Receptor-Mediated Monocytoid Differentiation of Human Promyelocytic Cells by Tumor Necrosis Factor: Synergistic Actions With Interferon-γ and 1,25-Dihydroxyvitamin D₃

By J. Brice Weinberg and James W. Larrick

Human myeloid leukemia cells respond to various signals by differentiating to more mature cells. This study was designed to evaluate the effects of a mononuclear phagocyte-derived factor, tumor necrosis factor/cachectin (TNF), on the proliferation and differentiation of the human cell lines HL-60 (promyelocytic) and U937 (monoblastic), and to characterize TNF receptors on these cells. TNF had no effect on HL-60 cell growth or thymidine incorporation, but it markedly inhibited that of U937 cells. HL-60 cells treated with TNF formed osteoclast-like polykaryons and developed nonspecific esterase positivity. In a dose-dependent fashion, TNF enhanced HL-60 cell nonspecific esterase activity. H₂O₂ production, NBT reduction, and acid phosphatase content. Together, TNF and interferon-γ (IFN-γ) additively and synergistically caused increases in these activities as well as the expression of HLA-DR and the monocyte antigens LeuM3 (CDw14) and OKM1 (CD11). TNF also synergistically enhanced the differentiating effects of 1,25-dihydroxyvitamin D₃. The potentiating actions of D₃ of IFN-γ on the TNF effect were maximal when the two agents were present together throughout the incubation, and pretreatment with TNF augmented the subsequent response to D₃, but not IFN-γ. HL-60 and U937 cells bound ¹²⁵I-labeled TNF specifically, rapidly, and reversibly with binding constants of 227 and 333 pmol/L and receptors per cell of 4,435 and 6,806 for HL-60 and U937, respectively. Scatchard plots were linear, which suggested single classes of receptors. HL-60 TNF receptors were not changed by a three-day treatment with IFN-γ or D₃, U937 and HL-60 cells internalized and degraded ¹²⁵I-labeled TNF to comparable degrees. TNF has differing effects on HL-60 and U937 cells that are apparently mediated through comparable high-affinity TNF receptors. The unique responses of different cell types to TNF may be due to postreceptor factors.

© 1987 by Grune & Stratton, Inc.

Materials and Methods

Medium and serum. All cultures were done in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with dextrose (1 mg/mL), 20 mmol/L HEPES buffer, penicillin (100 U/mL), and streptomycin (100 μg/mL). Fetal bovine serum (FBS) was from Sterile Systems (Logan, UT). By limulus amebocyte lysate testing, the materials contained <200 pg/mL of LPS.

Culture methods. The HL-60 and U937 cell lines were from the American Tissue Culture Collection (Rockford, MD). They were used during passages 30 to 68. Maintenance cultures were passed two to three times each week after initial seedings of 2.5 to 5.0 × 10⁵/mL in 10% FBS. In experiments, the cells were cultured at 1 to 2 × 10⁴/mL with the various additives at 37°C with 5% CO₂ and 100% humidity in 25- or 75-cm² surface area plastic culture flasks (Becton Dickinson, Oxnard, CA) for four to seven days. Cell viability was determined by trypan blue exclusion.

Hydrogen peroxide and nitroblue tetrazolium assays. The production of hydrogen peroxide (H₂O₂) in the presence or absence of 200 nmol/L phorbol myristate acetate (PMA) was determined by using horseradish peroxidase and phenol red and a microtiter plate reader as described previously. Nitroblue tetrazolium (NBT) reduction by the cells in response to 200 nmol/L PMA was assessed by visually counting safranin-counterstained slides.

From the Division of Hematology/Oncology, Veterans Administration and Duke University Medical Centers, Durham, NC; and Cetus Immune Research Laboratories, Palo Alto, CA.

Address reprint requests to J. Brice Weinberg, MD, Hematology/Oncology (151G), VA and Duke Medical Centers, Durham, NC 27705.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
Morphology and cytochemistry. After appropriate cultures, the cells were collected onto glass slides by using a Shandon cytocentrifuge (Shandon Southern, Astmoor, England). Wright's, nonspecific esterase (o-naphthylbutyrate esterase), and acid phosphatase (with and without 27 mmol/L sodium tartrate) stains were done as described before.32

Cell surface antigen studies. After cultures with the additives indicated, the cells were processed for indirect immunofluorescent staining and analysis on an EPICS V fluorescent cell analyzer (Coulter) as described before.3 Antibodies against HLA-DR, LeuM3 (CDw14), and LeuM2 were from Becton Dickinson (Mountain View, CA), and OKM1 (CD11) was from Ortho Diagnostics (Raritan, NJ).

Acid phosphatase and lysozyme assays. After cells were dissolved in 0.05% Triton X-100, acid phosphatase was measured by using p-nitrophenol phosphate as a substrate, and protein was measured as described before.13 Lysozyme activity was measured by using Micrococcus lysodeikticus as a substrate with egg white lysozyme as a standard.13

Thymidine incorporation assays. U937 and HL-60 cells were cultured at 0.8 × 10⁴ and 1.0 × 10⁴/microtiter plate chamber, respectively, in 0.2 mL RPMI 1640 with 10% FBS with different additives for 60 hours. Then, 1 μCi of tritiated thymidine (3H-TdR, 2 Ci/mmol) was added to each chamber, and after four further hours of culture, the cells were harvested by using a multiple automated sample harvester, deposited onto glass fiber filter paper, and washed with distilled water.1 The filter disks were then counted in liquid scintillant.

Receptor binding assay. TNF was labeled to a specific activity of approximately 0.9 μCi/μg protein by using 1,3,4,6-tetrachloro-

![Fig 1. HL-60 and U937 cell counts during incubation with IFN-γ and/or TNF. The counts are means of triplicate samples from one of five comparable experiments done with HL-60 and two with U937.](image)

Table 1. Induction of Multinuclearity in HL-60 Cells by TNF and IFN-γ

<table>
<thead>
<tr>
<th>TNF*</th>
<th>IFN-γ*</th>
<th>Multinucleated (%)</th>
<th>Nuclei/Polykaryon</th>
<th>Multinuclearity Index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>4.2 ± 1.5</td>
<td>2.7 ± 0.3</td>
<td>10.2 ± 3.2</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>3.7 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>1,000</td>
<td>0</td>
<td>6.2 ± 0.5</td>
<td>2.2 ± 0.2</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>2.4 ± 1.8</td>
<td>2.4 ± 0.2</td>
<td>5.4 ± 4.1</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>7.7 ± 3.0</td>
<td>3.3 ± 0.3</td>
<td>20.4 ± 6.9</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>10.2 ± 1.5</td>
<td>3.6 ± 0.2</td>
<td>29.1 ± 3.0</td>
</tr>
<tr>
<td>1,000</td>
<td>100</td>
<td>18.5 ± 4.1</td>
<td>3.4 ± 0.2</td>
<td>42.4 ± 7.4</td>
</tr>
</tbody>
</table>

*Units per milliliter of TNF or IFN-γ present through the five-day culture period.†Percentage of all nuclei that occur within multinucleated cells.‡Mean ± SEM of triplicate samples from one of five comparable experiments done.
Fig 3. Photomicrographs of HL-60 cells cultured with 0 (A and C) or 1,000 U/mL (B and D) TNF for four days. A and B are stained with Wright’s stain (original magnification × 600; current magnification × 396) and C and D with nonspecific esterase stain (original magnification × 300; current magnification × 198). Cells staining positive for nonspecific esterase are gray-black.

Fig 4. Nonspecific esterase positivity in HL-60 cells cultured four days with varying doses of TNF. The points show means ± SEM from triplicate samples of an experiment representative of 12 comparable experiments done. Note that 100 U/mL of TNF is 580 pmol/L.

Fig 5. Nonspecific esterase positivity (A), acid phosphatase content (B), PMA-stimulated NBT reduction (C), and PMA-stimulated H2O2 production (D) by HL-60 cells after four days of culture with IFN-γ and/or TNF. The bars show means ± 1 SEM of triplicate samples of experiments representative of several comparable experiments done (14 in A, three in B, five in C, and 14 in D).
internalized, 1,000 pmol/L $^{125}$I-labeled TNF was bound to the cells for two hours at 0°C. The cells were then washed twice with 0°C RPMI-10% FBS and incubated for varying periods of time at 0°C or 37°C. Then washed cells were treated for five minutes at 0°C with 0.05 mol/L glycine HCl/0.15 N NaCl buffer (pH 3) to elute the noninternalized ligand. Supernatants and cell pellets were counted for $^{125}$I. Supernatants were also analyzed for degraded $^{125}$I-labeled TNF by determining the proportion of $^{125}$I precipitable by 10% trichloroacetic acid.

**Materials.** $^{3}H$TdR and $^{125}$I-sodium were from New England Nuclear (Boston), purified, recombinant human IFN-γ from Genentech (S San Francisco, CA); and purified, recombinant human TNF$^{10,11}$ from Cetus Corp (Emeryville, CA). All other reagents were from Sigma Chemical Co (St Louis). As determined by limulus amoebocyte lyase assays, the IFN-γ contained <10 ng/mg protein and the TNF, <112 ng/mg protein, which signified final concentrations of LPS <112 pg/mL in assays using the highest doses of IFN-γ and TNF.

**RESULTS**

**Cell proliferation:** HL-60 proliferation was not affected by the TNF and/or IFN-γ (Fig 1A). When assessed for their abilities to incorporate $^{3}H$TdR on day 3, the HL-60 cells were affected none or little by TNF and only slightly by IFN-γ (Fig 2A). By day 3, TNF and/or IFN-γ slowed U937 proliferation as assessed by the rates of increase in cell number (Fig 1B) and the $^{3}H$TdR incorporation (Fig 2B). A concentration of approximately 1 U/mL of TNF (~6 pmol/L) inhibited U937 $^{3}H$TdR incorporation by 50%.

**Cell morphology and cytochemistry.** As we have reported before, IFN-γ induced multinuclearity in HL-60 and U937 cells. The TNF also induced polykaryon formation and appeared to work synergistically with IFN-γ in causing this phenomenon (Table 1 and Fig 3). The nuclear chromatin of the cells was slightly more coarse in the treated cells, but nucleoli were still visible in most cells. The cells did not become adherent after the treatments. In a dose-dependent fashion, TNF enhanced HL-60 expression of the enzyme nonspecific esterase (Fig 4), and TNF and IFN-γ were synergistic in their ability to cause this increase (Fig 5A). All U937 cells were natively positive for nonspecific esterase, but TNF and IFN-γ caused a qualitative increase in activity (data not shown). In the HL-60 cells, most of the polykaryons were positive for nonspecific esterase, but some were negative. The treatments with TNF and IFN-γ caused a qualitative increase in the expression of acid phosphatase in HL-60 and U937 cells. This was apparent in mononuclear as well as multinucleated cells. The acid phosphatase was totally inhibited by tartrate in the mononuclear and multinucleated cells. Acid phosphatase measured in cell lysates demonstrated the increases more quantitatively (Fig 5B).

---

**Table 2. Cell Surface Antigen Analysis of HL-60 Cells Treated With TNF With or Without IFN-γ or D3**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Percent Positive†</th>
<th>Mean Channel Fluorescence‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>IFN-γ</td>
<td>D3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

*TFN (U/mL), IFN-γ (U/mL), and/or D3 (nmol/L) present through the four-day culture.
†Percentage of cells with fluorescence higher than the cutoff based on P3 control.
‡Mean channel fluorescence of the positive cells (arbitrary units from logarithmic scale of fluorescence intensity).

---

**Fig 6.** PMA-stimulated $H_{2}O_{2}$ production by HL-60 cells after four days of culture with D3 and/or TNF. The bars show means ± SEM of triplicate samples from an experiment representative of four comparable experiments done.

**Fig 7.** Time course for binding of 500 pmol/L $^{125}$I-labeled TNF to HL-60 and U937 cells at different temperatures. The values represent the means (fmol/10⁶ cells) of triplicate samples. The value for the 1,080-minute sample at 4°C (not shown) was 2.3. This is representative of two comparable experiments done.
lysosome was demonstrated in the HL-60 cells with any of the different treatments.

*Reactive oxygen species generation.* As leukemia cells differentiate to more mature myeloid or monocytoid cells, they gain in their abilities to produce reactive oxygen species such as $\text{H}_2\text{O}_2$ and superoxide in response to PMA. After a three- to four-day incubation with TNF and IFN-$\gamma$, there was synergistic enhancement in ability to reduce NBT and produce $\text{H}_2\text{O}_2$ (Fig 5C and D). There was a slight increase in $\text{H}_2\text{O}_2$ production by the TNF/IFN-$\gamma$-treated cells even in the absence of PMA, but it was higher when the cells were stimulated with PMA in the assay (data not shown).

*Cell surface antigen changes.* After incubation of the HL-60 cells for four to seven days with the TNF and/or IFN-$\gamma$, there were increases in the numbers of cells expressing the class II antigen HLA-DR, the monocyte antigen LeuM3, and the C3bi receptor antigen OKM1 (Table 2). In addition, as reflected by the increase in the mean channel of fluorescence, there was an increase in the density of the antigens expressed on the cells.

**Table 3. Influence of Sequence of Addition of TNF, D$_3$, and IFN-$\gamma$ on HL-60 Cell Differentiation**

<table>
<thead>
<tr>
<th>Days 0-3</th>
<th>Days 4-6</th>
<th>H$_2$O$_2$ Production*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>TNF</td>
<td>0</td>
<td>7.8 ± 2.7</td>
</tr>
<tr>
<td>0</td>
<td>TNF</td>
<td>9.5 ± 0.9</td>
</tr>
<tr>
<td>IFN-$\gamma$</td>
<td>0</td>
<td>11.3 ± 0.8</td>
</tr>
<tr>
<td>0</td>
<td>IFN-$\gamma$</td>
<td>13.4 ± 0.7</td>
</tr>
<tr>
<td>TNF</td>
<td>IFN-$\gamma$</td>
<td>15.6 ± 5.4</td>
</tr>
<tr>
<td>IFN-$\gamma$</td>
<td>TNF</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>0</td>
<td>TNF/IFN-$\gamma$</td>
<td>13.9 ± 1.2</td>
</tr>
<tr>
<td>TNF</td>
<td>TNF</td>
<td>14.4 ± 0.3</td>
</tr>
<tr>
<td>IFN-$\gamma$</td>
<td>IFN-$\gamma$</td>
<td>9.9 ± 0.4</td>
</tr>
<tr>
<td>TNF/IFN-$\gamma$</td>
<td>TNF/IFN-$\gamma$</td>
<td>29.2 ± 2.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Days 0-3</th>
<th>Days 4-6</th>
<th>H$_2$O$_2$ Production*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>TNF</td>
<td>0</td>
<td>13.9 ± 2.5</td>
</tr>
<tr>
<td>0</td>
<td>TNF</td>
<td>16.7 ± 2.5</td>
</tr>
<tr>
<td>D$_3$</td>
<td>0</td>
<td>5.9 ± 3.2</td>
</tr>
<tr>
<td>D$_3$</td>
<td>D$_3$</td>
<td>12.3 ± 2.4</td>
</tr>
<tr>
<td>TNF</td>
<td>D$_3$</td>
<td>37.1 ± 5.4</td>
</tr>
<tr>
<td>D$_3$</td>
<td>TNF</td>
<td>4.3 ± 1.4</td>
</tr>
<tr>
<td>0</td>
<td>TNF/D$_3$</td>
<td>14.8 ± 2.0</td>
</tr>
<tr>
<td>TNF</td>
<td>TNF</td>
<td>24.2 ± 4.2</td>
</tr>
<tr>
<td>D$_3$</td>
<td>D$_3$</td>
<td>7.9 ± 4.8</td>
</tr>
<tr>
<td>TNF/IFN-$\gamma$</td>
<td>TNF/D$_3$</td>
<td>63.3 ± 4.5</td>
</tr>
</tbody>
</table>

Cells were cultured for three days with indicated additives (medium alone, 100 U/mL TNF, 100 U/mL IFN-$\gamma$, and/or 5 nmol/L D$_3$), washed, and then cultured another three days with the indicated additives. Results of two experiments are shown.

* Nanomoles H$_2$O$_2$ per milligram protein per hour produced in response to 200 nmol/L PMA (mean ± SEM of triplicate samples).

Fig B. Binding (fmol/10$^6$ cells) of $^{125}$I-labeled TNF to HL-60 and U937 cells as a function of ligand concentration. The binding was for four hours at 0°C. C and D shown Scatchard plots derived from the data in A and B. The data points shown are the means of triplicate samples of a representative experiment from 11 done with HL-60 and seven done with U937 cells.
induce receptors for TNF.\textsuperscript{38,40,41} We hypothesized that the synergistic effects seen might be explained by such a mechanism. To test this, a set of experiments was done in which HL-60 cells were incubated for three days with nothing, TNF, D\textsubscript{3}, and/or IFN-γ, washed three times, and then assessed for differentiation by measuring their ability to produce H\textsubscript{2}O\textsubscript{2} (Table 3). Results demonstrated that the maximal effect was seen when both TNF and IFN-γ or D\textsubscript{3} were together during the six days and that prior treatment with TNF augmented the subsequent response to D\textsubscript{3} but not IFN-γ.

*TNF receptors on HL-60 and U937 cells.* \textsuperscript{125}I-labeled TNF bound rapidly, specifically, and reversibly to HL-60 and U937 cells. The binding reached a maximum at about 30 minutes at 23°C and 37°C and at one to two hours at 4°C. But after the 30-minute peak binding at 37°C, cell-bound \textsuperscript{125}I-labeled TNF reduced progressively over a three-hour period, which suggested the possibility of degradation of the ligand (Fig 7). After incubation for two hours at 4°C with 400 pmol/L \textsuperscript{125}I-TNF, the subsequent addition of 500 nmol/L unlabeled TNF caused the elution of 52% of the \textsuperscript{125}I-TNF over a two-hour period at 4°C. Saturation binding done at 4°C for four hours with HL-60 and U937 cells demonstrated saturability, and Scatchard analysis showed comparable binding affinities and numbers of receptors per cell (dissociation constants \([K_d]\) of 226 and 328 pmol/L and receptors per cell of 4,435 and 6,806 for the HL-60 and U937 cells, respectively) (Fig 8). Scatchard plots were linear which suggested single classes of receptors in these cells. Pretreatment of selected cell types with IFN-γ has been shown to increase the numbers of TNF receptors.\textsuperscript{38,40,41} However, pretreatment of HL-60 cells for three days with 100 U/mL of IFN-γ or with 5nmol/L D\textsubscript{3} did not appreciably change the affinity or number of TNF receptors (Fig 9).

Differences in responses to TNF could be related to different degrees of TNF internalization and/or degradation. To determine the cellular handling of TNF, HL-60 and U937 cells were incubated with 1,000 pmol/L \textsuperscript{125}I-labeled TNF at 0°C for two hours, washed twice with cold RPMI-10% FBS, and incubated for varying periods of time at 0°C or 37°C. Then the washed cells were treated for five min at 0°C with 0.05 mol/L glycine HCl/0.15 mol/L NaCl buffer (pH 3) to determine the amount internalized. Internalized \textsuperscript{125}I-labeled TNF is resistant to removal by the low-pH, high-salt buffer, whereas external plasma membrane–bound ligand is removed. Supernatants were collected and analyzed for degraded ligand (\textsuperscript{125}I solvable in 10% trichloroacetic acid). As noted in Fig 10, both cell types rapidly internalized the \textsuperscript{125}I-labeled TNF at 37°C, and there was some degradation of the TNF at 37°C by both cell types.
Our results demonstrate that HL-60 and U937 cells have specific, high-affinity receptors for TNF and that TNF acts as a differentiating factor inducing monocytic differentiation in HL-60 cells and causing cell death in U937 cells. The mechanism by which TNF causes these effects is not known. TNF and IFN-γ, like D₃, are able to cause HL-60 cell differentiation with little or no inhibition of proliferation. This is in contradistinction to many of the other differentiating agents such as PMA. It is possible that the TNF acts by inducing other cellular endogenous substances with differentiating capabilities. These would include TNF itself, IFN-γ, or IFN-β₂. In fibroblasts, IFN-β₂ has been noted to be induced by TNF and to influence cell proliferation. In mouse myeloid leukemia M₁ cells and human U937 cells, lung conditioned medium and PMA have been shown to cause differentiation and to induce mRNA for IFN-β₂. These possibilities indicate that autocrine factors may play a role in controlling leukemia cell differentiation.

The TNF induction of monocytic surface antigens and nonspecific esterase activity are indicators of monocytic differentiation, whereas the increases in H₂O₂ production and acid phosphatase content are not specific for monocytes/macrophages. Multinucleated macrophages are seen in physiological settings in osteoclasts and in pathological settings in various inflammatory states such as viral, mycobacterial, and fungal infections; foreign body reactions; sarcoidosis; arthritis; and cancer. The mechanism by which TNF and IFN-γ cause the multinuclearity in the cells is not known. We have shown before that IFN-γ causes polykaryon formation in nondividing human monocytes by a process of cell fusion. In the rapidly dividing leukemia cells, this is more difficult to determine; it could either be fusion of mononuclear cells or nuclear division without cellular division (endomitosis). The multinucleated cells have some characteristics of osteoclasts, cells thought to be in the mononuclear phagocyte system. However, as opposed to osteoclasts, these multinucleated HL-60 cells were not consistently positive for nonspecific esterase, and their acid phosphatase was not tarsate resistant, a typical feature of osteoclasts. It is of special interest that TNF or lymphotoxin increases the number of multinucleated osteoclasts in fetal rat bone and increases bone resorption. This indicates that our in vitro observations with human leukemia cell lines may relate to those seen in whole bone in vitro cultures and that these leukemia cells might be useful models for studying these phenomena.

HL-60 and U937 cells have TNF receptors of comparable affinity and number despite differing dramatically in their phenotypic responses to the TNF. TNF was toxic for U937 cells in very small doses, whereas it had little toxic effect on HL-60. The toxicity of TNF for U937 cells made it difficult to do refined characterization of these treated cells. TNF receptors previously described for different cell types vary widely in numbers (a few hundred to 12,000/cell), with the binding affinities in the picomolar range. Differences in temperatures of the assays as well as length of time at 0 or 37°C to determine the amount internalized. Internalized ¹²⁵I-labeled TNF is resistant to removal by the low-pH, high-salt buffer, whereas plasma membrane-bound ligand is removed. Supernatants were collected and analyzed for degraded ligand (¹²⁵I soluble in 10% trichloroacetic acid). The points represent means of triplicate samples from one of two comparable experiments done.
TNF-INDUCED MYELOID LEUKEMIA DIFFERENTIATION

References


23. Bachwich PR, Chensue SW, Larrick JW, Kunkel SL: Tumor

response to subsequent incubation with D3. It is possible that TNF modulates D3 receptors in causing this.

TNF has been shown to inhibit the growth and differentiation of the murine Friend erythroleukemia cell line.13,16 TNF also inhibits human CFU-GM, BFU-E, and CFU-GEMM growth in normal bone marrow cells.17 The differentiating effects of TNF for myeloid cells may play a role in the physiological control of myeloid/monocytic differentiation of hematopoietic cells and may provide a useful approach to the treatment of hematopoietic disorders characterized by defects in myeloid/monocytic differentiation.19

Acknowledgment

We thank Mary Ann Misukonis for excellent technical work, Dr Dana Devine for assistance in labeling the TNF, David Leslie for assistance with the flow cytometer, Cetus Corporation for supplying the recombinant TNF, Genentech, Inc, for supplying the recombinant IFN-γ, and the VA Medical Department. We thank Dr Don Granger and Dr Jim Niedel for critically reviewing the manuscript.
55. Sassa S, Kawakami M, Cerami A; Inhibition of the growth and differentiation of erythroid precursor cells by an endotoxin-induced mediator from peritoneal macrophages. Proc Natl Acad Sci USA 80:1717, 1983

JB Weinberg and JW Larrick