Expression of Cytokine Genes, Cytokine Receptor Genes, and Transcription Factors in Cultured Hodgkin and Reed-Sternberg Cells

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ABSTRACT

The present study was carried out by Northern blot analysis and enzyme linked immunosorbent assay to determine the expression of cytokine genes and their receptors in cultured Hodgkin and Reed-Sternberg cells (HD and RS). The study showed that HD and RS cells express a variety of cytokine genes, including those for granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-8, and transforming growth factor-beta (TGF-β). The expression of these cytokines and their receptors is consistent with the concept that HD cells are derived from T-cells. The study also demonstrated that HD cells express a variety of transcription factors, including NF-κB, which is involved in the activation of cytokine genes.

INTRODUCTION

HD is a malignant disorder characterized by the presence of multinucleated RS and the mononuclear H cells in a stromal background consisting of lymphocytes, plasma cells, histiocytes, and eosinophils. Both the etiology of HD and the precursor identity of its presumed malignant component, the RS and H cells, have remained uncertain.

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The abbreviations used are: HD, Hodgkin's disease; RS, Reed-Sternberg; H, Hodgkin; CSF, colony-stimulating factor; M-CSF, macrophage CSF; IFN, interferon; TNF, tumor necrosis factor; GM-CSF, granulocyte-macrophage CSF; G-CSF, granulocyte CSF; IL, interleukin; LIF, leukemia inhibitory factor; TGF, transforming growth factor; JE/MCAF, JE/macrophage chemotactant; and activating factor; cDNA, complementary DNA; AP, activation protein; NF, nuclear factor; NFAT, nuclear factor of activated T-cells; EMSA, electrophoretic mobility shift assay; TPA, 12-O-tetradecanoylphorbol-13-acetate; mAb, monoclonal antibody; TCR, T-cell receptor; ATCC, American Type Culture Collection; Sf, sodium dodecyl sulfate; SSC, standard sodium citrate; IL-2R (IL-6R), IL-2 (IL-6-R), IL-3 (IL-3-R) genes; c-fos and c-jun, which are involved in heterodimeric formation of the transcription factor activation protein 1, as well as for the NF-κB/KBF1 gene.

2 for review). Various cell types have been proposed as the originators of HD including lymphoid cells (3–6), mononuclear phagocytes (7–11), interdigitating reticulum cells (12), follicular dendritic cells (13, 14), and granulopoietic cells (15). Unfortunately, the analysis of RS-H cells is hampered by the scarcity of this neoplastic component and contamination with bystander cells in HD-involved tissues. The advent of improved tissue culture methodology for the establishment of immortalized cell lines has enhanced the possibilities of studying neoplastic cells.

Recently, a number of cell lines have been established from tissues or pleural effusions of patients with HD (16–21), mostly with the nodular sclerosis variant. These in vitro derived cell lines may represent the in vivo RS-H cells having identical or very similar characteristic features. Therefore, they might be operationally regarded as in vitro representatives of RS-H cells, although it has to be considered that cell lines cannot be derived from the vast majority of cases of HD, and therefore, HD tumors that allow establishment of cell lines may somehow bear atypical features.

Recent studies aimed at determining the precursor identity of RS and RS-H cells and the pathogenesis of HD have focused on phenotypic studies (see Ref. 22 for review) and molecular genetic studies of rearrangements of the T-cell receptor and immunoglobulin genes (23–26), have analyzed possible alterations of the genomic structure and expression levels of certain protooncogenes including those of the ras gene family (27, 28), of the c-myc gene (16, 29) and the c-fms gene (30, 31), and have also explored the presence of EBV-Barr virus DNA in RS-H cells. In most of these studies, HD-derived cell lines were used. Despite these extensive efforts, there is still no consensus concerning either the etiology of HD or the identity of RS-H cells. Characterization of further features of HD-derived cell lines would thus be important in increasing our understanding of HD.

A number of features of HD are consistent with characteristics of a tumor of cytokine-producing cells, including occurrence of "B" symptoms, of sclerosis, eosinophilia, and polycarbon formation. Several investigators have assessed the capacity of RS-H cells to express cytokine genes. For instance, Paietta et al. (31) have shown that the L428 cell line, obtained from a nodular sclerosis HD patient, disclosed transcripts for the M-CSF and its receptor, encoded by the c-fms protooncogene (32). CSF secretion by cultured RS-H cells has also been shown by Burrichter et al. (33) and Byrne et al. (34). Naumovski et al. (35) have described secretion of IFN-γ by the SUP-HD1 cell line. The expression of in situ hybridization, transcripts for IL-5 were detected in RS cells of primary HD tissues by Samozuk and Nansen (36). Hsu et al. (37–39) have analyzed expression of IL-1, TNF-α, and TNF-β in the HDLM-1 and HDLM-1d Hodgkin lines (37–39). Similarly, Kretschmer et al. (40) have shown the ability of L428 and L540 Hodgkin cells to produce TNF. TGF-β and IL-6 secretion by RS-H cells has also been demonstrated in both cultured and primary Hodgkin tissues (41–43). Also, expression of IL-9 by cultured and primary RS-
H cells has been shown (44, 45).

Given these first observations, the aim of the present study was to examine the spectrum of cytokines released by the two well-defined HD-derived permanent cell lines HDLM-2 (46) and KM-H2 (47) in more detail. To this end, an extensive panel of cytokines was analyzed at the mRNA and protein levels, including GM-CSF, G-CSF, M-CSF, IL-1-α and IL-1-β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, LIF, TNF-α, TNF-β, TGF-β, IFN-γ, the gene coding for the JE/MCAF protein, and the early response genes c-jun and c-fos, known to be involved in cytokine signaling (48, 49). In addition, expression of the p55 IL-2Rα and p75 β chain of the p80 IL-6R, and of the M-CSF receptor (c-fms) was assessed by Northern blotting using specific cDNA probes. The presence of transcription factors known to bind to consensus sequences in regulatory 5′-flanking regions of many cytokine genes (50), such as the AP 1, the NF κB, and the NFAT 1, previously shown to be a T-cell-specific molecule (51), were analyzed in nuclear extracts of both cell lines, of peripheral blood T-cells, T-cell leukemia lines, lymphoblastoid B-cell lines, and of monocytes by EMSA.

MATERIALS AND METHODS

Cell Lines and Culture Conditions. Mycoplasma-free HDLM-2 (46), KM-H2 (47), embryonic lung fibroblast FH 109 (52), the T-cell leukemia lines Jurkat, Rex, and Molt-14 (kindly provided by S. Meuer, German Cancer Research Center, Heidelberg, Germany), and the Epstein-Barr virus transformed B-lymphoblastoid cell lines LUNDAK (53) and Laz 506 (kindly provided by S. Meuer) were grown in standard culture medium [RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% low endotoxin fetal calf serum (Hazelton, Vienna, UT), 2 mM l-glutamine, 1% penicillin/streptomycin (GIBCO)] at 37°C in a 5% CO₂ atmosphere in air. Only cultures in the log phase of growth were investigated. In some experiments, cell lines were cultured for 12–24 h with TPA (Sigma, Munich, Germany) at a concentration of 24 nM. T-cells and monocytes were prepared as previously described in detail (54).

Cell Surface Immunophenotyping and Monoclonal Antibodies. Expression of surface and nuclear markers was examined either by indirect immunofluorescence using flow cytometry (FACS Star; Becton Dickinson, Sunnyvale, CA) or on fixed cytospin slides according to standard procedures (55). mAbs used in these studies included anti-CD2, CD3, -CD4, -CD8, -CD10, -CD11b, -CD19, -CD20, -CD21, -CD33, -CD71 (from Coulter Electronics, Hialeah, FL), anti-CD14, -CD15, -CD34, HLA-DR (from Becton Dickinson), anti-CD30, KI-67 (kindly provided by H. Stein, University of Berlin, Germany), polyclonal anti-human α or β light-chain (from Tago, Burlingame, CA), anti-p55 IL-2R (CD25) mAb Tc (kindly provided by T. Waldman, National Institutes of Health, Bethesda, MD), anti-p75 IL-2R mAb (Oncogene Science, Inc., Manhasset, NY), and anti-p80 IL-6R mAb MT 18 (kindly provided by T. Hirano, Osaka University, Osaka, Japan). An mAb against the monomorphic portion of the TCR-β chain (BF-1) was kindly provided by S. Meuer.

cDNA Probes. For hybridization, the following specific cDNA probes were used: GM-CSF, 1.0-kilobase PstI fragment of pBR322-hGM-CSF (kindly provided by P. Habermann, Hoechst, Frankfurt, Germany); G-CSF, the 0.6-kilobase EcoRI/HindIII fragment of pUC8-hG-CSF (kindly provided by L. Souza, Amgen, Thousand Oaks, CA); M-CSF, the 1.0-kilobase PstI/BglII fragment of pUC18-hM-CSF (kindly provided by P. Ralph, Cetus Corp., Emeryville, CA); IL-1-β, the 0.6-kilobase BamHI/Smal fragment of pYPEsec-1-hIL-1β (ATCC, Rockville, MD); IL-2, the 0.7-kilobase EcoRI fragment of PC9/hIL-2 (kindly provided by W. Sikora, Medizinische Hochschule, Hannover, Germany); IL-4, the 0.6-kilobase Hpal/Xhol fragment of pGembl-hIL-3 (P. Habermann); IL-6, the 0.4-kilobase PstI fragment of pcD-HL-4 (kindly provided by S. Gillis, Immunex Corp., Seattle, WA); IL-5, the 0.9-kilobase BamHI fragment of pIL-5115.1 (ATCC); IL-6, the 0.55-kilobase TaqI/BamHI fragment of pGEM4-hIL-6 (kindly provided by T. Hirano, University of Osaka, Osaka, Japan); IL-7, the 0.51-kilobase PvuII fragment of pGembl-hIL-7 (S. Gillis); IL-8, the 0.4-kilobase BgII/EcoRI fragment of pBBG44 (British Bio-Technology, Oxford, United Kingdom); IL-10, the 0.76-kilobase BglII/HindIII fragment of pcDSRA (kindly provided by P. Vieira, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA); JE/MCAF, the 0.74-kilobase KpnI fragment of pGEM-HJ34 (kindly provided by B. Rollins, Dana-Farber Cancer Institute, Boston, MA); LIF, the 0.6-kilobase HindIII/EcoRI fragment of pBBG 46 (British Bio-Technology); TNF-α, the 1.0-kilobase PstI fragment of pSP64-hTNF-α (kindly provided by G. Adolph, Boehringer International, Vienna, Austria); TNF-β, the 0.6-kilobase Ndel/BglII fragment of pBBGS (British Bio-Technology); IFN-γ, the 0.7-kilobase BglII/HindIII fragment of pBR322-hIFN-γ (Genentech, San Francisco, CA); TGF-β, the 1.04-kilobase EcoRI fragment of pSP 64 (Genentech); c-fms, the 1.23-kilobase EcoRI fragment of pcms 104 (ATCC); NFκB/KBF1, the 3.9-kilobase Notl insert of pBKS+βF1 (kindly provided by S. Gosh, Whitehead Institute, Cambridge, MA); c-jun, the 1.2-kilobase EcoRI/BamHI fragment of pSHKJ (ATCC); c-fos, the 0.9-kilobase EcoRI fragment of pBR322-hfos (ATCC); IL-2Rα chain, the 0.9-kilobase EcoRI fragment of pSP65-hIL-2R (W. Sikora); IL-2Rβ chain, the 1.9-kilobase Sall/BamHI fragment of pUC10-b30SB (kindly provided by A. Kawahara, Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan); IL-6R p80, the 1.7-kilobase HindIII/Xhel fragment of pBl176-hIL-6R (T. Hirano); and actin, the 0.8-kilobase BamHI/PstI fragment of pBR322 chicken α-actin (kindly provided by J. Ramadori, University of Mainz, Mainz, Germany). IL-1-α was probed with an oligonucleotide as previously described (56).

Isolation of Total Cellular RNA and Northern Blot Analysis. Medium- or TPA-treated HDLM-2, KM-H2 cells, and FH109 lung fibroblasts, TPA (32 nm)- or phytohemagglutinin (1 mg/ml)-activated T-lymphocytes, or TPA (32 nm)-activated blood monocytes were harvested by centrifugation after the appropriate culture time. Cells were resuspended in 0.1 M sodium acetate, 1 mM EDTA, pH 5.2, then lysed with 0.5% SDS, and extracted with an equal volume of acetate/EDTA-equilibrated phenol (60°C). This mixture was incubated at 65°C for 20 min with frequent vortexing. The aqueous phase was recovered after centrifugation and extracted once with an equal volume of phenol/chloroform and twice with chloroform. The resulting RNA was precipitated overnight at −20°C with 2.5 volumes of ethanol. The total RNA from each sample was then electrophoresed in a 1% agarose gel containing 20 mM sodium borate, pH 8.3-0.5 mM EDTA-3% formaldehyde. The RNA was then transferred to nitrocellulose paper (Schleicher and Schuell, Dassel, Germany) in 10x SSC (1.5 M sodium chloride and 150 mM sodium citrate) using capillary blotting overnight. The blots were then back and prehybridized at 65°C in 7% SDS-10x Denhardt’s solution (1x Denhardt’s = 0.2% Ficoll-0.2% bovine serum albumin-0.02% polyvinylpyrolidone)-5x SSC-20 mM salmon sperm DNA (Sigma). The blots were probed with specific cDNA probes radiolabeled by random priming with a [α-32P]CTP (>6000 Ci/mmol) (Amersham, Arlington Heights, IL). Hybridization with oligonucleotides were performed essentially as previously described (56). The blots were washed at 55°C in 1% SDS-1x SSC and were autoradiographed with Kodak XAR film (Eastman Kodak, Rochester, NY) at −70°C with an intensifying screen.

Isolation of DNA and Southern Blot Analysis. Genomic high molecular weight DNA was isolated from HDLM-2 and KM-H2 cells, and Southern analysis was performed according to standard procedures (57). DNA probes were used to detect possible rearrangements of immunoglobulin heavy chain genes (BglII-restricted genomic DNA-Jκ probe), immunoglobulin light chain genes (EcoRI-restricted genomic DNA-Cλ probe or BamHI-restricted genomic DNA-Cλ, probe), and TCR-γ genes (HindIII-restricted genomic DNA-Tγ probe). An M-CSF and c-fms probe (see above) were also used in Southern blot analysis of DNA digested with various restriction endonucleases (Bethesda Research Laboratory, Gaithersburg, MD).

Cytokine Determination in Culture Supernatants. Cell-free supernatants of medium- or TPA (32 nm, 24 h)-treated cells were assessed for GM-CSF, G-CSF, M-CSF, IL-1-α, IL-1-β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-18, IFN-γ, IL-23, IL-27, IL-28B, IL-29, TNF-α, TNF-β, LIF, CSF-1, G-CSF, M-CSF, and Th1 cytokines (IL-12, IFN-γ) by ELISA.
8, TNF-α, TNF-β, and TGF-β synthesis. Enzyme-linked immunoabsorbent assay kits were purchased from the following suppliers and performed as indicated by the manufacturer: R&D Systems, Minneapolis, MN (IL-1-α, IL-1-β, IL-3, IL-4, IL-6, IL-8, TNF-α, and TNF-β); Amgen, Thousand Oaks, CA (G-CSF); Medical Resource Limited, Ohlsson and Edlund (58) with modifications as described in Ref. 59. Other cytokines. Some samples required dilution up to 1:10 prior assay. Extraction of Nuclear Proteins and Electrophoretic Mobility Shift Assay. Nuclear extracts were prepared from 10^7 cells by the method of Ohlsson and Edlund (58) with modifications as described in Ref. 59. Protein concentrations were measured according to a standard protocol (60), and 10 µg protein was used for EMSA. EMSA was performed using 10,000 cpm of the end-labeled double-stranded oligonucleotide (NF-κB, TCGAAGAGGAGCTTTCGAGG; AP1, GGGAGCCCTGACTCTAACAGCT; NFAT1, GGTTAAAAGAAGGGGAAAAACTGTGTCCA; the consensus binding sites are underlined) and 20 ng specific or nonspecific competitor oligonucleotide. The components were incubated for 30 min at room temperature before separation on a 5% polyacrylamide gel. The gels were dried and autoradiographed at —70°C using an intensifying screen.

UV Cross-Linking. UV cross-linking was performed as previously described (61). Briefly, the NF-κB oligonucleotide was continuously labeled with the Klenow large fragment polymerase (Boehringer-Mannheim, Germany) in the presence of 5 mM dGTP-5 mM dATP (Boehringer)-5 mM 5'-bromodeoxyuridine triphosphate (Sigma)-[32P]dCTP (Amersham, Arlington Heights, IL). Nuclear proteins from treated monocytes, T-cells, HDLM-2 KM-H2, and Lundak B-cells were incubated with 100 fmol continuously labeled oligonucleotide as described above and then subjected to UV irradiation (254 nm) for 20 min at 4°C. Proteins were separated under reducing conditions on a 12% SDS gel. A rainbow marker (Amersham) was used as a size marker. The gel was dried and autoradiographed at —70°C using an intensifying screen.

RESULTS

Phenotypic and Genotypic Features of HD-derived Cell Lines. Table 1 shows the phenotypic profile of HDLM-2 and KM-H2 cells. Both lines express CD15, CD30, Ki-67, CD71, CD25, and HLA-DR. In comparison to HDLM-2 cells, CD25 expression in KM-H2 was weak and detectable in a minority of cells only. Similarly, HDLM-2 cells exhibited the IL-2Rβ chain on their surface, while KM-H2 cells did not. HDLM-2 cells were stained with anti-CD2 but not with anti-CD4 mAb, while KM-H2 cells expressed CD4 but not CD2. Upon exposure to TPA for 24 h, KM-H2 cells displayed binding sites for anti-CD33 and anti-CD34 mAbs, while HDLM-2 cells did not. HDLM-2 cells failed to react with anti-B-cell antibodies (CD10, CD19, CD20, CD21, κ, λ). In contrast, KM-H2 cells stained positively for the CD21 monoclonal antibody but were unlabeled by other anti-B-cell mAbs. IL-6R p80 surface expression was seen in a subpopulation of HDLM-2 cells but not in KM-H2 cells. There was no difference in the morphological appearance of CD25-positive and -negative KM-H2 cells or of IL-6R p80-positive and -negative HDLM-2 cells. Analysis of gene rearrangements in DNA obtained from HDLM-2 cells revealed germline configuration of the immunoglobulin heavy- and light-chain genes, but the TCR-β chain showed a biallelic rearrangement. Genotypic analysis of KM-H2 cells demonstrated rearrangement of the immunoglobulin heavy-chain gene but not of the κ and λ light-chain genes probed with EcoRI-digested genomic DNA-C, or BamHI-digested genomic DNA-C, (not shown).

Constitutive Expression of Cytokine mRNA in HD-derived Cell Lines. Next, the cell lines were tested for constitutive mRNA production of a series of cytokines, cytokine-associated protooncogenes, and cytokine receptors. As shown in Table 2, expression of cytokine mRNA in both lines was heterogeneous. The spectrum of constitutive mRNA accumulation of HDLM-2 cells resembled that of activated T-cells, i.e., synthesis of transcripts for IL-1-α, IL-5, IL-6, ILF, TNF-α, and TNF-β. Transcripts for the IL-2R p55 and p75 chains, the IL-6R p80
chain, c-jun, c-fos, and NF-κB were also synthesized. Unlike activated T-cells, however, HDLM-2 cells failed to produce IL-2, IL-4, IFN-γ, IL-7, and IL-10 mRNA. Exposure to TPA also did not lead to expression of these genes. Detection of M-CSF and c-fms mRNA in both cell lines was surprising, because expression of these genes was previously found in monocytes/macrophages and placenta tissue only. Recently, c-fms expression was also identified in the HD-derived L428 line (31) and was interpreted as being indicative of the affiliation of RS-H cells with the phagocytic lineage. To determine whether c-fms and M-CSF transcript synthesis by HDLM-2 and KM-H2 cells was due to an aberrant expression resulting from a rearrangement, Southern blot analysis of restriction enzyme fragment length polymorphism for BamHI, RsaI, HindIII, EcoRI, PstI, and BglII was performed but did not reveal any gross rearrangement or amplification of either gene upon probing with the respective genomic probes. In addition, typical monocyte products such as IL-1-β, G-CSF, and JE/MCAF (62, 63) were not detectable in 15 μg RNA collected from HDLM-2 and KM-H2 cells. TGF-β transcripts were, however, constitutively expressed in both cell lines.

Effect of TPA on Expression of Cytokine mRNA by HD-derived Cell Lines. Since most of the cytokine genes investigated here contain TPA-responsive elements in their regulatory 5' sequences (50) conferring transcriptional activation upon binding, the effect of TPA treatment on mRNA accumulation of various genes that were not constitutively expressed by either line [GM-CSF, G-CSF, IL-1-β, IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, and LIF (for KM-H2 cells only) and IFN-γ, JE, IL-6R p80, and IL-2R p75] was investigated. To this end, HDLM-2 and KM-H2 were exposed to TPA (24 nm) for 12 and 24 h. In all experiments, cell viability in TPA treated cultures was >95% by trypan blue dye exclusion. As shown in Table 2, GM-CSF and IL-3 mRNA, albeit not constitutively expressed by either cell line, was induced in HDLM-2 and KM-H2 cells by TPA. LIF and IL-8, as well as IL-2R β chain, mRNA not detectable in unstimulated KM-H2 was inducible upon TPA treatment, as was IL-8 mRNA in HDLM-2 cells. TPA failed, however, to elicit synthesis of transcripts for G-CSF, IL-1-β, IL-2, IL-4, IL-7, IL-10, IFN-γ, and JE/MCAF in both cell lines and also did not induce IL-6R p80 mRNA expression in KM-H2 cells.

Identification of Proteins of Various Cytokines in Supernatants of Cultures of HD-derived Cell Lines. Given the finding of mRNA synthesis of various cytokines by HDLM-2 and KM-H2 cells, release of the corresponding proteins was assessed in cell-free culture supernatants of both lines. Cultures of HDLM-2 and KM-H2 cells were harvested and were subjected to analysis of cytokine proteins by enzyme-linked immunoabsorbent assay (GM-CSF, G-CSF, IL-1-α, IL-1-β, IL-2, IL-3, IL-4, IL-6, IL-8, TNF-α, and TNF-β), Western blot analysis (TGF-β). As shown in Table 3, HDLM-2 and KM-H2 cells secreted M-CSF, IL-6, TNF-α, and TNF-β in the absence of TPA treatment. Upon exposure to TPA, secretion of GM-CSF, IL-1-α, IL-3, and IL-8 protein became detectable. G-CSF, IL-1-β, IL-2, and IL-4 protein was, however, not detectable in supernatants of HDLM-2 and KM-H2 cultures irrespective of the presence or absence of TPA.

Effect of Anti-M-CSF and Anti-IL-6 Antibody on Growth Characteristics of KM-H2 Cells. Since both cell lines coexpressed M-CSF and its receptor and HDLM-2 cells exhibited the IL-6R p80 chain and secreted IL-6, the possibility of auto-crine growth stimulation by both factors was investigated. HDLM-2 and KM-H2 cells were seeded at 10^5 cells/mL into 96-well flat bottom plates (Greiner, Nütrtingen, Germany) in standard culture medium with or without neutralizing moAbs to recombinant human M-CSF (kindly provided by P. Ralph) or recombinant human IL-6 (kindly provided by J. Content, University of Brabant, Brussels, Belgium) at final dilutions of 1:100. This concentration neutralized >1000 units/mL of M-CSF and >50 ng/mL of IL-6 in pilot experiments investigating the effect of M-CSF and IL-6 on growth of the myeloid leukemia WEHI 3BD and the hybridoma B9.9 cells. Cultures were performed for a period of 24, 48, and 72 h with [3H]thymidine (37 mBq/well) being present for the last 6 h. The cells were then collected onto glass fiber strips with a semiautomatic cell harvester (Cambridge Technology Inc., Cambridge, MA) and dried. The amount of [3H]thymidine incorporated into DNA was determined by liquid scintillation counting. Since no difference in the cultures with or without anti-M-CSF or anti-IL-6 moAbs could be detected (not shown), M-CSF and IL-6 may not have served as autocrine growth factors for either cell line, at least so far as the secreted form of these molecules is concerned. However, internal association of the M-CSF and IL-6R with their growth factors might be the mode of autocrine loop existent in the Hodgkin lines, but this was not investigated here.

Assay of Nuclear Proteins Obtained from HD-derived Cell Lines. Nuclear extracts from medium- or TPA (24 nm, 1 h)-treated HDLM-2, KM-H2 cells, T-cells, LUNDAK B-cells, and monocytes were analyzed for the expression of the transcription factors AP 1, NFAT 1, and NF-κB using EMSA (Table 4). HDLM-2 and KM-H2 cells constitutively produced a protein that bound an oligonucleotide containing the AP 1 consensus sequence from the collagenase enhancer. Both T-cells and HDLM-2 cells exhibited low AP 1-binding activity in an unstimulated state that was enhanced upon exposure to TPA by 7- to 8-fold and 3- to 4-fold, respectively. KM-H2 cells constitutively showed high AP 1-binding activity that was not further enhanced by TPA treatment. Normal peripheral blood T-cells, leukemic T-cell lines, and HDLM-2 and KM-H2 cells also displayed constitutive binding activity to an oligonucleotide containing the NFAT 1 consensus sequence, a nuclear factor previously demonstrated to be T-cell specific. Binding was further enhanced in all T-cell types and HDLM-2 cells upon exposure to TPA but was downregulated in KM-H2 cells after TPA treatment. Normal human monocytes and lymphoblastoid
Table 4 Expression of transcription factors by HD-derived cell lines, T-cells, monocytes, and B-cells

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*B* = no binding detectable; +, weak constitutive binding; ++, strong constitutive binding; ++++, enhanced binding upon treatment with TPA (for 1 h, 24 nM).

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**DISCUSSION**

We have shown the expression of a variety of cytokines (i.e., GM-CSF, M-CSF, IL-1α, IL-3, IL-5, IL-6, IL-8, LIF, TNF-α, TNF-β, TGF-β, cytokine receptors (IL-2R p55 and p75, IL-6R p80, M-CSF receptor), cytokine-associated protooncogenes, and transcription factors (NF-κB, NFAT1, c-fos, and c-jun which form the heterodimeric transcription factor AP 1) in HD-derived cell lines. The lines we have investigated (HDLM-2 and KM-H2) can be regarded as representatives of the neo-
plastic component of HD based on many characteristics of “true” RS-H cells (46, 47, 64), although it has to be considered that cell lines cannot be derived from the vast majority of cases of HD, and therefore, HD tumors that allow establishment of cell lines may bear atypical features. To establish concordance between primary RS-H cells and those in the HD-derived cell lines and to establish a relationship between cytokine expression by these cells and the pathophysiology of HD, future studies of the expression of the respective genes in primary HD-involved tissues will be clearly needed. A direct comparison between the primary tumor cells from which the cell lines were originally derived and both lines revealed, however, an almost identical morphological, cytochemical staining profile, immunophenotype, and genotype (46, 65, 66).

Constitutional B symptoms, elevations of acute phase reactants in the serum, the presence of mild thrombocytosis, or certain histopathological manifestations such as sclerosis, polycarion formation, plasmacytosis, and eosinophilia are common features in active HD (1) and may relate to an abnormal or unbalanced secretion of cytokines. Indeed, several of the cytokines identified here as products of RS-H cells exert biological effects that resemble distinct clinical and morphological features seen in HD. For instance, IL-6 is the major hepatocyte-stimulating factor that induces release of acute phase proteins (67). In addition, IL-6 exhibits thrombopoietin activity, an activity shared by LIF (68), and induces terminal differentiation of B-lymphocytes into immunoglobulin-producing cells. Injection of TNF-α and IL-1 can cause fever and sweats (69), clinical findings similar to B symptoms in HD. TNF-α also causes elevation of fibrogenin serum levels, also frequently seen in HD. LIF and TGF-β are known to be involved in collagen synthesis and, thus, in sclerosis formation (70, 71). Both IL-5 and GM-CSF are potent eosinopoietic growth factors and activators. Our results may therefore explain the prominent infiltration by eosinophils in many cases of HD. In addition, the supernatant obtained from cultures of HDLM-2 cells has been shown to contain a differentiation activity for myelomonocytic cells (35) that may be ascribed to the various CSFs and IL-6 produced by these cells. GM-CSF and IL-3 are involved in the induction of histamine release. The generalized itching often experienced by HD patients may be linked to secretion of these factors.

The presence of M-CSF/c-fms on HDLM-2 and KM-H2 cells and of IL-6/IL-6R p80 on HDLM-2 cells suggests autocrine growth stimulation. However, we were unable to detect such an autocrine loop generated outside the cells. Based on our experiments we cannot exclude any internal association of the M-CSF receptor and IL-6R with their respective ligands. Injection of TNF-α and IL-1 can cause fever and sweats (69), clinical findings similar to B symptoms in HD. TNF-α also causes elevation of fibrogenin serum levels, also frequently seen in HD. LIF and TGF-β are known to be involved in collagen synthesis and, thus, in sclerosis formation (70, 71). Both IL-5 and GM-CSF are potent eosinopoietic growth factors and activators. Our results may therefore explain the prominent infiltration by eosinophils in many cases of HD. In addition, the supernatant obtained from cultures of HDLM-2 cells has been shown to contain a differentiation activity for myelomonocytic cells (35) that may be ascribed to the various CSFs and IL-6 produced by these cells. GM-CSF and IL-3 are involved in the induction of histamine release. The generalized itching often experienced by HD patients may be linked to secretion of these factors.

The spectrum of cytokines being produced by HDLM-2 and KM-H2 cells and of IL-6/IL-6R p80 on HDLM-2 cells suggests autocrine growth stimulation. However, we were unable to detect such an autocrine loop generated outside the cells. Based on our experiments we cannot exclude any internal association of the M-CSF receptor and IL-6R with their respective ligands. It might also be possible that only a minor subpopulation of cells participates in autocrine growth stimulation that is not detectable by [3H]thymidine incorporation assay. In this regard, the observation that only the L428KSA variant but not the parental L428 HD line autocrinously generated M-CSF is of note. Remarkably, cell cycle kinetic studies revealed that L428KSA was derived from the proliferatively active cell population of the parental line (31).

The spectrum of cytokines being produced by HDLM-2 and KM-H2 may also serve to define the precursor identity of RS-H cells. The HDLM-2 cells have rearranged the gene for the β chain of the TCR, which appears to be nonfunctional because neither cytoplasmic nor surface expression of the TCR-β chain could be detected by using a mAb against a monomorphic portion of TCR-β. HDLM-2 cells also produce IL-2R transcripts and express the p55 and p75 IL-2R chain on the cell surface. The HDLM-2 cells produce mRNA of cytokines which are normally products of activated T-cells, including GM-CSF, IL-3, IL-5, IL-6, LIF, and TNF-β (which has been found in lymphocytes only). High levels of IL-1-α were also recently found in activated T-cells (72). Similarly, also M-CSF expression by activated T-cells has been reported (73). Clinical heterogeneity is apparent in HD, and thus, HD-derived cell lines show heterogeneity. Extrapolation of the information gained from KM-H2 cells would also support a T-cell origin rather than an affiliation with the macrophage or B-cell lineage, although KM-H2 cells nonproductively rearrange the immunoglobulin heavy-chain gene and display the B lineage-associated CD21 antigen. Interestingly, both cell lines expressed the IL-8 gene, previously observed in monocytes and fibroblasts only. Other typical monocyte products such as IL-1-β, JEC/4A, and G-CSF were, however, not detectable in cytoplasmic RNA of HDLM-2 and KM-H2 cells. Expression of c-fms by both cell lines must be ranked as abnormal. Abrupt expression of c-fms has been reported in some solid tumor cells (32, 74, 75) and also in the HD-derived L428 line (31) and may be the result of an altered activation of the c-fms promoter. Nevertheless, restriction enzyme analysis of DNA obtained from both lines failed to disclose a gross DNA rearrangement of the c-fms gene (and of the M-CSF gene). Therefore, we are currently investigating the c-fms gene in KM-H2 in more detail by SI-nuclease assay.

Both KM-H2 and HDLM-2 cells display a pattern of nuclear proteins binding to the NF-κB oligonucleotide which resembles that of T-cells in EMSA. UV cross-linking experiments confirm that HD-derived NF-κB proteins have the same molecular weight as T-cell-derived NF-κB-binding proteins, while monocytes (and also B-cells) show a NF-κB-binding complex lacking the 85,000 moiety. One might argue that the pattern of NF-κB expression may be related to levels of monocyte differentiation. We have, however, never observed the appearance of p85 protein regardless of mode (TPA, IL-1, TNF-α, endotoxin) and duration (30 min-6 h) of monocyte stimulation. In addition, the nuclear transcription factor NFAT 1, previously characterized as being T-cell specific, is constitutively expressed in both HDLM-2 and KM-H2 cells and binding activity is enhanced in response to TPA. Human monocytes and B-cells, however, failed to show NFAT 1-binding activity in the presence or absence of appropriate stimulation.

REFERENCES


Expression of Cytokine Genes, Cytokine Receptor Genes, and Transcription Factors in Cultured Hodgkin and Reed-Sternberg Cells

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