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Destruction of tumor cells by monokines released from activated human blood monocytes: evidence for parallel and additive effects of IL-1 and TNF

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Summary. The incubation of human peripheral blood monocytes with endotoxins activates the cells to lyse tumorigenic targets directly and also induces the production and release into the culture medium of factors that produce lysis of mouse-transformed fibroblasts L-929 (tumor necrosis factor (TNF)-sensitive) and human A-375 melanoma cells (interleukin-1 (IL-1)- and TNF-sensitive). Immunoblotting analysis revealed that the culture medium of endotoxin-activated but not of control monocytes contained both IL-1 and TNF with a molecular weight of 17,000 daltons each. TNF activity was determined by lysis of L-929 cells, and IL-1 activity was measured by the proliferation of D-10 cells. The production of IL-1 and TNF was concentration-dependent, and the amounts of these monokines were paralleled. The antitumor activity of the culture supernates from endotoxin-treated monocytes was significantly decreased by incubation with heterologous antisera to IL-1, TNF, or both. Recombinant human IL-1 and TNF were used in parallel experiments and as positive controls. Each monokine used produced cytotoxic effects in susceptible targets. The combination of IL-1 and TNF, which more likely resembles culture supernates of activated macrophages, produced an additive antitumor cytotoxicity effect.

Introduction

The exact mechanism by which activated macrophages destroy tumor cells is still controversial. This is due in large part to lack of standardization in the assays; variations occur in the source of macrophages (rodent versus human), type of activating signal (bacterial product versus lymphokines), type of target cells (adherent versus nonadherent), and type of the cytotoxicity assay (cytostasis versus cytolysis, short-term assay versus long-term assay) [10, 13, 22]. Despite these difficulties, the majority of investigators agree that in general, macrophage-mediated antitumor toxicity can be categorized into two major mechanisms: direct and indirect [14]. The direct mechanism involves the binding of activated macrophages to susceptible target cells, destabilization of the target cell membrane, and vacuolization and lysis of the target cells [4, 12]. The indirect mechanism involves the interaction of macrophage-produced factors that diffuse into their vicinity and produce toxicity in adjacent susceptible target cells [13, 14]. Some of the factors that have been reported to produce this effect are metabolites of oxygen [19], neutral proteases [1, 23], breakdown products of complement [8], arginase [6], tumor necrosis factor (TNF) [5, 30], interleukin-1 (IL-1) [15, 20], and tumor cytotoxic factor (TCF) [27, 28].

Previous studies from our laboratory demonstrated that treatment of human blood monocytes with either endotoxins (lipopolysaccharide, LPS) or recombinant human interferon gamma and muramyl dipeptide induce the release of factors into the culture medium with tumor cytotoxic properties (TCF) [27, 28]. These activation stimuli also induce monocytes to produce and release both IL-1 and TNF, two monokines shown independently to produce tumor cytotoxicity. Since both IL-1 and TNF are available as recombinant molecules for clinical application [16, 29], we wished to determine whether these monokines were the major cytotoxic molecules in human monocyte TCF and whether they operate independently. We demonstrated that the production of TNF and IL-1 occurred in parallel and in a concentration-dependent manner. Either monokine can independently be cytostatic and cytolytic to their respective susceptible target cells. In combination (the natural situation), IL-1 and TNF produce additive antitumor cytotoxicity.

Materials and methods

Reagents. Eagle's minimal essential medium (MEM), Roswell Park Memorial Institute (RPMI) 1640 medium, and Hanks' balanced salt solution (HBSS) were purchased from Mediatech (Herndon, Va.), and fetal bovine serum (FBS) was purchased from M. A. Bioproducts (Walkersville, Md.). All of these media were endotoxin free, as determined by the limulus amebocyte lysate assay (detection limit of <0.125 ng/ml; Associates of Cape Cod, Inc., Woods Hole, Mass.). The LPS (*Escherichia coli* type; B5) was purchased from Difco Laboratories (Detroit, Mich.). Recombinant human IL-1-beta (500 half-maximal units in 500 µl, as determined by thymocyte proliferation assay) and rabbit anti-IL-1-beta serum were purchased from Cistron (Pine Brook, NJ). Recombinant human TNF (5×10^6 units /0.5 mg protein, as determined by cytotoxicity assay using L-929 cells) and rabbit anti-TNF (1:100 dilution can neutralize 4000 units/ml of TNF) serum were generous

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gifts from Dr. L. Lin, Cetus Corporation (Emeryville, Calif.).

Cell cultures. The A-375 cell line, derived from a human melanoma, and L-929 murine transformed fibroblast cells were used as tumor target cells. The cell lines were free of mycoplasma and were maintained on plastic in MEM supplement with sodium pyruvate, vitamins, L-glutamine, nonessential amino acids, and 5% heat-inactivated FBS (complete MEM; CMEM) at 37° C in a humidified atmosphere containing 5% CO_2 and air.

Isolation and culture of human peripheral blood monocytes. Human peripheral blood monocytes were purified from fresh dextran-sedimented leukocytes obtained from the Gulf Coast Regional Blood Center (Houston, Tex.). Subsequent to Ficoll-Hypague centrifugation, the monocytes were isolated by countercurrent elutriation, as described previously [26]. The fraction of monocytes isolated by countercurrent elutriation contained >95% monocytes (nonspecific esterase staining and morphological criteria), with >97% viability (trypan blue dye exclusion). These cells were resuspended in RPMI-1640 supplemented with heat-inactivated 5% FBS (CRPMI-1640) at a concentration of 5×10^5 monocytes/ml, and then 1×10^5 monocytes were plated into each well of 96-well Microtest II plates (Flow Laboratories, McLean, Va.). After a 2-h incubation, the nonadherent cells were removed by a gentle wash with medium. At this point, the purity of monocytes was >99%, as assessed by morphology and nonspecific esterase staining.

In vitro activation of monocytes. Monocytes were incubated at 37° C in medium alone or in medium containing various amounts of LPS. After 24 h, and before the addition of radiolabeled target cells, the cultures were thoroughly washed with medium.

Monocyte-mediated cytotoxicity. Cytotoxicity was assessed by measuring the release of radioactivity from target cell DNA, as described previously [10]. A-375 melanoma cells or L-929 cells in their exponential growth phase were incubated in the appropriated medium with ^{125}I iododeoxyuridine (IdUrd) (0.3 μCi/ml, sp. act. >2000 Ci/mmol; New England Nuclear, Boston, Mass.) for 24 h. The cells were then washed twice to remove unbound radioiodine, harvested by a 1-min trypsinization (0.25% Difco trypsin; 0.02% EDTA), and washed. The radiolabeled cells were resuspended in CMEM and 1×10^4 cells were plated into each culture well to obtain an initial target-to-effector cell ratio of 1:10. After 72 h, the cultures were washed twice with HBSS, and the adherent, viable cells were lysed with 0.1 ml of 0.1 *N* NaOH. The radioactivity of the lysate was monitored in a gamma counter, and the cytotoxic activity of the monocytes was calculated as follows:

percentage of specific cytotoxicity mediated by activated monocytes =

$$[A\text{-}B]/A \times 100,$$

where A represented the counts per minute in cultures of untreated monocytes and target cells, and B the counts per minute in cultures of LPS-treated monocytes and target cells.

Production of TCF by human monocytes. Monocytes isolated by centrifugal elutriation were plated into 38-mm^2 culture wells, and 2 h later all nonadherent cells were removed by washing with medium. The adherent monocyte monolayers were incubated in CRPMI-1640, with or without LPS. After 24 h, the cell-free culture supernates were harvested, filtered through 0.22-μm millipore membranes, and stored at −20° C until use.

TCF or recombinant monokine-mediated cytotoxicity in vitro. A total of 10×10^3 ^{125}I IdUrd-labeled A-375 cells or L-929 cells were plated into 96-well Microtest II plates, and 14 h later, the nonadherent target cells (<10%) were removed and the cultures were given fresh CMEM. Culture supernates of control or LPS-activated monocytes or various concentrations of IL-1 or TNF were added to the target cell monolayers. In a neutralizing test, the culture supernates or the recombinant monokines were preincubated with appropriate concentrations of control serum, anti-IL-1 serum or anti-TNF serum at 4° C for 16 h before their addition to the target cell monolayers. As a control group, radiolabeled target cells were incubated in medium alone. At 72 h after the addition of culture supernates or recombinant monokines, the cultures were washed twice with HBSS, and adherent cells lysed with 0.1 ml of 0.1 *N* NaOH. The lysate was monitored for radioactivity in a gamma counter, and the cytotoxic activity of culture supernates (TCF) or the monokines was calculated as follows:

$$\text{percentage cytotoxicity} = [A\text{-}B]/A \times 100,$$

where A represented the counts per minute in target cells cultured with medium alone, and B the counts per minute in target cells cultured with TCF or with the recombinant monokines. The cytotoxic assay using culture supernates against the L-929 cells was referred to as the assay for TNF activity.

Assay for IL-1 activity. To assay IL-1 activity, the monocyte culture supernates were added to cloned murine T cell line D10.G4.1 cells, which are dependent on IL-1 for blastogenic response to a mitogen, as reported previously [2]. In brief, 1×10^4 D10 cells previously stimulated with C57BL/6 spleen cells were plated into 96-well Microtest II plates and incubated with RPMI-1640 supplemented with 10% FBS and 2×10^{-5} *M* 2-mercaptoethanol in the presence of 2.5 μg/ml concanavalin A and various dilutions of test culture supernates for 48 h. ^{3}H Thymidine (TdR) (0.5 μCi/well, 6.7 Ci/mmole; ICN, Irvine, Calif.) was added to each well 6 h before the end of the incubation, and then the D10 cells were harvested on glass fiber in a cell harvester. Incorporation of ^{3}H TdR was assessed by liquid scintillation counting.

Immunoblotting analysis. The culture supernates of control monocytes (2×10^6/ml) or monocytes incubated for 24 h at 37° C in CRPMI-1640 containing 1 μg/ml of LPS were collected by centrifugation and concentrated 50-fold by YM10 membrane (Amicon Corp., Danvers, Mass.). Polyacrylamide gel electrophoresis (PAGE) and immunoblotting were done essentially as described previously [7]. Samples (30 μl) were mixed with 10 μl of a nonreducing solution consisting of 0.5 *M* Tris, pH 6.8, 10% sodium dodecyl sulfate (SDS), 20% glycerol, and 1% bromophenol blue.

The mixture was boiled for 5 min, applied to a 13%–18% gradient concentrated 0.75-mm thick SDS-PAGE and electrophoresed for 5 h with SDS-PAGE running buffer consisting of 0.5 *M* Tris, 3.5 *M* glycine, and 10% SDS, pH 8.3, at a constant current of 35 mA. After electrophoresis, the gel was equilibrated for 1 h with the transfer buffer consisting of 0.25 *M* Tris and 1.92 *M* glycine, pH 8.3, and transferred to a nitrocellulose membrane for 3 h in the transfer buffer, using a constant voltage of 29 mV (current 260 mA). After the proteins were transferred to the membrane, IL-1 and TNF molecules were visualized using rabbit anti-human IL-1 and rabbit anti-human TNF, as the first antibody, and goat anti-rabbit immunoglobulin conjugated with horseradish peroxidase as the second antibody (Bio-Rad Laboratories, Richmond, Calif.). Trypsin inhibitor (20×10^3 daltons), myoglobin (17.8×10^3 daltons), α lactoalbumin (14.4×10^3 daltons), and ribonuclease (13.7×10^3 daltons) were used as molecular weight markers.

Statistical analysis. The statistical significance of difference between test groups was analyzed using the Student's *t*-test (two-tailed).

Results

Characterization of A-375 cells and L-929 cells for sensitivity to recombinant IL-1 and TNF

The human melanoma cell line A-375 is commonly used as a target to asses the cytotoxic activity of human-activated monocytes [9]. In the first set of experiments, the susceptibility of A-375 cells to monokines such as IL-1 and TNF was confirmed. The data shown in Fig. 1 are from a representative experiment of five. A-375 cells were lysed by either IL-1 or TNF in a concentration-dependent manner. The murine transformed fibroblast cell line L-929 was confirmed by the data shown in Fig. 1 to be highly susceptible to TNF. In fact, the cytolytic level of TNF in our 72 h assay was compatible with that reported in the standard TNF assay with cells coincubated with actinomycin D for 18 h [24]: 0.1 ng/ml recombinant TNF is equal to 1 unit/ml TNF in standard L-929 TNF assay producing 50% cytotoxicity (information from Cetus Corporation). In contrast, recombinant IL-1 did not produce cytotoxicity to ^{125}I IdUrd-labeled L-929 cells.

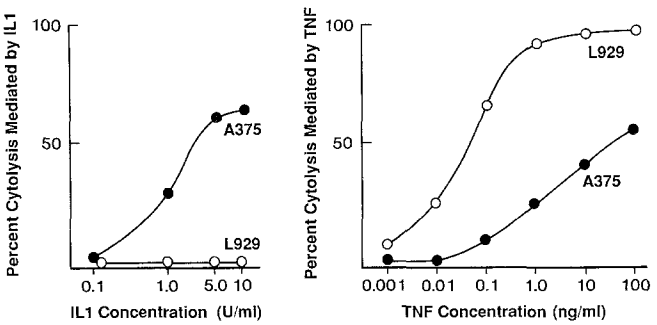

Fig. 1. Cytolysis of A-375 human melanoma and L-929 murine transformed fibroblasts by recombinant human interleukin-1 (IL-1) and recombinant human tumor necrosis factor (TNF). ^{125}I Iododeoxyuridine (IdUrd)-labeled A-375 (●) or ^{125}I IdUrd-labeled L-929 (○) cells were incubated with the monokines for 72 h. Percentage of cytolysis was calculated as compared with target cells incubated in medium alone

Table 1. Direct lysis of A-375 melanoma cells by LPS-activated human monocytes

Treatment of monocytes[a]	Radioactivity in viable tumor cells after 72 h of cocultivation
Medium	2161 ± 46[b]
LPS 1.0 μg/ml	860 ± 78 (60)[c]
100 ng/ml	806 ± 69 (63)
10 ng/ml	920 ± 70 (57)
1 ng/ml	767 ± 63 (65)
100 pg/ml	836 ± 83 (61)
10 pg/ml	1462 ± 91 (32)
1 pg/ml	2030 ± 45
Tumor cells alone	2103 ± 51

[a] Human monocytes (1×10^5) were incubated for 24 h in endotoxin-free medium or in medium containing LPS at the indicated concentrations. ^{125}I IdUrd-labeled A-375 melanoma cells (1×10^4) were added to the monocyte monolayers. The assays were terminated 72 h after the cocultivation

[b] Mean cpm ± SD of triplicate cultures

[c] Numbers in parentheses are the percentage of cytotoxicity as compared with control monocytes at corresponding ratio (density) to target cells ($P < 0.001$). One experiment of five

Induction of tumoricidal properties in human blood monocytes by LPS

In the present studies as well as in previously published results [27], highly purified human blood monocytes that were isolated by centrifugal elutriation from the blood of healthy donors were not spontaneously cytotoxic against allogenic tumor cells. The direct monocyte-mediated cytotoxicity and the indirect TCF-mediated cytotoxicity against A-375 melanoma cells are shown in Table 1 and Fig. 2, respectively. Concentration response experiments established that elutriated human monocytes could be rendered tumoricidal by in vitro interaction with at least 10 pg/ml LPS. Similarly, control elutriated monocytes that did not release detectable TCF activity into the culture supernates could be activated by LPS to release TCF into the culture supernate. The minimum concentration of LPS necessary to activate monocytes for release of TCF ranged between 1.0 and 100 pg/ml (five experiments).

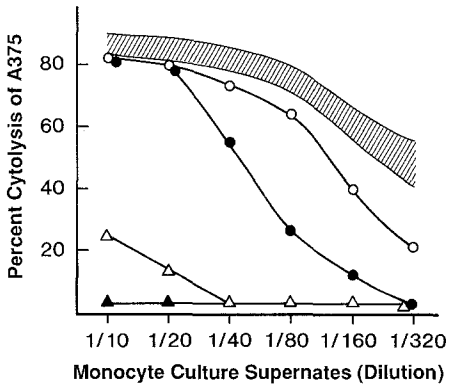

Fig. 2. Level of tumor cytotoxic factor activity in culture supernates of monocytes incubated with different concentrations of lipopolysaccharide (LPS). Samples of culture supernates were added to ^{125}I IdUrd-labeled A-375 cells for a 72-h incubation. Percentage cytolysis was calculated as compared with target cells incubated in medium alone. LPS concentrations: 1.0 μg/ml–1.0 ng/ml (▨); 100 pg/ml (○); 10 pg/ml (●); 1 pg/ml (△); 0.1 pg/ml (▲)

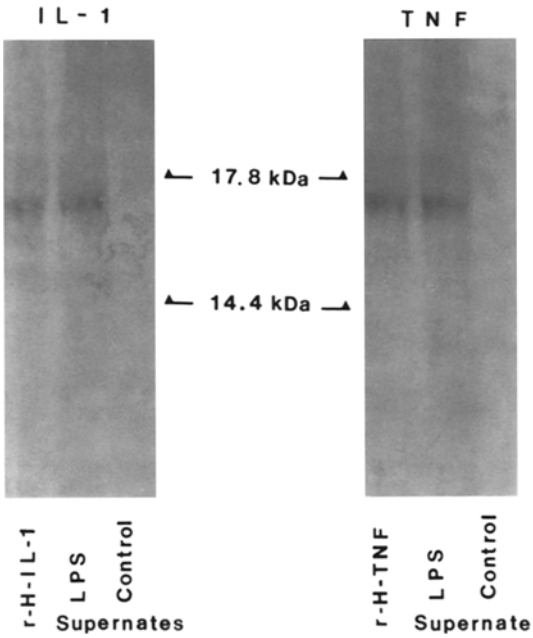

Fig. 3. Presence of IL-1 and TNF in the culture medium of LPS (1.0 μg/ml)-activated but not normal human blood monocytes, immunoblotting analyses

Evidence for IL-1 and TNF in monocyte-culture supernates with TCF activity by immunoblotting analysis

In these experiments, we confirmed the existence of IL-1 and TNF in monocyte-culture supernates with TCF activity by immunoblotting analysis. Monocyte culture medium with or without TCF activity, IL-1 and TNF, were processed through SDS-PAGE. Proteins were transferred to nitrocellulose membranes and stained with anti-IL-1 or anti-TNF serum followed by goat anti-rabbit immunoglobulin peroxidase. The data in Fig. 3 show the presence of two molecular weight bands of about 17,000 daltons in culture supernates of LPS-activated monocytes with TCF activity but not in culture supernates of control nonactivated monocytes devoid of TCF activity. These two molecules in the monocyte culture supernates rich in TCF activity were compatible with recombinant human IL-1 and TNF, respectively.

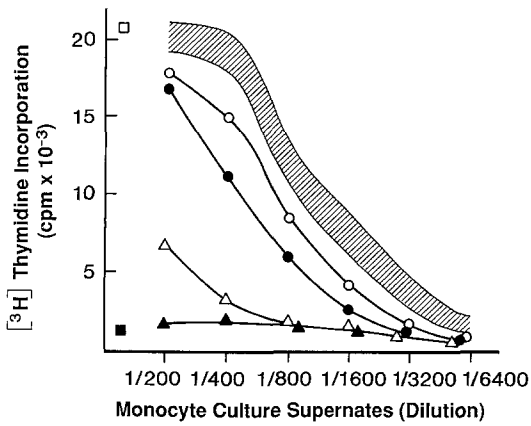

Fig. 4. Level of IL-1 activity in culture supernates of monocytes treated with different concentrations of LPS. Samples of culture supernates were added to D10.G4.1 cells and incubated for 48 h in the presence of concanavalin A. The IL-1 activity was expressed as ^{3}H thymidine incorporation. LPS concentrations: 1.0 μg/ml–1.0 ng/ml, (▨); 100 pg/ml, (○); 10 pg/ml, (●); 1 pg/ml (△); 0.1 pg/ml (▲). Radioactivity of medium control (■) and of IL-1 (1.0 units/ml) (□) were the negative and positive controls, respectively

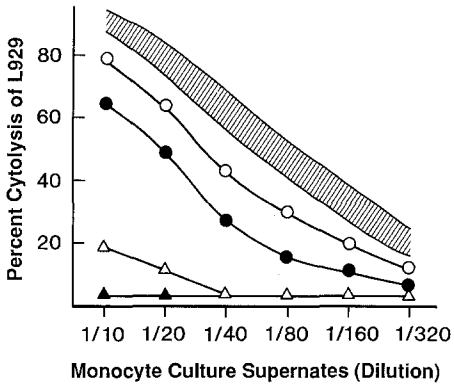

Fig. 5. Level of TNF activity in culture supernates of monocytes treated with different concentrations of LPS. Samples of culture supernates were added to ^{125}I IdUrd-labeled L-929 cells for a 72-h incubation. Percentage cytolysis was calculated as compared with target cells incubated in medium alone. LPS concentrations: 1.0 μg/ml–1.0 ng/ml (▨); 100 pg/ml, (○); 10 pg/ml (●); 1 pg/ml (△); 0.1 pg/ml (▲)

Levels of IL-1 and TNF in monocyte culture supernates

In the next two sets of experiments, we measured the level of biological activities of IL-1 and TNF in the monocyte culture supernates by using the D10 cell proliferation assay (Fig. 4) and L-929 cytotoxicity assay (Fig. 5), respectively. The sensitivity of the TNF assay is shown in Fig. 1. The minimum concentration of IL-1 for induction of D10 cell proliferation is 0.0001 units/ml. Untreated control monocytes did not release any detectable IL-1 and TNF into the culture medium. Treatment of monocytes with LPS produced concentration-dependent and parallel release of significant levels of IL-1 and TNF into the culture medium, which correlated with production of TCF activity shown in Fig. 2. Specifically, LPS concentration exceeding 1.0 ng/ml stimulated the release of all three (TCF, IL-1, and TNF) activities into the culture supernatant fluids.

Neutralizing effect of anti-IL-1 and anti-TNF sera for TCF activity in the culture medium of activated monocytes

Culture medium (1:40 dilution) of monocytes preincubated with 1 μg/ml LPS, IL-1 (10 units/ml) or TNF (10 ng/ml; equal to 100 units/ml) were each preincubated with medium or with medium containing nonspecific rabbit serum, rabbit anti-IL-1 serum, or rabbit anti-TNF serum for 16 h prior to cytotoxicity assays against A-375 cells or L-929 cells. The data of one experiment of four shown in Fig. 6A demonstrate that anti-IL-1 serum and anti-TNF serum specifically and completely neutralized the cytotoxic effect of recombinant human IL-1 and TNF on A-375 cells, respectively. The concentrations of anti-IL-1 serum and anti-TNF serum used here completely abolished 10 units/ml of IL-1 activity in D10 cells proliferation assay (data not shown) and 100 units/ml TNF activity in L-929 cytolysis assay (Fig. 6B), respectively. When these dilutions of anti-IL-1 or anti-TNF sera were incubated separately with TCF, each decreased the percentage of lysis of

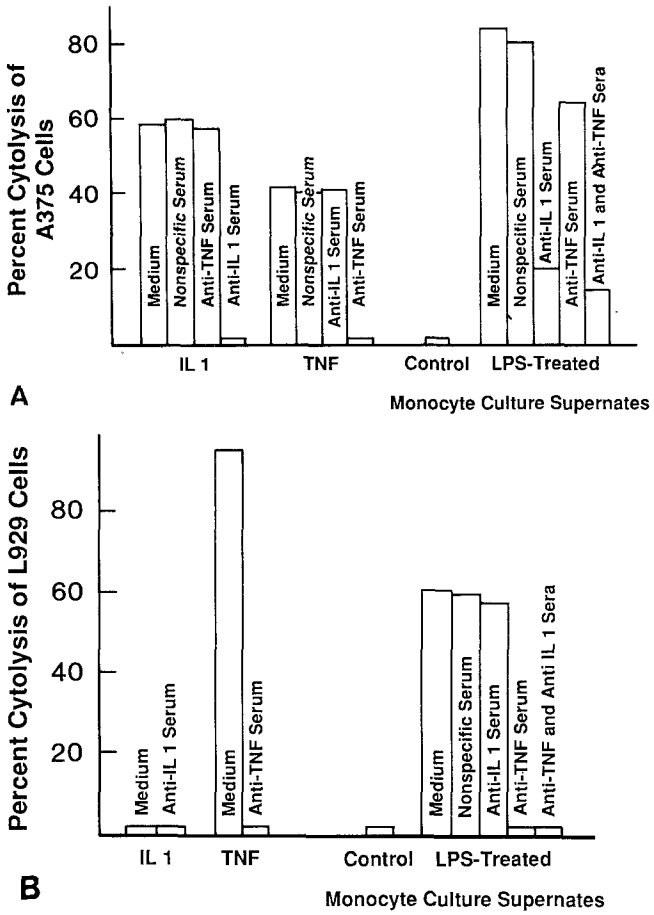

Fig. 6. Neutralizing effects of anti-IL-1 and anti-TNF sera on tumor cell lysis produced by monocyte culture medium with TCF activity. The IL-1 (10 units/ml), TNF (10 ng/ml = 100 units/ml), and culture supernates (1:40 dilution) of LPS (1.0 μg/ml)-treated monocytes were preincubated with medium alone, nonspecific serum (1:10 dilution), anti-IL-1 serum (1:4 dilution), or anti-TNF serum (1:100 dilution) for 16 h at 4° C. These mixtures were added to plated ^{125}I IdUrd-labeled A-375 cells **(A)** or L-929 cells **(B)**. Cytotoxicity was determined 72 h later. Percentage cytotoxicity was calculated as compared with target cells cultured in medium alone. Deviation from the mean did not exceed 5%

the A-375 cells. When TCF was incubated with both antisera, we observed additive decrease in lysis of A-375 cells. However, even the combination of anti-IL-1 and anti-TNF sera did not completely eliminate the cytotoxicity of TCF. Under identical conditions, anti-IL-1 serum and anti-TNF serum completely abolished the IL-1 activity and TNF activity in the monocyte culture supernates as measured in the D10 cell proliferation and L-929 cytolysis assays, respectively.

Additive effect of recombinant human IL-1 and TNF on cytotoxicity against A-375 cells

In the final set of experiments, we examined whether IL-1 and TNF lyse A-375 cells in an additive or synergistic manner. To do so, combinations of IL-1 and TNF at various concentrations were added to ^{125}I IdUrd-labeled A-375 melanoma cells. The data shown in Table 2 demonstrate that IL-1 and TNF produced additive cytolytic effects against the human target cells.

Table 2. Additive cytolytic activities of recombinant human IL-1 and TNF and against A-375 melanoma cells

Recombinant human IL-1 concentration (units/ml)[a]	Recombinant human TNF concentration (ng/ml)[a]				
	–	0.01	0.1	1.0	10
–	0[b]	2	10	22	44
0.01	−2	3	11	21	47
0.1	7	5	12	25	49
1.0	25	20	39	48	69
10	62	59	75	81	78

[a] ^{125}I IdUrd-labeled A-375 cells were plated in 96-well culture plates and incubated for 3 days with IL-1 and TNF at the indicated concentrations. Triplicate cultures

[b] Percentage cytotoxicity was calculated as compared with target cells incubated in medium alone. One experiment of six

Discussion

The incubation of human peripheral blood monocytes with endotoxins, lymphokines, or both can render the cells cytotoxic to tumorigenic but not to normal cells [9]. Subsequent to interaction with various stimuli, macrophages are known to produce more than 100 distinct molecules ranging from 32 daltons (superoxide anion) to 444×10^3 daltons (fibronectin) and in biological activity from stimulation of cell division and differentiation to tumor cell lysis [18]. Diffusible mediators produced by activated macrophages that can produce damage to tumor cells include IL-1 [15, 20] and TNF [5, 30]. Recombinant DNA technology has made these two monokines available in large quantities for both basic and clinical studies. Our present studies were undertaken to investigate whether these monokines act independently or can produce additive antitumor effects.

We have previously reported that human blood monocytes release TCF into their environment subsequent to interaction with LPS or interferon [27, 28]. The TCF can produce lysis in several tumor cell lines but not in normal cells. The cytotoxicity of the TCF was not due to superoxide anion, hydrogen peroxide, arginase, or other proteinases reported to produce tumor cytotoxicity [1, 5, 6, 19, 23, 30]. The present study shows that the major active antitumor molecules in TCF are, in fact, TNF and IL-1. This conclusion is based upon several lines of evidence: (a) immunoblotting analysis revealed the presence of both IL-1 and TNF in culture supernates of endotoxin-activated but not normal human monocytes; (b) the culture supernates of endotoxin-activated human blood monocytes were active in a biological assay measuring IL-1 activity (D10 proliferation) and TNF activity (lysis of L-929 cells); (c) both IL-1 and TNF activities were neutralized by the use of appropriate antisera; and (d) TCF containing both IL-1 and TNF molecules (17×10^3 daltons) [16, 17, 21, 29] was very active against the early passage of A-375 melanoma cells sensitive to both IL-1 and to TNF.

In this study L-929 cells were not sensitive to the effects of IL-1 (Fig. 1). This finding does not agree with a previous report [20] where L-929 cells were sensitive to cytolytic effects of highly purified IL-1 obtained from cultures of human monocytes. The discrepancy in the results could therefore be due to either differences in the sensitivity of the target cells [11] or to the fact that we used recombinant material.

The production of IL-1 and TNF by endotoxin-stimulated human blood monocytes occurred in parallel. The production of IL-1 by LPS-treated human monocytes occurs subsequent to synthesis of RNA and increased transcription of IL-1 [17]. Similarly, LPS-activated mouse macrophages express increased amounts of TNF mRNA and secrete large quantities of cachectin-TNF into the culture medium [3]. Since the culture supernates of normal blood monocytes incubated in endotoxin-free conditions were devoid of IL-1 or TNF activities, it is reasonable to assume that LPS induces de novo the production of these monokines.

Although it is tempting to study the interaction of a single cytotoxic molecule with its target cell, it may not approximate to the in vivo reality. Endotoxin-activated monocytes release both IL-1 and TNF and a plethora of other molecules [18]. Low concentrations of IL-1 and TNF have been reported to act synergistically for production of tumor cell cytostasis [25]. Our present data show that when combined, IL-1 and TNF can produce additive antitumor activity measured as lysis of susceptible target cells. Since both IL-1 and TNF are released by activated monocytes and are the major diffusible mediators of tumor cell lysis, we plan to study administration of both monokines to experimental animals with syngeneic, local, and disseminated cancer.

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