGTCGGTTGTGGCTC
IL-7(26)	402	CATGGTCAAAACGGATTAGGG	CTCTCTTATTCTCACCGGC	CTCTAAAACATCTCCAGCACAGAAGAAGAC
IL-7R(27)	605	GCCCTCGTGGAGGTAAAGTGCC	GGGAGACTGGGCCATACGATAG	GCTGATGGTTAGTAAGATAGGATCCATCTCC
IL-8(28)	308	GGACAAGAGCCAGGAAGAAACC	CTTCAAAAACTTCTCCACAAC	GTTCTTTAGCACTCCTTGGCAAAACTGCAC
IL-8R(29)	440	GGGGGACGCTATAGGATGTGGG	CACAAGGCCAGTTGGCAGGTTG	CACCTGAGATGGGAAAACCACCCCCCTGAG
IL-10(30)	467	CCCTGACCTCCCTCTAATTTATC	CAGGCTGGAGTACAGGGGCAC	GTGCTGGGACTACAGGCGTGAGCCACCGCG
GM-CSF(31)	424	ATGTGGCTGCAGAGCCTGCTGC	CTGGCTCCCAGCAGTCAAAGGG	GCTGGCCATCATGGTCAAGGGGCCCTTGAGC
TNF-α(32)	450	GACAAGCCTGTACGGCATGTTG	GGGCAATGATCCCAAAGTAGAC	GGGCCGATTGATCTCAGCGCTGAGTCGGTC
TNF-β(33)	421	CCTCAAACCTGCTGCTCACCTC	AGTTCTACAGAGCGAAGGCTCC	AGGGCTGAGGACTAGGTGGGGGATGCCATC
TNF-RI(34)	601	GTGGAAGTCCAAGCTCTACTCC	CGATGTCCTCCAGGCAGCCCAG	GCTTGAAGGAACTACTACTAAGCCCCTGG
TNF-RII(35)	468	CTGCCGATAAGGCCCGGGGTAC	GCTTCATCCCAGCATCAGGCAC	GCTCCAGCAGCAGCTCCCTGGAGAGCTCG
c-*myc*(36)	564	CCGCCCGCGGCCACAGCGTCTG	CCTCGGTGTCCGAGGACCTGGG	GCAGGATAGTCCTTCCGAGTGGAGGGAGGC
c-*myb*(37)	610	GCCTCCTTTAACTTCCACCCCC	CTGCTCCTCCATCTTTCCACAG	CTGCTACAAGGCTGCAAGGGGCTCGCCAGG
c-*fgr*(38)	520	GGCGGTGAAGACGCTGAAGCCG	GCCTGGGTAGGGGATTCGGCCC	GAGCTCAGTGAGCAGGATCCCAAAGGACC
N-*ras*(39)	464	CCCACCATAGAGGATTCTTAC	GGTAATCCCATACAACCCTGAG	CTTCAACACCCTGTCTGGTCTTGGCTGAGG
bcl-1(40)	444	CGGGAAAGGCCACCTGTCCCAC	CATCCTGGCAATGTGAGAATGC	GGCACGCAGCCTCCCAAACACCCCACCTCC
bcl-2(41)	441	GGTGCCACCTGTGGTCCACCTG	GTTCACTTGTGGCCCAGATAGG	AGGGCGTCAGGTGCAGCTGCCTGGACATC
p53(42)	576	GGCCCCTGTCATCTTCTGTCCC	GCCGGTCTCTCCCAGGACAGGC	CCTCAAAGCTGTTCCGTCCCAGTAGATTAC
TGF-β(43)	530	GCGCCCACTGCTCCTGTGACAGC	GGCGGGGCGGGGCGGGGCGGG	GGGGCAGGGCCCGAGGCAGAAGTTGGCATG
restin(44)	420	GGCAGCTCAGCAGCTCCTCAGGT	AAGGTTTCGTCGTCATTGCAGTTGG	GGGCGTTCCTCACCCCGACTGCCATGGTG
IL-1α(17)	308	GTCTCTGAATCAGAAATCCTTCTATC	CATGTCAAATTTCACTGCTTCATCC	GATTGATGCATGGACCCTTGATGGAGTGGGCC
IL-1RI(45)	548	GGTACAGGGATTCCTGCTATG	GGTGACCGTCGCTGGACTGGC	CATGGCTATTTGCTCTTCAGATGAACCACC
c-*kit*(46)	452	CGGGAAGCCCTCATGTCTGAAC	GCGAGGAAAGCCATGCCCTTTG	GGCCAACTCGTCATCCTCCATGATGGCGGG
c-*fms*(47)	491	CCATGGATGGGGCGACACGGGG	GGAGTGAAGGCGGCGTATAGGG	CCTGCTCCAAGGGGCCTGAGCTGAGTGTGG

Results

Cell Culture and Cytology. Mono- and multinuclear cells with characteristics of H and RS cells were isolated from the pleural fluid by Ficoll-Hypaque separation and were transferred into culture medium as described. The majority of the cells grew adherently. Large multinucleated RS-like cells (as shown in Fig. 1 *a*) account for ~10% of the cell population at a stable frequency. Cells were grown at a concentration of 10^6 cells/ml, and culture medium was replaced every other day.

Cells have been in continuous culture without morphological and phenotypical changes since November 1991. Cytologic appearances of the cell line HD-MyZ demonstrating the typical morphological features of RS and H cells are shown in Fig. 1 *a*. Fig. 1, *b* and *c*, show hematoxyline/eosin staining of patient's bone marrow sections with infiltration by H and RS cells.

HD-MyZ Cells Display Myelomonocytoid Features. Phenotypic analysis of the cell line was performed by immunofluorescence using a flow cytometer and cytocentrifugation (Table 2). RT-PCR was used for analysis of mRNA expression. Cells strongly expressed CD13 (My7), MHC class II (HLA-DR), and CD68, but did not express either B or T cell lineage–associated antigens, thus indicating myelomonocytoid differentiation.

Cells were also positive for MHC class I, transferrin receptor (CD71), CD10, and CD29 (integrin β1 chain). The activation antigen CD30 (Ki-1), which is strongly expressed on

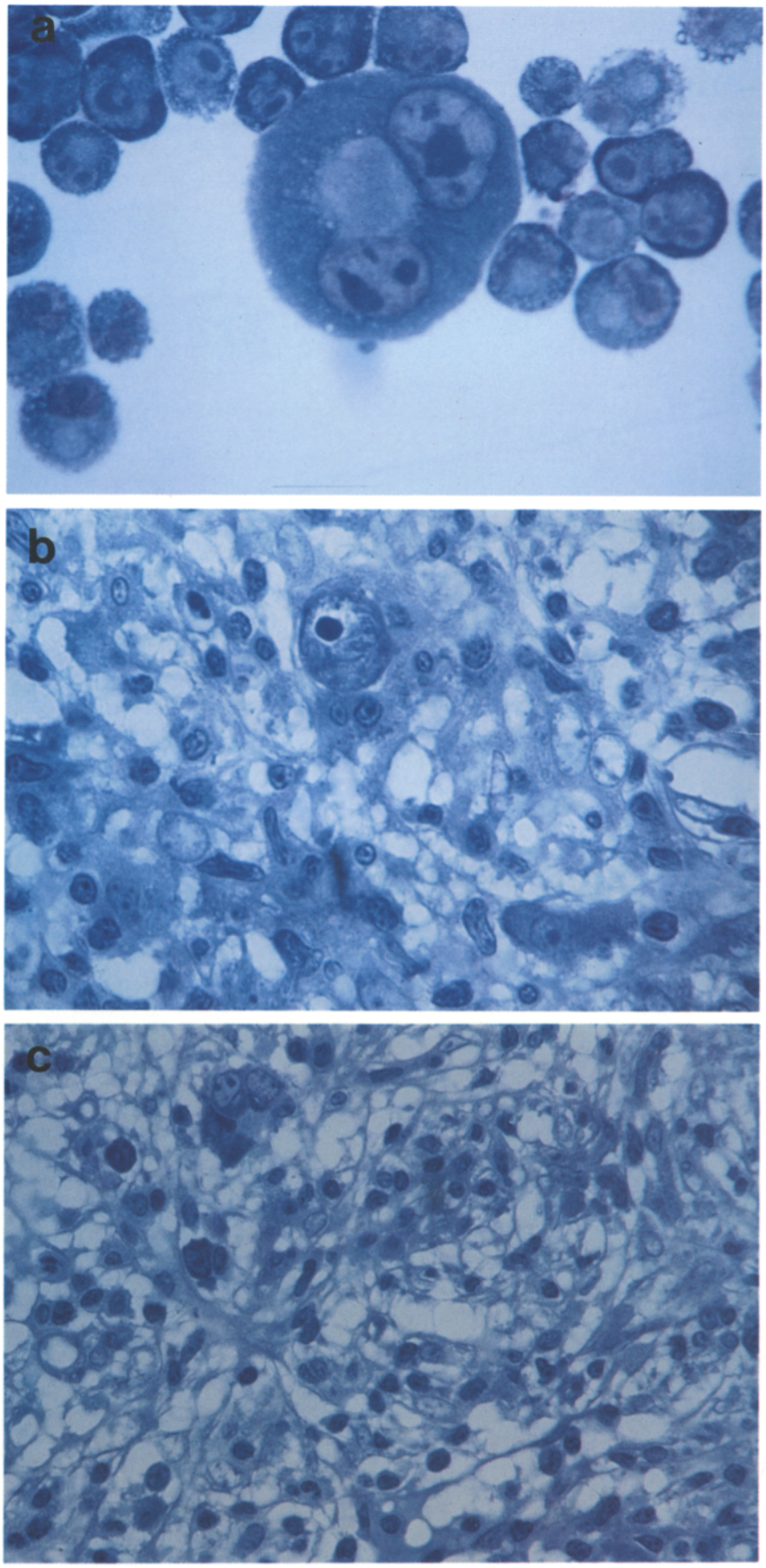

Figure 1. Morphology of HD-MyZ cells: (*a*) Cytospin preparations. HD-MyZ cells (mono- and multinucleated cells) were stained with hematoxylin/eosin (×63). (*b* and *c*) Bone marrow sections from patient MZ were stained with hematoxylin/eosin. Infiltration of the patient's bone marrow by H and RS cells (×100).

Table 2. *Immunophenotype of HD-MyZ Cell Line*

B cell lineage		T cell lineage		Myeloid lineage		Other	
CD antigen	Expression	CD antigen	Expression	CD antigen	Expression	CD antigen	Expression
CD19(HD37)	–	CD1a(T6)	–	CD13(My7)	+ + +	TNF-RI(utr-1)	–
CD20(1F5)	–	CD2(T11)	–	CD14(Mo2)	–	TNF-RII(htr-9)	–
CD21(HB5)	–	CD3(Leu-4)	–	CD11b(Mo1)	–	CD25(TAC)	–
CD37(HD28)	–	CD4(Leu-3)	–	CD33(My9)	–	CD10(J5)	+ +
CD22(HD39)	–	CD7(Leu-9)	–	CD34(My10)	–	CD56(L19)	–
CD23(HD50)	–	CD8(Leu-2)	–	CD15(L16)	–	CD71	+
CD38(T10)	–	CD5(Leu-10)	–	CD68(cytopl)	+ +	CD29(4B4)	+ + +
CD39(AC-2)	–					MHC II(HLA-DR)	+ + +
CD40(G28-5)	–					MHC I(W6/32)	+ +
CD76(HD66)	–					CD30(Ki-1)	–
CD77(BLA-1)	–					APO-1/Fas	–
IgG	–					HEA-125	–
IgD	–						
IgM	–						

H and RS cells in most cases of HD, could not be detected on the cell surface. A weak cytoplasmic staining, however, was repeatedly observed.

Recently, a novel intermediate filament-associated protein, designated restin, has been described, which is found to be expressed in in vitro cultured human peripheral blood monocytes. This novel marker appears to be abundantly expressed in RS cells (44). Using RT-PCR analysis we were able to detect restin mRNA expression in HD-MyZ cells (Fig. 2).

Germ Line Configuration of the IgH and the TCR-β Loci in HD-MyZ. Since HD-MyZ cells were lacking cell surface markers of lymphoid differentiation but revealed instead myelomonocytoid features (coexpression of CD13, HLA-DR, CD68, and restin), we were interested in further studying lymphoid commitment on a molecular genetic level. Using BamHI- or EcoRI-digested DNA from HD-MyZ cells no rearranged bands were detected with IgJ_H region of TCR β probes (Fig. 3).

Absence of EBV Genomes in HD-MyZ. No EBV-specific sequences were detected in HD-MyZ (H) as well as the EBV-negative cell lines BJAB (B) and Jurkat (J). In the human Burkitt's lymphoma line Namalwa(N), which carries two integrated copies of the EBV genome (51), the 3.1-kb BamHI fragment (W) is clearly detected (Fig. 4).

mRNA Expression of Oncogenes, Cytokines, and Cytokine Receptors. We studied the mRNA expression of a number of well-characterized proto-oncogenes and suppressor genes. As shown in Fig. 5 *a*, specific transcripts of p53, c-*myc*, and *bcl*-1 could be detected. In addition expression of N-*ras* and c-*myb* was also detected, whereas mRNA expression of *bcl*-2 and c-*fgr* oncogenes was not observed. Expression of the proto-oncogene c-*fms* (CSF-1 receptor) could be observed after PMA stimulation for 8 h (Fig. 6 *b*). Table 3 summarizes the expression of these genes in HD-MyZ cells.

Since part of the clinical symptoms and of the pathology of HD might be explained by the unbalanced production of tumor cell–derived cytokines, we were interested in studying the constitutive mRNA expression of cytokines and their receptors. HD-MyZ cells were found to constitutively express transcripts of IL-1α, IL-1β, IL-5, IL-6, IL-7, IL-8, IL-10, as well as IL-1 (type 1) receptors (Fig. 5 *b*). In contrast, the cells did not express TNF-α, IL-3, or GM-CSF (Fig. 5 *c*). Table 3 summarizes the data on cytokines and cytokine receptors.

In addition, we studied the regulation of cytokine and cytokine receptor expression after in vitro stimulation with phorbol esters. In vitro stimulation with PMA for 24 h induced the de novo expression of IL-8R mRNA (Fig. 6 *a*).

Secretion of Cytokines. We assessed the secretion of IL-1β, IL-6, and IL-8 into the cell-free supernatant of our cell line by ELISA. In accordance with our mRNA expression data, high levels of IL-8 and moderate levels of IL-6 and IL-1β could be detected in the supernatant of unstimulated HD-MyZ cells. Stimulation with PMA for 40 h led to a marked increase in IL-6, IL-8, and IL-1β secretion (Table 4).

Xenotransplantation into SCID Mice. 10^6 cells were in-

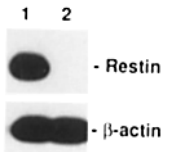

Figure 2. Restin expression in HD-MyZ. Autoradiographs of blotted and hybridized RT-PCR products. Constitutive restin mRNA expression is observed in HD-MyZ cells (lane *1*), whereas freshly isolated human PBL did not express restin mRNA (lane *2*). β-actin expression in both cell lines was used as control.

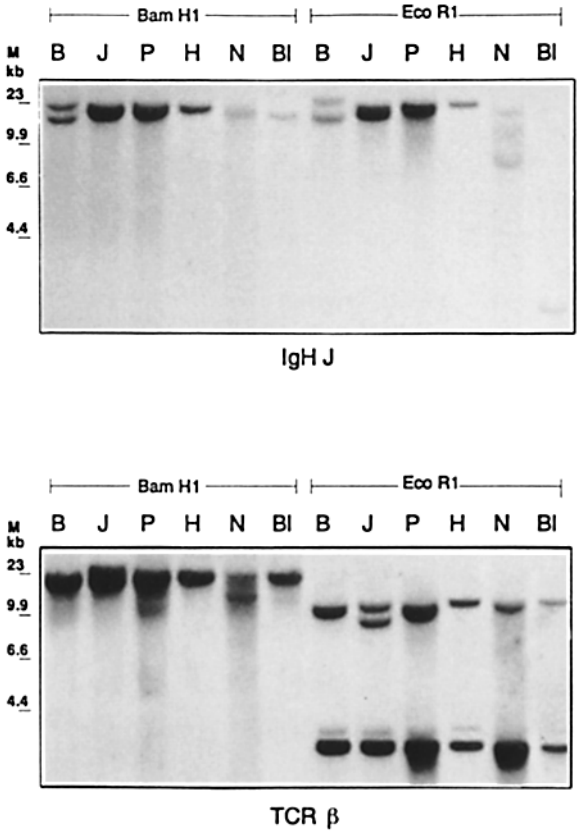

Figure 3. Germline configuration of the IgH and the TCR-β loci in HD-MyZ. Approximately 10 μg of cellular DNA was digested with BamHI and EcoRI restriction endonucleases, respectively, separated by electrophoresis in 1% agarose gels, transferred on nylon membranes, and hybridized with a ^{32}P-labeled IgH J region–specific DNA probe and autoradiographed (*top*). After removal of the IgH J probe the same blot was rehybridized with a cDNA probe specific for the TCR β gene (*bottom*). The IgH J region probe detected germ line fragments of 16 kb (BamHI) and 13 kb (EcoRI) in HD-MyZ (*H*) as well as in human placenta DNA (*P*) and the human T cell line Jurkat (*J*). In control DNA from B lymphoma lines BJAB (*B*)-, Namalwa (*N*)-, and BL60 (*Bl*), rearranged fragments were present. The TCR β gene probe detected germ line fragments of ~20 kb (*BamHI*) and 11, 4, and 3.5 kb (*EcoRI*) in HD-MyZ (*H*) as well as in all control cell lines, except for the T cell line Jurkat (*J*), which contains an additional rearranged 9-kb EcoRI fragment.

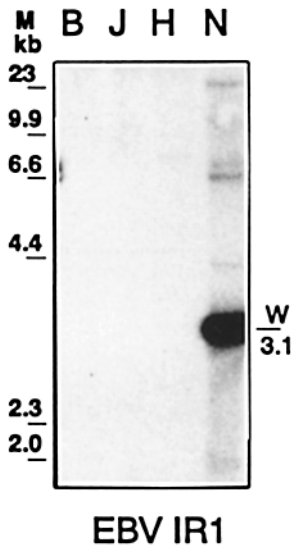

Figure 4. Absence of EBV genomes in HD-MyZ. A Southern blot of BamHI-digested DNA was prepared as described in Fig. 3 and was hybridized with a probe specific for the EBV internal repeat 1 (*EBV IR1*). No EBV-specific sequences were detected in HD-MyZ (*H*) as well as the EBV-negative cell lines BJAB (*B*) and Jurkat (*J*). In the human Burkitt's lymphoma line Namalwa (*N*), which carries two integrated copies of the EBV genome (53), the 3.1-kb BamHI W fragment (*W*) is clearly detected.

jected either intravenously or subcutaneously into SCID mice. Animals from both groups developed fatal disease within 5 wk after injection. At autopsy massive tumors were observed at the site of injection (subcutaneous group). In addition, infiltration and enlargement of axillary, inguinal, and retroperitoneal lymph nodes and spleen as well as pleural effusion were noticed. In contrast to subcutaneous application, intravenous injection led to a more prominent infiltration of the visceral organs, a massive pleural effusion, and psoas muscle infiltration. Primary tumor tissue consisted of large RS- and H-like cells. The spleen was found to be infiltrated by tumor cells (Fig. 7, *a* and *b*).

For detection of human cells within single cell suspensions of tumor cells obtained from the primary tumor (subcutaneous group), metastatic lymph nodes, spleen, and pleural effusion by flow cytometry (FCM) analysis, a PE-labeled anti–human HLA-DR antibody was used that does not crossreact with mouse cells. HLA-DR$^+$ cells were gated. FCM analysis of these SCID mice–derived tumor cells with double immunofluorescence was performed using PE-labeled anti–human HLA-DR and FITC-labeled CD13, CD19, CD25,

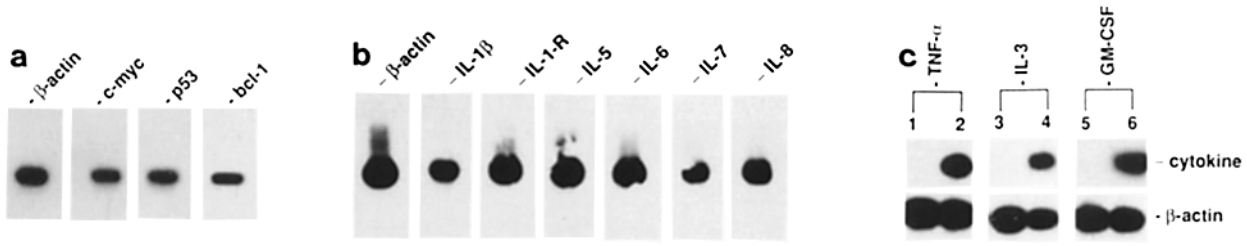

Figure 5. mRNA expression of cytokines, cytokine receptors, and proto-oncogenes in HD-MyZ cells. (*a*) RT-PCR products were blotted, autoradiographed, and hybridized as described above. Specific transcripts for c-*myc*, p53, and *bcl*-1 were detected in unstimulated HD-MyZ cells. (*b* and *c*) Autoradiographs of blotted and hybridized RT-PCR products are depicted. (*b*) Specific transcripts for IL-1β, IL-1 receptor(type 1), IL-5, IL-6, IL-7, and IL-8 were observed, whereas mRNA products of TNF-α, IL-3, and GM-CSF could not be detected in HD-MyZ cells (lanes *1*, *3*, and *5*). Raji cells (lanes *2* and *6*), PHA plus PMA-stimulated human PBL (lane *4*), as well as β-actin expression were used as controls (*c*).

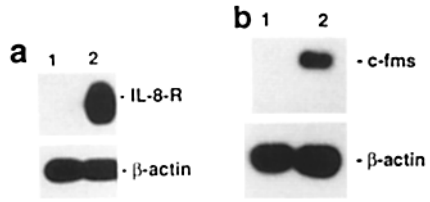

Figure 6. Expression of IL-8 receptor and proto-oncogene c-*fms* (CSF-1 receptor) is induced in HD-MyZ by PMA treatment. (*a*) HD-MyZ cells were in vitro activated with 10 ng/ml PMA for 24 h (lane *2*). Control cells were cultured for the same period in culture medium alone (lane *1*). In control cells no IL-8 receptor mRNA expression could be detected. In contrast, significant amounts of IL-8 receptor transcripts were observable after 24 h of PMA stimulation. β-actin expression served as control. (*b*) HD-MyZ cells were stimulated with 10 ng/ml PMA. Unstimulated cells did not reveal c-*fms* expression (lane *1*), whereas c-*fms* transcripts could be detected after 8 h of PMA stimulation.

and CD71, and revealed a phenotype corresponding to that of HD-MyZ cells (Fig. 8). Cells from all these tumor sites were transferred into culture medium and showed the same growth characteristics.

Discussion

The pathogenesis of HD and, at its center, the cellular origin of the H or RS cells still remain enigmatic. Almost every hematopoietic cell, including both B and T lymphocytes, monocytes/macrophages, myeloid cells, and interdigitating reticulum cells, has been speculated to be the normal counterpart (for review see reference 2). With the advent of several HD-derived tumor cell lines it has been possible to carry out detailed studies on the biology and the origin of these cells. Due to the fact that H or RS cells account only for a minority of cells within the bystander cells in HD tissue and that many attempts to cultivate H or RS cells failed, one has to bear in mind that those cell lines, which were successfully derived from HD, might only reflect a certain aspect of the disease. Thus, one has to be careful in drawing generalizations from these cell lines about the in vivo biology and origin of H or RS cells.

We describe the successful establishment of a novel H cell line, which was derived from the pleural fluid of a patient with HD of nodular sclerosing type. The morphological analysis of the cells within the pleural effusion disclosed the typical features of RS and H cells, i.e., large multinucleated and mononucleated cells with prominent nucleoli. These cells were transferred into culture medium, grew adherently, and have been in continuous culture since November 1991. The immunophenotyping disclosed that the HD-MyZ cell line was devoid of B or T lineage–associated markers (Table 2). HD-MyZ cells were found to strongly express CD13, a cell surface antigen expressed on myelomonocytic cells. MHC HLA-DR, CD71, CD10, and cytoplasmic CD68 were also detected. This phenotype ($CD13^+$, $CD68^+$, $HLA\text{-}DR^+$) is compatible with myelomonocytic differentiation (54, 55). Repeated FCM analysis did not show any change in cell surface antigen expression. CD13 (identical to aminopeptidase-*N*) and CD10 (identical to neutral endopeptidase) are both metalloproteases. Both proteases are thought to be involved in the inactivation of regulatory peptides at the cell surface (56).

CD30 expression was not observed on the cell surface of HD-MyZ cells. Interestingly, another HD-derived cell line (SU/RH-HD-1) displaying myelomonocytic features, i.e., CD14 expression, was also described to be devoid of the HD-associated activation antigen CD30 (3).

Analysis of IgH and TCR β locus confirmed our assumption that HD-MyZ cells lack lymphoid commitment. To our knowledge HD-MyZ is the only HD-derived cell line that is devoid of Ig or TCR rearrangement. Little is known about

Table 3. *Constitutive mRNA Expression of Selected Cytokines, Cytokine Receptors, and Proto-oncogenes*

mRNA expression	HD-MyZ
IL-1α	+
IL-1β	+
IL-2	–
IL-3	–
IL-4	–
IL-5	+
IL-6	+
IL-7	+
IL-8	+
IL-10	+
GM-CSF	–
TGF-β	–
TNF-α	–
TNF-β	–
IL-1R(type 1)	+
IL-2R(CD25)	–
IL-3R(α-chain)	–
IL-4R	–
IL-6R (p80)	+
IL-7R	–
IL-8R*	+
TNF-R (type I)	–
TNF-R(type II)	–
c-myc	+
c-myb	+
bcl-1	+
bcl-2	–
c-kit	–
N-*ras*	+
p53	+
c-fgr	–
*c-fms**	+

* Induction after PMA stimulation.

Table 4. *Secretion of Cytokines by HD-MyZ*

	1 h M	1 h PMA	40 h M	40 h PMA
		pg/ml		
IL-1β	8	13	15	40
IL-6	45	70	150	670
IL-8	350	400	2,800	5,950

10^6 cells/ml were incubated with culture medium in the presence or absence of 10 ng/ml PMA. After the indicated incubation period, cells were pelleted, cell-free supernatants were collected, and cytokine concentration was measured by ELISA.

the involvement of specific oncogenes in the pathogenesis of HD. Studies on oncogene expression in different HD-derived cell lines revealed a heterogeneous pattern. Frequently expressed proto-oncogenes were c-*myc*, c-*myb*, c-*raf*, and N-*ras* (57). In our cell line specific transcripts for c-*myc*, c-*myb*, *bcl*-1, N-*ras*, and p53 tumor suppressor gene were detected.

Since HD-MyZ cells showed phenotypical characteristics of myelomonocytoid differentiation (CD13, CD68, MHC class II), we were interested in studying the expression of a recently described intermediate filament-associated protein designated restin (44), which is found to be expressed at high levels in H and RS cells and in in vitro cultivated human peripheral blood monocytes. Specific restin transcripts were detected in HD-MyZ cells. Expression of the c-*fms* proto-

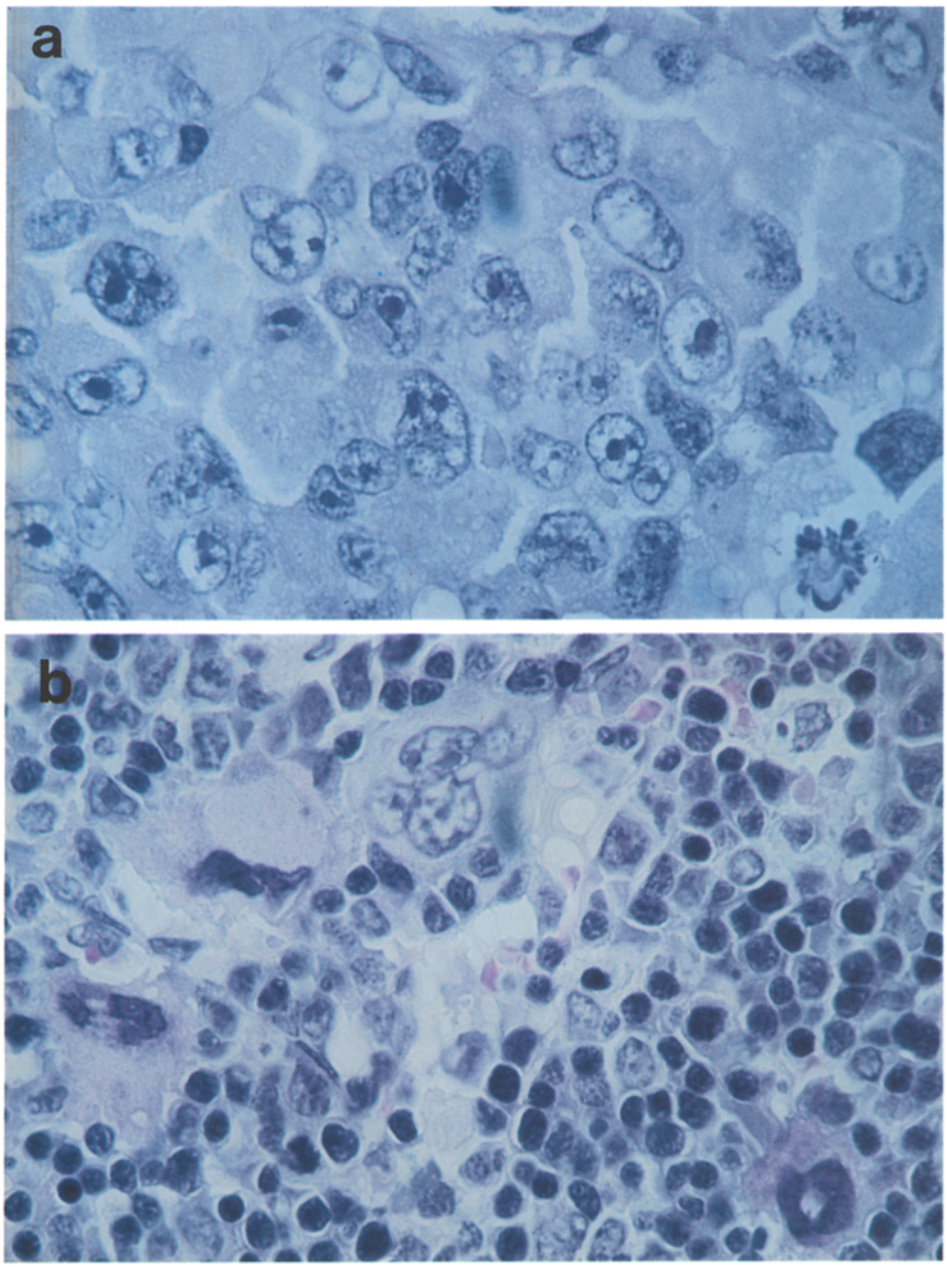

Figure 7. Infiltration of spleen and metastatic lymph node by tumor cells. Tumors were dissected from SCID mice. Hematoxylin/eosin-stained sections from metastatic lymph node tumor are shown (*a*). The tumor consists of large anaplastic multi- and mononucleated cells resembling RS or H cells. (*b*) Hematoxylin/eosin-stained spleen sections demonstrating infiltration by tumor cells.

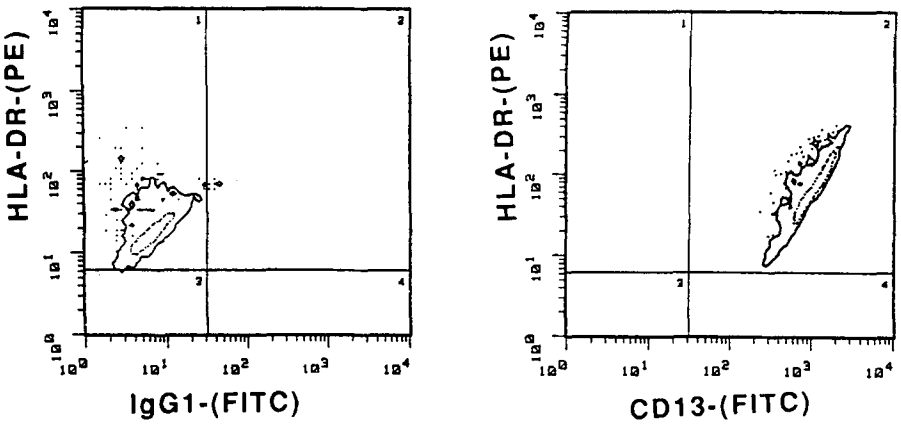

Figure 8. FCM® analysis of SCID mice derived human tumor cells. 10^6 tumor cells were used for FCM analysis with double immunofluorescence using PE-conjugated anti-HLA-DR mAb and FITC-labeled CD13 mAb or FITC-labeled IgG1 control mAb. For detection of human cells HLA-DR-positive cells were gated. Contour plots are depicted. Coexpression of CD13 and HLA-DR is observed in all tumor cells.

oncogene (CSF-1 receptor) could be induced in HD-MyZ cells by stimulation with PMA. *c-fms* expression is correlated with monocytic differentiation (58).

HD is clinically characterized by B symptoms, elevated acute phase reaction proteins, thrombocytosis, and histopathologically by sclerosis, eosinophilia, plasmocytosis, lymphocytic, and neutrophil reaction. These features might reflect the effects of tumor cell–derived cytokines. We therefore studied mRNA expression of various cytokines and cytokine receptors as well as the release of these cytokines into the supernatants. The pattern of cytokine production observed in HD-MyZ cells fits into the setting of monocytoid differentiation of this cell line and could explain some of the clinical and histopathological features of HD. Autocrine production of growth factors has been suggested to be involved in the pathogenesis of various hematologic malignancies. Thus, TNF-α has been proposed as autocrine growth factor in hairy cell leukemia (59), IL-1β in acute myeloid leukemia (60), and IL-6 in plasmocytoma (61). Since some cytokines (IL-1α, IL-1β, IL-6) were expressed constitutively in conjunction with their receptors, an autocrine growth model might also exist in HD-MyZ cells.

Recently IL-6 secretion as well as IL-6 receptor expression were observed in several H cell lines (HDLM-2, KM-H2) as well as in HD tissue (62, 11). Neutralizing antibodies to recombinant IL-6, however, did not inhibit the growth of the cell lines tested (11).

Using immunoperoxidase staining of tumor tissue, IL-1 expression was observed in 20 patients with HD and in patients with true histiocytic malignancies, whereas B or T cell lymphomas were devoid of IL-1 expression (63). The authors argued that this observation might provide further evidence for the relationship between RS cells and interdigitating reticulum cells. In the mean time IL-1 expression has been described in a variety of tumors. IL-1 has been discussed as an autocrine growth factor in acute myeloblastic leukemia (see above). Since HD-MyZ cells display monocytic features and simultaneously express IL-1α, IL-1β, and IL-1 receptors, IL-1 appears to be a likely candidate for an autocrine growth factor. Further studies using neutralizing antibodies or antisense oligonucleotides are warranted to clarify this model.

Due to the fact that HD-MyZ constitutively secretes large amounts of IL-8 in the absence of IL-8 receptors (which, however, can be induced by PMA treatment), IL-8 might have an important role as a paracrine rather than an autocrine cytokine. IL-8 might be responsible for inflammatory symptoms in patients with HD.

One important criterion for defining an in vitro growing cell as malignant is the tumorigenic growth in nude or SCID mice. In addition SCID mice provide a useful in vivo model for studying human lymphoma growth and in vivo therapy strategies (64). The subcutaneous and intravenous inoculation of HD-MyZ cells into SCID mice led to the development of large tumor masses with infiltrative, destructive growth and to the evolution of extensive lymphadenopathy and splenomegaly. Histologically RS and H cells could be detected in these tissues. FCM analysis of human cells derived from these SCID mice tumors revealed the same phenotype as the parental in vitro cell line.

Due to the fact that the patient-derived cell line led to the development of disseminated tumors in SCID mice mimicking the patient's clinical picture concerning the localization of disease manifestations, we have reason to assume that this cell line indeed represents the disease-causing tumor cell. Taken together our data might suggest the following pathogenetical model: an activated myelomonocytic tumor cell with morphological characteristics of H or RS cells furnished with a broad repertoire of cytokines might cause the tissue reaction, as well as the biological heterogeneity observed in HD, and might sustain its growth in an autocrine fashion. The model using SCID mice might be of great value for understanding the biological heterogeneity of HD and for testing novel therapeutic interventions in vivo.

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Address correspondence to Bernd Dörken, Free University of Berlin, Universitätsklinikum Rudolf Virchow, Robert-Rössle-Klinik, Lindenbergerweg 80, O-1115 Berlin-Buch, Germany.

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