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Production of Tumor Necrosis Factor-α and Lymphotoxin by Cells of Hodgkin's Neoplastic Cell Lines HDLM-1 and KM-H2

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The production of tumor necrosis factors (TNF) from cells of two Hodgkin's Reed-Sternberg (H-RS) lines, HDLM-1 and KM-H2 was examined. The culture supernatant from these two types of H-RS cells exerts a cytotoxic effect on L929 cells. Both tumor necrosis factor (TNF-α) and lymphotoxin (TNF-β) are responsible for this activity. This was confirmed by the presence in the cells of proteins and m-RNAs of TNF-α and TNF-β, as determined with immunoperoxidase staining and Northern blot hybridization. Approximately 20% of HDLM cells and 5% of KM-H2 cells were positively stained by a monoclonal anti-TNF-α antibody, and this staining was inhibited by preabsorption of the antibody with recombinant TNF-α. Staining with anti-TNF-β, however, showed an intense reaction in more than 60% of HDLM-1 cells, but only in 5% to 10% of KM-H2 cells. The abundant expression of TNF-β in HDLM-1 cells is consistent with approximately 10 times the TNF activity in HDLM-1-conditioned medium as compared with that of KM-H2. The rich secretion of TNF-β in HDLM-1 cells was also validated by the inhibition of most of the TNF activity in HDLM-1-conditioned medium with anti-TNF-β antibody, and by the presence of abundant TNF-β mRNA in HDLM-1 cells. The reason for the abundant production of TNF-β in HDLM-1 cells is not yet known, but may be attributable to a chromosomal abnormality in the 6p21 region. The expression of TNF-α, but not TNF-β, by H-RS cells was demonstrated in lymph nodes from patients with Hodgkin's disease. The capacity of H-RS cells to secrete TNF as well as other cytokines, such as interleukin-1, colony-stimulating factors, and transforming growth factors, may contribute to the unique clinical and histopathologic alterations in patients with Hodgkin's disease. (Am J Pathol 1989, 135: 735–745)

We proposed that Hodgkin's neoplastic cells [Hodgkin's Reed-Sternberg (H-RS) cells] can produce various types of cytokines that form a complex interacting and overlapping network of signals that orchestrate the unique clinical and histopathologic responses in patients with Hodgkin's disease (HD). We and others previously showed that H-RS cells can produce interleukin-1 (IL-1), colony-stimulating factors (CSFs), transforming growth factor-β, and metabolites from the arachidonic-acid pathway.[1–10]

We speculated that, among other known cytokines, tumor necrosis factor-α (TNF-α) may be produced by H-RS cells. TNF-α was previously reported to be unique to cells of monocyte/histiocyte lineage.[11,12] It appeared to us that, because H-RS cells often possess functions and phenotypes similar to those of histiocytes/interdigitating reticulum (IR) cells,[1,4,8,13–20] these cells might be engaged in the production of TNF-α. Recently, however, expression or secretion of TNF-α was observed in T and B cells.[21,22] Thus, the production of TNF-α in H-RS cells, if confirmed, will be significant for the pathophysiology of this lymphoma, but will not offer an indication of its cell lineage.

In this study, to examine the possibility that TNF-α is produced in H-RS cells, we used immunocytochemical staining, Northern blot hybridization, and an L929 cytotoxicity assay. With the L929 assay, the activity of both TNF-α and TNF-β was detected. The latter type of TNF is also called lymphotoxin because it was initially discovered in lymphoid cells.[23,24] Whether TNF-β is specific for lymphoid cells was not established. Because of the lack of lineage specificity and the overlapping of biological activities of the two types of TNFs, we also included the production of TNF-β in H-RS cells in our study. We confirmed that both TNF-α and TNF-β are produced in two types of cultured H-RS cells, HDLM-1, and KM-H2. The production of TNF-β, however, is enhanced in HDLM-1 cells. In addition, we demonstrated the expression of TNF-α, but not TNF-β, in H-RS cells in tissues.

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Materials and Methods

Tissue Culture and Preparation of Conditioned Medium

We examined the TNF activity in culture supernatant (CM, conditioned medium) of HDLM-1 and KM-H2 cells with an L929 cytotoxicity assay. The HDLM-1 cells (provided by Dr. M. S. Lok, Denver General Hospital, CO) were established from the pleural effusion of a 74-year-old male patient with a nodular sclerosing subtype of HD. The KM-H2 cells (provided by Dr. S. Fukuhara, Kyoto University, Japan) were established from the pleural effusion of a 32-year-old male patient with a mixed cellular subtype of HD. The phenotypes, gene rearrangements, and biologic properties of these cell lines were reported in detail previously.[9,25-29] To prepare H-RS-CM, we cultured HDLM-1 and KM-H2 cells at 4×10^5 to 2×10^6 cells/ml in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 μm 2-mercaptoethanol, and 50 μg/ml gentamicin at 37 C in a humidified, 5% CO_2 atmosphere.[20] The viability of these cells was maintained at 90% to 95%. The medium was collected every 3 days and filtered through a 0.45 μm filter.

Phorbol Ester Induction

Phorbol myristic acetate (PMA) has significant effects on the differentiation, proliferation, and protein synthesis of H-RS cells. To study the effect of PMA on the production of TNFs, we treated H-RS cells with PMA (Sigma Chemical Co., St. Louis, MO) for 3 days. The protocol that we used for PMA induction of H-RS cells was described previously.[19,20] Briefly, PMA dissolved in DMSO (14 μg/ml) was added at a final concentration of 2 ng/ml to cultures. The medium was collected 3 days after PMA induction and dialyzed extensively before being used in a biologic assay.

Cell Cytotoxicity Assays for TNF Activity in H-RS-CM

The activity of the TNFs was measured on d-actinomycin-treated murine L929 cells as described previously.[30] Briefly, L929 cells were seeded into flat-bottom 96-well microtiter plates at a density of 5×10^4 cells/well in 100 μl Eagle's minimal essential medium (MEM). After overnight incubation, spent medium was removed, and 200 μl of MEM containing d-actinomycin (final concentration, 2.5 μg/ml) and H-RS-CM in serial dilutions (5–0.001% V/V) were added to each well. Plates were reincubated for 20 hours, medium was removed, and the cells were stained with crystal violet for 15 minutes. The staining intensity on rinsed and dried plates was measured as absorbance at 595 nm. All assays were done in octuplicate. The number of cytotoxic units was defined as the reciprocal of the supernatant dilution that causes a 50% decrease in absorbance. The TNF unit was standardized for recombinant TNF obtained from Amgen (Thousand Oaks, CA).

Neutralization of TNF Activity

We used a rabbit anti-TNF-β antibody (Genzyme, Boston, MA) to block the cytotoxic activity of L929 cells in H-RS-CM. We showed in our laboratory that this antibody does not inhibit the activity of recombinant TNF-α. The antibody (200 neutralizing units) was added to H-RS-CM (100 units) in MEM for 30 minutes at 37 C. The treated H-RS-CM was then serially diluted and added to L929 cell cultures. All tests were performed in triplicate. The remaining L929 cytotoxic activity (not blocked with anti-TNF-β) was attributed to TNF-α or a TNF-like substance other than TNF-β in H-RS-CM.

Detection of TNF-α and TNF-β in H-RS Cells

We used a specific anti-TNF-α monoclonal antibody, a rabbit anti-human TNF-β antibody, and immunoperoxidase staining to confirm the presence of TNFs in both untreated and PMA-treated H-RS cells. The monoclonal anti-TNF-α (Boehringer Mannheim, Indianapolis, IN) was shown to react with both natural and recombinant TNF-α, but not with TNF-β.[31] The rabbit antibody to human TNF-β was generated against homogeneous, purified human lymphotoxin (Genzyme). This antibody binds to both neutral and recombinant TNF-β and does not cross react with IL-1, CSFs, or TNF-α.

For immunostaining, cytospin smears of cultured H-RS cells were fixed in acetone at room temperature for 5 minutes. After being washed in Tris-buffered saline (TBS), 0.01 M, pH 7.6, the smears were incubated with anti-TNF-α (2.5 μg/ml, or 1:40) or anti-TNF-β (1:800), and then with biotin-labeled horse anti-mouse Ig or goat anti-rabbit Ig (1:200) and avidin-biotin-peroxidase complex.[32-34] Each step lasted 30 to 60 minutes, with an interval of 5 minutes for washing with TBS. The slides were developed in a DAB-hydrogen peroxide-nickel chloride solution.[33] Absorption of antibody with recombinant TNF-α (100 units, Amgen) served as a staining control. Absorption with recombinant TNF-β was not done because this reagent was not available to us.

Because it is generally not well known whether TNF-β is expressed in cells of monocyte/histiocyte lineage, we

examined the expression of TNF-β in the following cells: HL-60, THP-1, U-937, KG-1, and CTV-2. All these cells are related to monocytes or granulocytes.[35] The cells were cultured as described for H-RS cells.

Northern Blot Hybridization with TNF-α and TNF-β Probes

To confirm the production of TNFs in H-RS cells, we hybridized mRNAs from H-RS cells with a TNF-α synthetic probe or a TNF-β cDNA probe. The TNF-β cDNA clone was obtained from Dr. G. Wong (Genetics Institute, Cambridge, MA).[23,36] The oligonucleotide 24 mer probe 5'-TACAGGCTTGTCACTCTCGGGGTTCG for human TNF-α was synthesized by the phosphoramide method and purified by C-18 reverse-phase HPLC.[21] Both TNF-α and TNF-β are highly specific and do not cross hybridize.[21-23,36]

RNA from cell pellets was prepared by ultracentrifugation of the guanidinium thiocyanate cell lysate on a CsCl cushion as described previously.[3] Poly(A)$^+$-RNA was isolated on oligo(dT)-cellulose columns. For Northern blot hybridization, RNA was fractionated in 1.4% agarose gels containing 2.2 M formaldehyde. Hybridization of TNF-α or TNF-β to the RNA blots was performed at 42 C in 35% formamide, 1% SDS, 1 M NaCl, 10 mM EDTA, 10% dextran sulfate, 50 mM sodium phosphate, pH 7.0, and 100 μg/ml sheared and heat-denatured salmon sperm DNA (Sigma, St. Louis, MO). After overnight hybridization, the blots were washed in three changes of 2× SSC at room temperature (5 minutes each), followed by two 30-minute washes at 60 C in 2× SSC and 1% SDS. The blots were exposed to X-Omat XAR-5, XK-1 (Kodak) X-ray film.

Cytogenetic Study of HDLM-1 Cells

To relate the cytokine production by H-RS cells to their possible cytogenetic abnormalities, we carried out a cytogenetic study of HDLM-1 cells. The karyotype of KM-H2 cells has been described previously.[26] We cultured the cells in fresh RPMI medium and Colcemid (0.2 μg/ml) for 90 minutes, so that they were arrested in metaphase. The cell pellet was resuspended in a hypotonic solution (0.075 M KCl and 1% sodium citrate, 1:1) for 20 minutes at room temperature, fixed with modified Carnoy's fixative solution (absolute ethanol and glacial acetic acid, 3:1) for 15 minutes, and then washed with two changes of the fixative solution. The air-dried slides, aged for 1 week, were stained with a conventional Giemsa stain for scoring of chromosome numbers and for detection of chromosome aberrations, or with trypsin-Giemsa stain for chromosomal banding analysis.

Expression of TNFs in H-RS Cells in Tissues

We used the avidin-biotin-peroxidase technique,[32-34] as described above, to examine the expression of TNFs in H-RS cells in ten frozen tissue sections. These included seven sections of nodular sclerosis (NS) and three of the mixed cellularity (MC) form of HD. The diagnosis of HD in each of these cases was confirmed by the expression of CD30, but not of other T or B cell markers.[14] In addition, five tonsils and five lymph nodes from patients with reactive hyperplasia, sinus histiocytosis, or dermatopathic lymphadentitis were included for evaluation of TNFs in cells of non-neoplastic tissues. The dermatopathic nodes were included because these tissues contain numerous interdigitating reticulum (IR) cells.[18] The nature of IR cells is confirmed by their characteristic enzyme and immunohistochemical staining patterns with anti-IRac, which are distinct from those of histiocytes.[15]

We performed control tests for staining specificity by omitting the primary antibody, replacing the primary antibody with nonimmune serum, or absorbing the primary antibody with recombinant TNF-α.

Results

Cytotoxic Effect on L929 Cells

The culture supernatant from HDLM-1 and KM-H2 cells elicited cytotoxic activity when assayed with L929 cells. The substances responsible for this activity were TNF-α and TNF-β, based on immunoperoxidase staining and Northern blot hybridization (see below). By using recombinant TNF-α as a standard, we found that the amounts of TNF in culture supernatant ranged from 750 to 1000 U/ml (average, 800 U/ml) for HDLM-1 cells and 100 to 200 U/ml (average, 150 U/ml) for KM-H2 cells. The TNF titers in the supernatant were increased approximately twofold to fourfold after H-RS cells were treated with PMA for 2 days.

Approximately 80% to 90% of the L929 cytotoxic activity in HDLM-1-CM was abolished by incubation of the conditioned medium with anti-TNF-β antibody. However, the antibody neutralized only approximately 30% to 40% of the L929 cytotoxic activity in KM-H2-CM.

Demonstration of TNF-α and TNF-β in Cells

By using a monoclonal anti-TNF-α antibody and immunoperoxidase staining, we demonstrated that TNF-α was present in approximately 20% of HDLM cells and 5% of KM-H2 cells. After PMA induction, the number of cells positive for TNF-α increased to 60% to 70% and to 15% to 25% in HDLM-1 and KM-H2 cells, respectively (Figure

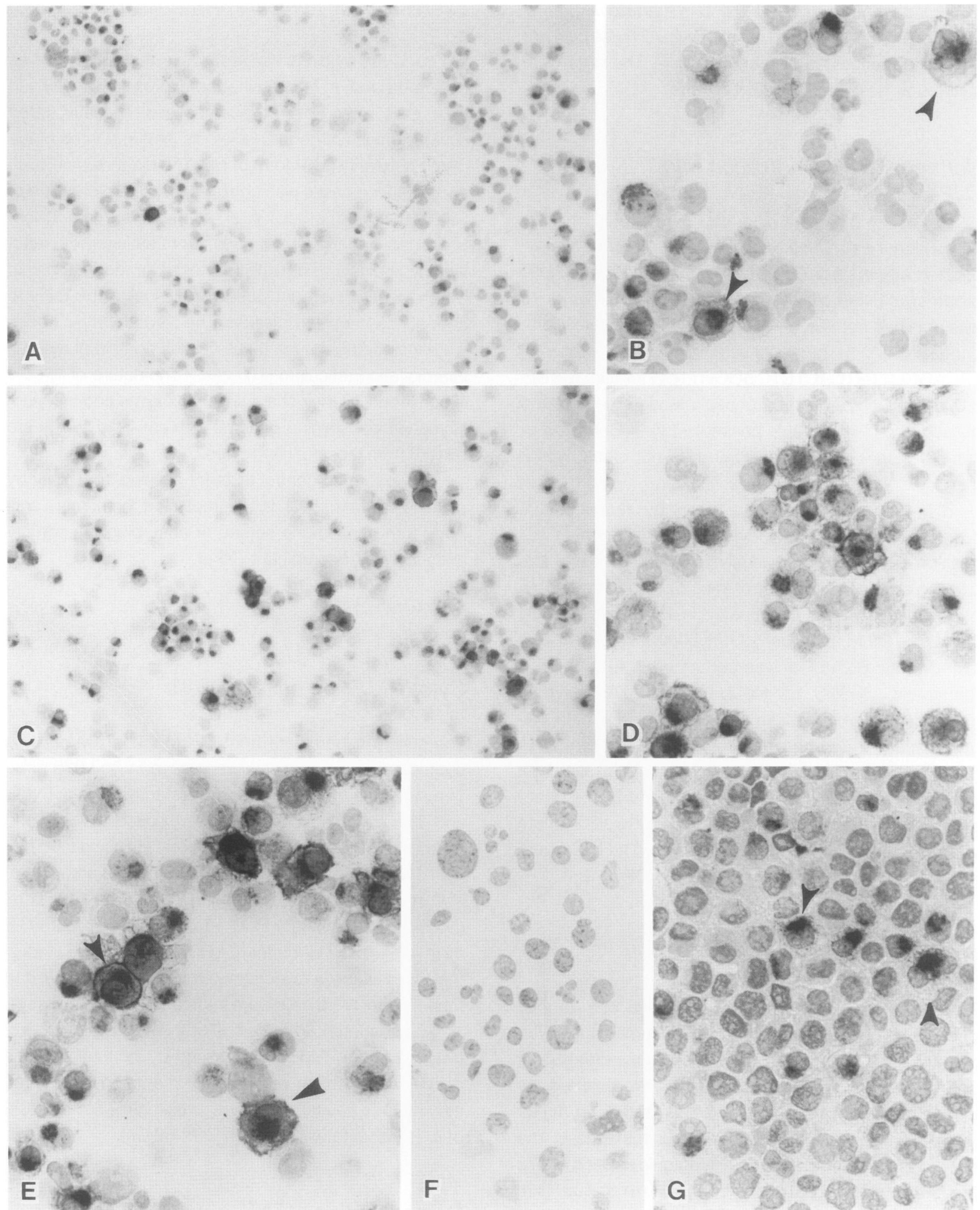

Figure 1. *Expression of TNF-α in cultured H-RS cells.* **A** *and* **B**: *HDLM-1 cells without PMA induction.* **C**, **D**, *and* **E**: *HDLM-1 cells 2 days after PMA induction.* **G**: *KM-H2 cells without PMA induction. The number of cell stained with anti-TNF-α and the staining intensity were increased when the cells were treated with PMA. In both types of H-RS cells, TNF-α is located mainly in the paranuclear region. However, a considerable number of HDLM-1 cells also elicited a membranous staining pattern (arrows in B and E). In* **F**, *anti-TNF-α, which had previously been absorbed with recombinant TNF-α, was added to HDLM-1 cells. No staining was observed, indicating that the staining reaction is highly specific (Original magnification: A and C, ×100; B and D–G, ×250).*

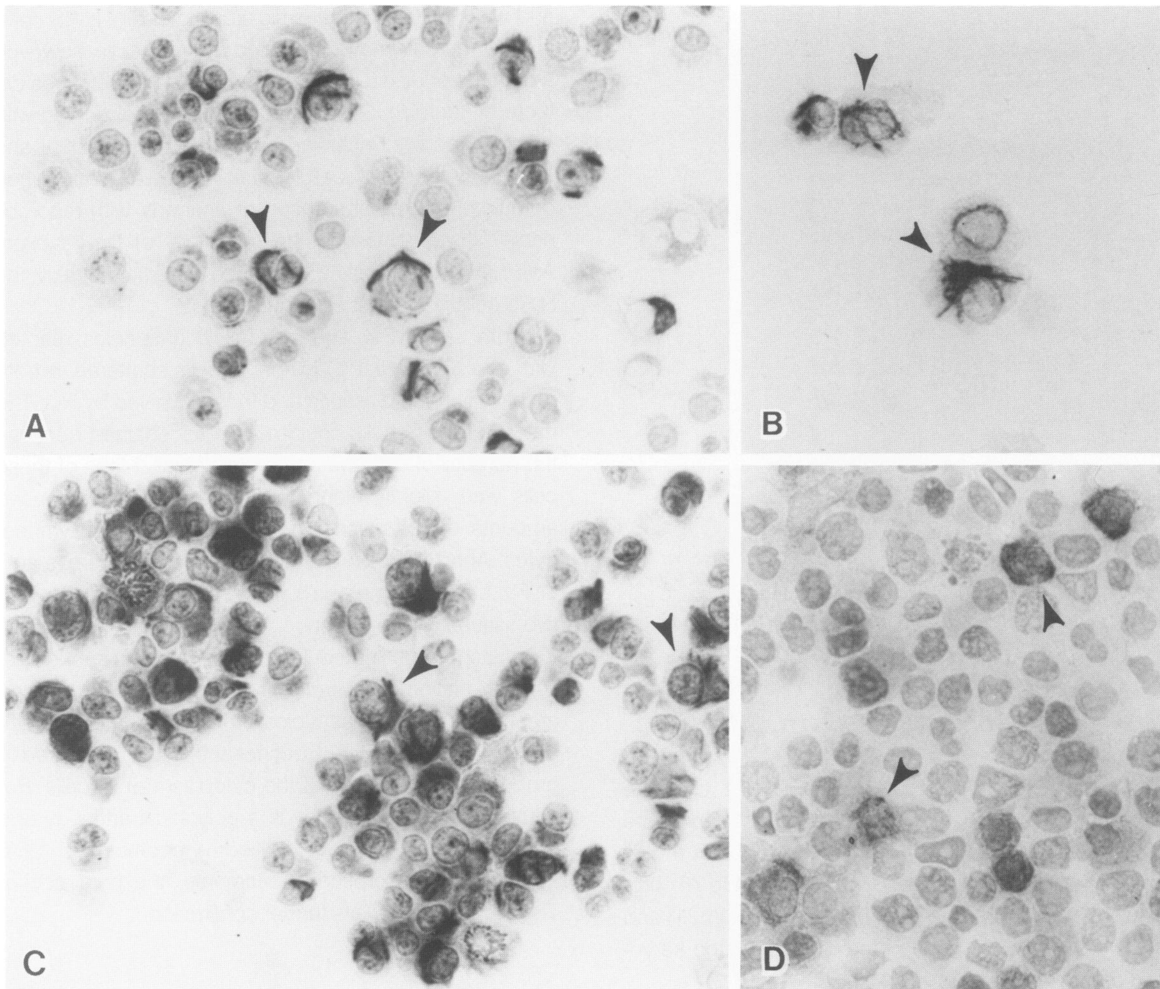

Figure 2. *Expression of TNF-β in cultured H-RS cells.* A *and* B: *HDLM-1 cells without PMA induction.* C: *HDLM-1 cells 2 days after PMA induction.* D: *KM-H2 cells without PMA induction. The number of cells stained with anti-TNF-β and the staining intensity were increased on treatment of the cells with PMA. In both types of H-RS cells, the staining was localized mainly in the cytoplasm. Approximately 10% to 20% of HDLM-1 cells also showed a filamentous staining pattern (arrows in B and C); this pattern indicates that TNF-β protein, after being synthesized, binds to filaments of an unknown type in HDLM-1 cells (Original magnification ×250).*

1). The staining intensity in PMA-induced cells was also enhanced. The TNF was localized in the cytoplasm and in approximately 10% of the stained cells on the membrane. The anti-TNF-α staining could be blocked completely by the absorption of antibody with recombinant TNF-α.

TNF-β was abundant in the cytoplasm in 60% of the HDLM-1 cells. In PMA-treated cells, the staining intensity and the number of TNF-β-positive cells increased to 70% to 80% of the cells. No staining of cell membranes was detected, but in 10% to 20% of cells, a filamentous distribution was observed (Figure 2). TNF-β was present in the cytoplasm in 10% of the KM-H2 cells. The staining was diffuse and weak, and occasionally it was granular and appeared to be located in the Golgi apparatus. Induction of cells with PMA generally increased the staining intensity.

Approximately 1% or 2% of U-937 and CTV-2 cells were positive for TNF-β. Staining of cell membranes was observed in U-937 cells.

Northern Blot Hybridization

Poly(A)$^+$-mRNA from HDLM-1 and KM-H2 cells hybridized with TNF-α and TNF-β cDNA probes. With these probes, we identified a 1.7-kb and a 1.4-kb band for TNF-α and TNF-β, respectively (Figures 3 and 4). These sizes are in agreement with the results of Northern blot analyses of monocytic or lymphoid cell lines.[21,22] HDLM-1 cells appeared to produce excess amounts of TNF-β mRNA compared with the production of TNF-α and of β-actin in these cells (Figure 4).

Figure 3. *RNA was prepared from HDLM-1 cells. Northern blot was probed with synthetic TNF-α. The size of the m-RNA hybridized was approximately 1.7 kb. A similar hybridization was obtained with KM-H2 cells, although the intensity was weaker (not illustrated).*

Chromosome Abnormalities in HDLM-1 Cells

Representative cytogenetic data on HDLM-1 cells are shown in Figure 5. Most HDLM-1 cells had chromosome numbers between 30 and 42 (hypodiploid), with modal numbers having two peaks, at 37 or 38 and 74 to 76 chromosomes. Two significant abnormalities, del(6)(p21) and der(5)t(1;5)(q21,q35), were noted in most of the karyotyped cells.

Expression of TNFs in H-RS Cells in Tissues

In all ten tissues examined, we detected a granular staining pattern in approximately 10% to 30% of H-RS cells and weak diffuse cytoplasmic staining in rare H-RS cells. In most regions heavily infiltrated by H-RS cells, however, a granular or diffuse extracellular staining pattern was detected (Figure 6). Such a pattern was not observed with other antibodies, including anti-CD3, CD30, CD20, and anti-TNF-β. The extracellular staining could be abolished completely by preabsorption of antibody with recombinant TNF-α, confirming the specificity of the reaction. Anti-TNF-β failed to stain, or stained extremely weakly, the H-RS cells in the tissues (Figure 6).

Both anti-TNF-α and anti-TNF-β antibodies reacted with rare (0.1% to 1%) lymphoid cells scattered in both reactive lymphoid tissues and those involved by HD. The nature of these cells could not be ascertained because the number of cells was extremely small. Many of these cells were slightly elongated and contained increased amounts of cytoplasm compared with that of lymphocytes. Anti-TNF-β did not stain histiocytes, whereas anti-TNF-α stained numerous histiocytes scattered in connective tissues and in the subepithelial region of the tonsils. Cytoplasmic staining could be observed in a subpopulation of histiocytes, and diffuse extracellular staining around histiocytes was typically present. In the dermatopathic nodes, anti-TNF-β, but not anti-TNF-α, appeared to produce weak staining of the cytoplasm of IR cells. Because anti-TNF-β antibody is a polyclonal antibody and because we did not have purified or recombinant TNF-β available for absorption experiments, the presence of TNF-β in IR cells awaits further confirmation.

Discussion

We showed that H-RS cells cultured *in vitro* can produce TNFs that are cytotoxic to L929 cells. That these substances were TNFs was confirmed by positive immunoperoxidase staining with anti-TNF-α and TNF-β antibodies

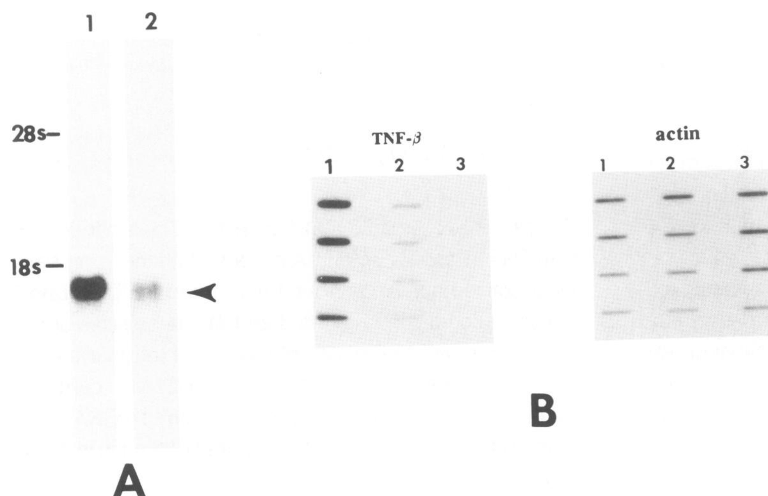

Figure 4. A: *RNA was prepared from HDLM-1 (lane 1) and KM-H2 (lane 2) cells. Northern blots were probed with TNF-β. Abundant expression of TNF-β was noted in HDLM-1 cells. The size of the m-RNA hybridized was approximately 1.4 kb.* **B:** *Dot hybridization of mRNAs prepared from HDLM-1 (lane 1), KM-H2 (lane 2), and MS-1 (lane 3; mesothelioma cell line, which served as a negative control). mRNA (1, 0.5, 0.25, 0.1 μg/ml) was spotted onto the nitrocellulose paper. The left panel shows hybridization with TNF-β, and the right panel with β-actin (as standard control). The similar hybridization intensity in the right panel confirms that a similar amount of mRNA was used. The abundant expression of TNF-β (lane 1, left panel) by HDLM-1 cells cannot be attributed to the use of excess mRNA.*

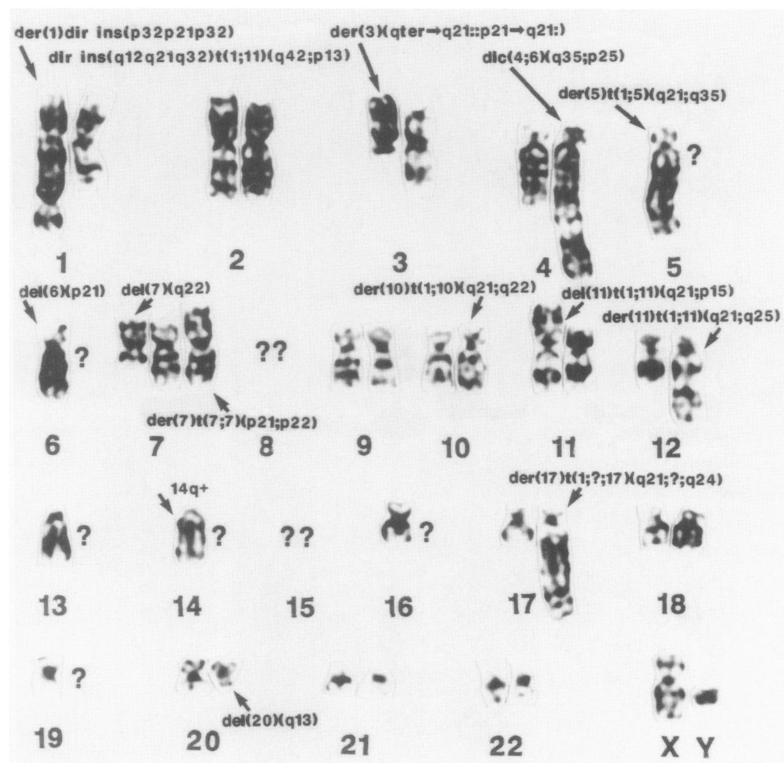

Figure 5. *Representative cytogenetic findings in HDLM-1 cells. Chromosomal abnormalities are labeled.*

and by the presence of 1.7-kb and 1.4-kb mRNAs that could be hybridized to TNF-α and TNF-β cDNA probes. The TNF activities were approximately 800 and 150 units/ml in HDLM-1 and KM-H2 culture supernatants, respectively. These activities increased when cells were treated with PMA. We also confirmed the expression of TNF-α by H-RS cells in tissues.

The production of TNFs by H-RS cells was not totally unexpected. Although it was known that TNF-α is produced by histiocytes and related cells, and TNF-β by lymphoid cells,[11,12,23,24] it has recently become clear that TNF-α can be produced, on appropriate stimulation, by a wide variety of cells including T and B lymphocytes, as well as by cells of epithelial origin.[21,22,37] Whether lymphoma/leukemia cells of monocyte/macrophage lineage can secrete TNF-β has not yet been studied systematically because these cell lines are rare. However, by using an anti-TNF-β antibody, we showed that a small percentage of U-937 and CTV-2 cells expressed TNF-β. Furthermore, a subpopulation of IR cells in dermatopathic nodes appeared to express TNF-β. Thus, it is likely that TNF-β can be produced by cells of monocyte/histiocyte lineage. The lack of lineage specificity in TNF production makes it impossible to draw conclusions regarding the origin of H-RS cells based on the pattern of TNF secretion. However, the constitutive secretion of TNFs by H-RS cells is significant because such secretion by normal lymphoid cells or monocytes is rare.[38]

The constitutive secretion of TNFs, especially the abundant production and the filamentous distribution of TNF-β in HDLM-1 cells, is interesting. The expression of TNF-β, however, could not be confirmed in H-RS cells in tissues. Because the mechanism for the transcriptional regulation of both types of TNFs has not yet been completely elucidated, we do not know why TNF-β, but not TNF-α, is overproduced. Both TNF-α and TNF-β genes may have arisen from a common ancestral gene through tandem duplication. They are located on chromosome 6 (p21-p23) near the major histocompatibility locus in close proximity to one another.[39–41] By using a TNF-β cDNA probe, we identified several corresponding cDNA clones in the HDLM-1 cDNA bank. Two of the cDNA clones were found to have similar 3′-untranslated regions, as previously reported.[39–41] However, one of the cDNA clones appeared to have a sequence divergence at its 3′ end (manuscript in preparation). Thus, the abundant expression of TNF-β mRNA and an unusual distribution of TNF-β in HDLM-1 cells may be a consequence of a chromosome abnormality at 6p21 in these cells. Alternatively, the production of TNF-β in H-RS cells may be acquired after adaptation in culture.

The expression (staining intensity) of TNFs, especially TNF-α, in HDLM-1 and KM-H2 cells varied from cell to cell. This was also the case for H-RS cells and histiocytes in tissues. The staining of TNF-α in the extracellular space surrounding H-RS cells and histiocytes suggests that TNF-α is secreted rapidly after its synthesis in the cells. Despite this heterogeneity, the discovery of TNFs in H-RS cells is significant for the following reasons: 1) The function of TNFs is synergistic with that of other cytokines, eg,

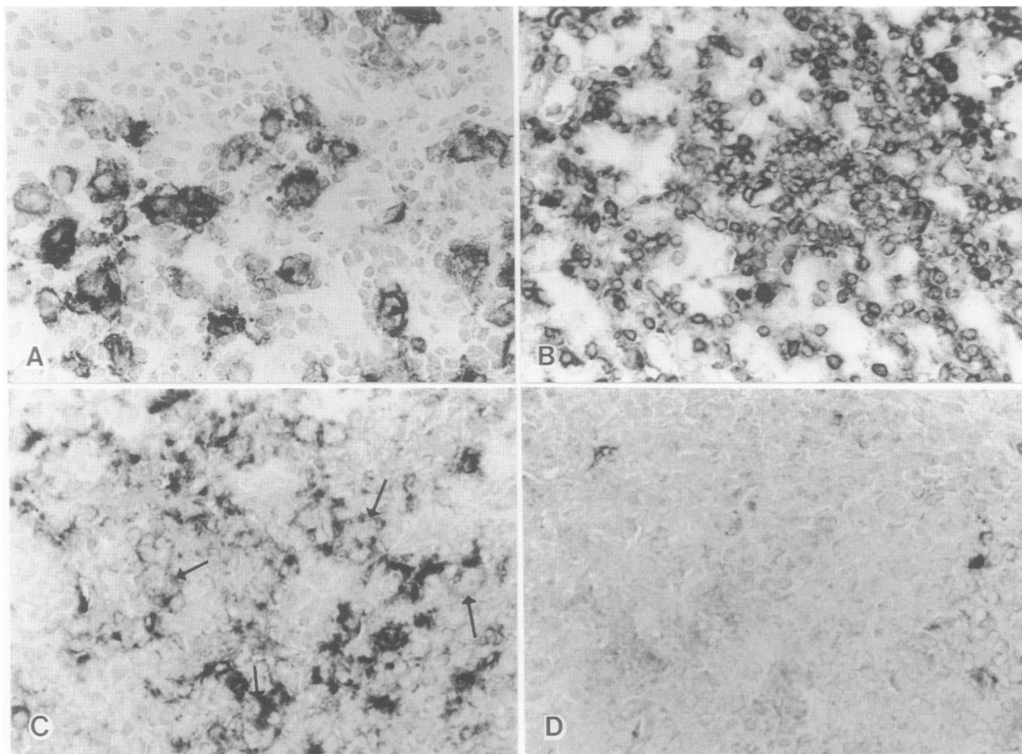

Figure 6. *Expression of TNF in H-RS cells. Adjacent sections were stained with anti-CD30* (A), *anti-CD3* (B), *anti-TNF-α* (C), *and anti-TNF-β* (D). *The H-RS cells were positively stained by anti-CD30 and TNF-α, but not with anti-CD3 and anti-TNF-β. Granular staining, especially in extracellular spaces surrounding H-RS cells (arrows in C), was detected with anti-TNF-α. A similar extracellular staining pattern in a histiocyte-rich area can also be observed in the subepithelial region of the tonsil (not illustrated). The anti-TNF-α staining can be inhibited by preabsorption of antibody with recombinant TNF-α. The results suggest that TNF-α is released from cells immediately after its synthesis.*

IL-1, M-CSF, and IFN-γ.[42-46] The activity of TNFs may be due to their capacity to augment the synthesis of other cytokines, eg, IL-1, GM-CSF, IFN-γ, and IL-6 (IFN-β_2).[45-47] These other cytokines may, in turn, stimulate the production of TNF.[11,43,46-50] The interactions among these cytokines, therefore, allows them to play an even more powerful role in the regulation of cell growth and function. The two types of cultured H-RS cells studied are known to produce, in addition to TNFs, IL-1 and M-CSF.[1-4] Another H-RS cell line, L-428, has been shown to produce GM-CSF or G-CSF.[5,6] The capacity of H-RS cells to secrete several different cytokines clearly indicates that these cells can exert a strong influence on the surrounding reactive cells.

In the normal immune response, the secretion of cytokines by macrophages is regulated and well balanced, so that an optimal defense against foreign antigens can be achieved. The production of one cytokine can elicit the secretion of other cytokines or immunoregulatory substances (eg, PGE_2) that act antagonistically. Because H-RS cells are neoplastic, the constitutive production of cytokines in these cells may not be controlled by feedback inhibition.[51] Prolonged secretion of TNF and IL-1 (and of other cytokines as well) by H-RS cells, even when combined in suboptimal doses, may contribute to metabolic derangements such as those observed in cancer patients with cachexia.

TNF injected into rats can result in increased uptake of hepatic amino acid, decreased serum trace metal (Zn) concentrations, increased serum levels of acute-phase reactants, and chronic wasting.[52] TNFs also have biologic and immunologic properties that include stimulation of thymocyte/T cell proliferation,[53] regulation of B cell differentiation, activation of macrophages, promotion of NK cells and of granulocyte activity,[45,46,54-57] induction of bone absorption, promotion of angiogenesis, regulation of phopholipid metabolism, induction of prostacyclin and PGE_2 synthesis, fibroblast proliferation, induction of fever, antiproliferation and cytocidal effects on some types of tumor cells, and regulation of the expression of HLA antigens.[58-63] We did not intend to explain the whole clinical picture of HD based on the secretion of TNFs, IL-1s, or both by H-RS cells or reactive cells; however, several of the physiologic properties associated with TNFs and IL-1, such as decreased serum levels of Zn, increased amounts of acute-phase reactants, and proliferation of T cells and fibroblasts, are observed frequently in patients with HD.[64]

In conclusion, TNFs mediate a variety of actions that are important in host defense against inflammation and in autoimmunity. However, their uncontrolled production by tumor cells may be more harmful than helpful. A number of histopathologic alterations in HD can be ascribed to the capacity of H-RS cells to produce IL-1s, TNFs, CSFs, and PGE_2. It should also be noted that many types of reactive cells (ie, T cells and fibroblasts) in tissues can be stimulated by cytokines released by H-RS cells to secrete more cytokines. This may explain the profound cellular reaction that occurs in tissues involved by HD although only small numbers of H-RS cells are present. The functional exhaustion of T cells, as a result of continuous cytokine stimulation, and the inhibition of T cells by PGE_2 could result in the immunosuppression that is often present in patients with HD.

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