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Research Article

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Effects of Human Interleukin 1 and Human Tumor Necrosis Factor on Human T Lymphocyte Colony Formation

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Abstract

The growth of T lymphocyte colonies in agar is dependent on the cooperation of a number of different cell types and their products. Among these interacting cells are monocytes and/or macrophages. Monocytes are capable of producing both interleukin 1 (IL-1) and tumor necrosis factor (TNF). These two factors display a positive influence on T cell colony formation. Recombinant IL-1 and recombinant TNF were both shown to stimulate T cell colony growth in a dose-dependent manner, and this stimulation could be blocked by prior incubation with anti-IL-1 or anti-TNF, respectively. In addition, conditioned medium obtained from monocytes cultured in the presence of endotoxin also stimulated T cell colony formation. This stimulation by monocyte-conditioned medium was partially suppressed by incubation with anti-TNF or anti-IL-1, while incubation with both antibodies together displayed greater suppression. In conclusion, monocytes produce at least two factors, IL-1 and TNF, which can stimulate T cell colony formation by peripheral blood lymphocytes.

Introduction

The signals involved in the proliferation of T cells were originally studied using liquid culture techniques. These studies indicated the necessity for the elaboration of soluble growth factors and a requirement for cellular cooperation. In addition, these studies indicated that at least two signals were required for T cell growth (1, 2). The in vitro growth of human T lymphocyte colonies has been shown to be dependent on the presence of a lymphokine produced by T lymphocytes and termed T cell growth factor or interleukin 2 (IL-2) (3). IL-2 is the product of a T cell subset which, in the presence of antigen or mitogen, becomes responsive to the monocyte product IL-1, which elicits the release of IL-2. Another subset of T lymphocytes acquires IL-2 receptors, and these cells proliferate in the presence of IL-2 (4). Thus, T cell colony formation requires the cooperation betweenT lymphocyte colony-forming cells and a heterogeneous population of helper cells (5–10). The nature of these accessory cells is controversial and includes both T cells (7, 9) and monocytes (8, 10). The exact role of these different cell populations and the nature of the factors they release that may be involved in T cell colony formation are still under investigation.

In the present study, we investigated the role of monocyte products on the stimulation of T cell colony formation by nonadherent, peripheral blood lymphocytes. Both recombinant IL-1 and human tumor necrosis factor (TNFα)[1] were shown to stimulate T cell colony formation by peripheral blood lymphocytes in a dose-dependent manner, and this stimulation could be suppressed by polyclonal anti-IL-1 and anti-TNFα, respectively. In addition, medium conditioned by monocytes in the presence of endotoxin contained both IL-1 and TNFα. This monocyte-conditioned medium (MCM) also stimulated T cell colony formation in a dose-dependent manner. The stimulation by MCM was partially suppressed by prior incubation with anti-TNFα or anti-IL-1, while both antibodies together displayed greater suppression. Thus, monocytes produce at least two factors, namely IL-1 and TNFα, which are capable of stimulating T cell colony formation by peripheral blood lymphocytes.

Methods

Materials. TNFα was expressed in *Escherichia coli* and purified to homogeneity as was described previously (11). This material had 200 pg or less endotoxin per mg recombinant TNFα (rTNF) when assayed using the Limulus lysate assay. Human recombinant IL-1 was also expressed in *E. coli* and purified by sequential ion-exchange chromatography and either gel-filtration or high performance liquid chromatography as previously described (12, 13). Enzymatic digestion removed 41 amino acids of the precursor peptide, yielding IL-1 with an NH_2-terminus at position 112 and a molecular weight of 17,500. This recombinant form of IL-1 was homogeneous on sodium dodecyl sulfate–polyacrylamide gel electrophoresis with an isoelectric point of 6.8–7.2 (14).

Polyclonal antibodies to TNFα were a kind gift from Dr. Leo Lin (Cetus Corp., Emeryville, CA). Polyclonal antibodies to IL-1 were produced in rabbits and the IgG fraction obtained. These antibodies were capable of neutralizing TNFα and IL-1, respectively, and displayed no crossreactivity (11, 15).

Isolation of peripheral blood monocytes. Peripheral blood was obtained from normal donors who had given informed consent. Peripheral blood leukocytes were isolated after semi-continuous flow pheresis (16). The buffy coat was diluted with 2 vol Hanks' balanced salt solution without calcium and magnesium and was centrifuged at 150 *g* for 10 min to remove platelets. This cell suspension was centrifuged over Ficoll-Hypaque (specific gravity 1.078) to remove contaminating granulocytes and erythrocytes. The mononuclear cells at the interface were collected and separated into two populations, monocytes and

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1. *Abbreviations used in this paper:* MCM, monocyte-conditioned medium; rTNFα, recombinant human tumor necrosis factor; TNFα, human tumor necrosis factor.

lymphocytes, by elutriation centrifugation as previously reported (13, 17, 18). Briefly, counterflow centrifugation elutriation was performed using a Sanderson-designed separation chamber fitted into a Beckman model J-21C centrifuge. Up to 1.5×10^9 mononuclear cells were loaded into the chamber and elutriation was performed at 10°C with flow rates of 18 and 28 ml/min against a force of 867 *g* (3,000 rpm). Each cell fraction was collected by centrifugation (300 *g* for 10 min) and the cell volume profile determined with an 80-pulse height analyzer (Particle Data, Inc., Elmhurst, IL). Using this technique, monocytes (380 μm^3 modal volume) were collected as a homogeneous population. The separated monocytes were further subjected to 2-aminoethylisothiouronium bromide-treated sheep erythrocyte rosette depletion to remove contaminating T lymphocytes before use. Nonrosetting cells were separated by centrifugation through Ficoll-Hypaque. The purity of nonrosetting cells was determined using OKT3 monoclonal antibody and indirect immunofluorescence with fluorescein-conjugated goat anti-mouse IgG. The cells were analyzed using both fluorescence microscopy and a model C flow cytometer (Coulter Electronics, Inc., Hialeah, FL).

Background staining was determined on cells treated with nonimmune mouse immunoglobulins and second antibody. In addition, the monocyte monoclonal antibody LeuM3 was used for analysis of monocyte specificity and compared with histological and histochemical staining for monocytes.

Monocytes were identified on cytocentrifuge-prepared slides stained with Wright's Giemsa and nonspecific esterase using alpha naphthyl acetate as substrate (19). Nonspecific esterase-positive monocytes displayed a diffuse, cytoplasmic, red-brown reaction product. The monocyte populations used in all experiments described contained < 2% OKT3 positive and > 98% LeuM3 and > 98% nonspecific esterase-positive cells.

MCM studies. Various cell concentrations of separate fresh and cryopreserved monocyte populations were cultured for 2 d in alpha medium containing 5% fetal bovine serum. In some cultures, endotoxin (lipopolysaccharide B, *Salmonella typhosa,* Difco Laboratories, Inc., Detroit, MI) was added at the time of initiation of monocyte cultures. To produce MCM, monocytes were allowed to remain adhered to the dish and culture medium was added. The conditioned mediums were harvested, dialyzed, and stored frozen at −20°C until assay.

TNFα assay. The activity of TNFα was monitored by a previously described cell lytic assay (20). Briefly, mouse L-929 fibroblasts were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. For assay, 0.1 ml containing 3×10^5 cells/ml were plated in each well of a 96-well culture plate to establish a dense monolayer. Dilutions of standard rTNFα or MCM were added in 0.5 ml vol together with 1 μg/ml actinomycin D. After 18 h of incubation at 37°C under 5% CO_2, the samples were removed and the plates washed with phosphate-buffered saline. Cell lysis was detected by staining the plates with 0.5% crystal violet in methanol and water (1:4 vol/vol). The endpoint on the microtiter wells was determined by a Titertek multiscan autoreader (Flow Laboratories, Inc., McClean, VA) set for absorption at 550 nm. Cells exposed to culture medium alone represent 0% lysis, while those exposed to 3 M guanidine hydrochloride provide the 100% lysis end point.

T cell colony assay. A modification of the whole blood method was used for human T cell colony formation in vitro (9, 21). Briefly, peripheral blood was centrifuged over Ficoll-Hypaque (specific gravity 1.078) and the interface mononuclear cells collected. The mononuclear cells were depleted of monocytes by double adherence to culture plates for 1 h. The lymphocytes were counted and diluted in RPMI 1640 medium to obtain different concentrations of nucleated cells. Irradiated (2,000 rad) red blood cells and granulocytes obtained from the pellet of the Ficoll/Hypaque centrifugation, different dilutions of monocyte-depleted peripheral blood mononuclear cells, and 56°C heat-inactivated AB human serum were mixed with 1% low gel temperature agarose (Bio-Rad Laboratories, Richmond, CA) that had been warmed to 40°C and held at 37°C until plating occurred. The final concentrations of 5-ml mixtures were 2.4% irradiated red blood cells containing ~ 2×10^4/ml granulocytes, 20% heat-inactivated AB serum, 0.5% agarose, and different concentrations of monocyte-depleted peripheral blood mononuclear cells.

1-ml aliquots of these mixtures were plated in quadruplicate in 6-well flat-bottom tissue culture plates containing 0.5 ml of the various test materials. In addition, each well was pulsed with 25 μl phytohemmaglutinin-M (Difco Laboratories, Inc.). Colonies (aggregates of > 40 cells) were counted after 6 d of culture with the use of a projecting microscope (Bausch & Lomb Inc.) The total number of colonies per well was determined after four to six representative areas were counted in each well and the average number of colonies for the representative areas were corrected to reflect the total area of the well. Plating efficiency is the number of colonies per 10^5 lymphocytes plated. Significance was determined using Student's *t* test. In this culture system, irradiated red blood cells and granulocyte serve as a "feeder" layer, since these cells form no T cell colonies after 2,000 rad are given.

Neutralization studies. Human recombinant IL-1, TNFα, or MCM were incubated with anti-IL-1, anti-TNFα, or the IgG fraction of normal rabbit serum at 4°C overnight before addition to the T cell colony assay. As controls, anti-IL-1 or anti-TNFα alone were also added to the T cell colony assay.

Results

T cell colony formation of nonadherent peripheral blood lymphocytes in vitro is dependent on the presence of IL-2 or on the ability to induce IL-2 in the culture system by a source of IL-1 (Fig. 1). 1 U of recombinant IL-2, 200 ng/ml recombinant IL-1, or 10% conditioned medium from 10^6 monocytes/ml demonstrate a flat-line dose response in T cell colony formation between the lymphocyte plating concentrations of 0.88 and 5.00×10^4 nonadherent peripheral blood mononuclear cells which, in the present situation, contain ~ 2% nonspecific esterase-positive cells.

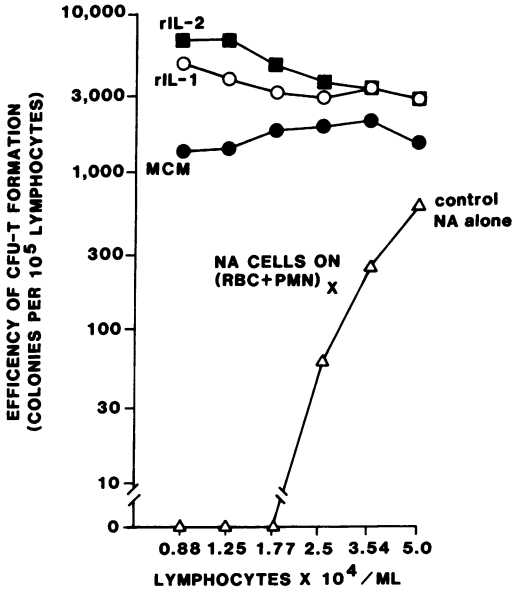

Figure 1. Efficiency of T cell colony formation between the lymphocyte plating concentrations of 0.88 and 5.00×10^4 nonadherent (NA) peripheral blood mononuclear (PMN) cells. Effect of addition of 1 U recombinant IL-2 (rIL-2), 1 U recombinant IL-1 (rIL-1), or 10% MCM. Data are presented from one of four representative experiments. CFU, colony-forming unit; RBC, red blood cells.

Addition of increasing concentrations of recombinant IL-1 produced a dose-dependent increase in T cell colony formation (Fig. 2). This increase could be blocked by prior incubation with anti-IL-1 ($P < 0.001$). In fact, incubation with anti-IL-1 alone produced a level of T cell colony inhibition below that seen when no stimulator was added ($P < 0.001$), indicating that anti-IL-1 may be inhibiting endogenous IL-1 present in the culture system.

TNFα is another product produced by activated human monocytes in culture (Table I). The TNF produced by human monocytes in culture is completely inhibited by prior incubation with anti-TNFα, whereas anti-IL-1 had no effect. Figs. 3 and 4 display the effects of recombinant TNFα on T cell colony formation in the presence and absence of 1 U of IL-2, respectively. rTNF demonstrated no effect on T cell colony growth when cultures were maximally stimulated with 1 U IL-2 (Fig. 3). Thus, TNFα did not directly suppress maximally stimulated T cell colony formation. In the absence of added IL-2 (Fig. 4), increasing concentrations of rTNF caused a dose-dependent increase in T cell colony formation that could be inhibited by prior incubation with anti-TNFα. Prior incubation of TNFα with anti-IL-1 also appears to result in suppression of T cell colony formation, suggesting that TNFα may exert its stimulatory influence through the action of IL-1 (Fig. 5). However, this suppression of T lymphocyte colony formation may also arise due to anti-IL-1 inactivation of IL-1 endogenous to the cultures (Figs. 2 and 5). This would also result in a lower T cell colony level as compared with TNFα alone.

*Table I. TNFα Assay of MCM**

Source of TNFα	A550
TNF‡ 2,500 pg/ml‡	0.004
625 pg/ml	0.032
156 pg/ml	0.098
39 pg/ml	0.191
0 pg/ml	0.272
MCM 1:2	0.007
1:8	0.030
1:32	0.124
1:128	0.257
MCM 1:2 + Anti-TNFα	0.249
MCM 1:2 + Anti-IL-1	0.009

* 3×10^4 mouse L-929 fibroblasts treated with 1 μg/ml actinomycin D were plated in 96-well culture plates for 18 h. Each well was stained with 0.5% crystal violet and absorbance measured at 550 nm using a Titerlek multiscan autoreader.
‡ rTNFα.

Monocytes are capable of producing and releasing both IL-1 and TNFα into culture medium. MCM obtained from two day cultures of 10^6 monocytes/ml contains ~ 2.5 ng/ml TNFα and ~ 1–10 ng/ml IL-1 when assayed for TNFα or IL-1, respectively. This MCM demonstrated a dose-dependent increase in T cell colony formation. To determine if this effect were due to IL-1 and/or TNFα production by monocytes,

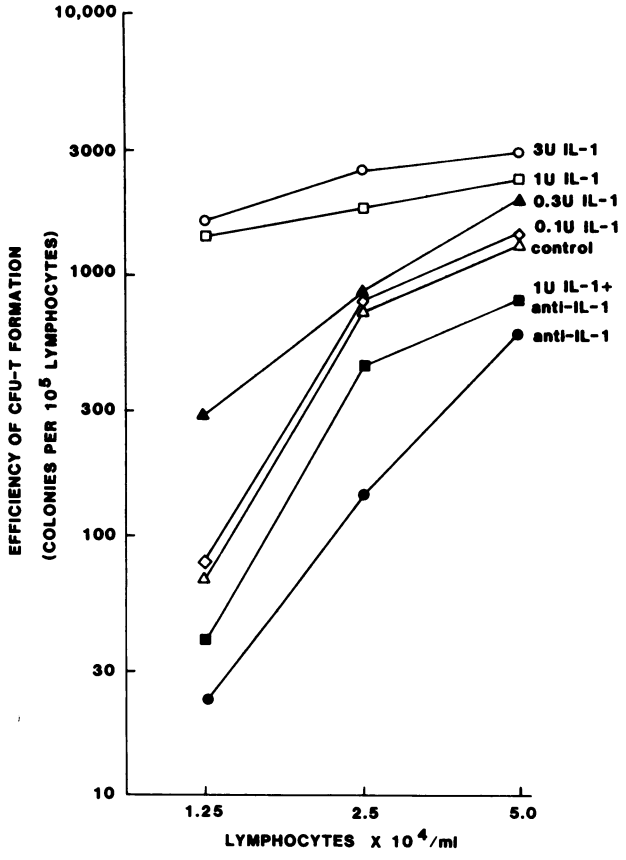

Figure 2. Influence of increasing concentrations of recombinant IL-1 on the efficiency of T cell colony formation when plating 1.25–5.00 × 10^4 lymphocytes/ml. Data are presented from one of four representative experiments. $P < 0.001$ when comparing control or 1 U IL-1 + anti-IL-1 vs. 0.3–3 U IL-1 at 1.25 × 10^4 lymphocytes/ml.

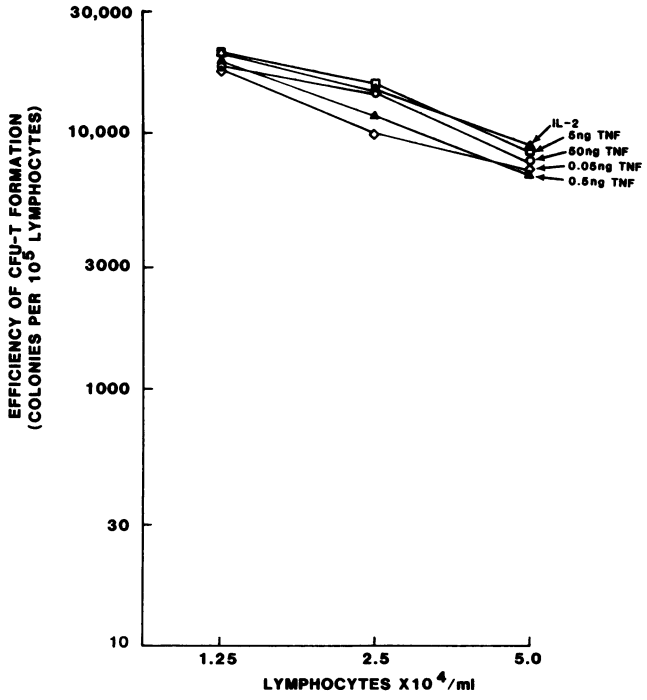

Figure 3. Effect of rTNFα on the efficiency of T cell colony formation in the presence of 1 U recombinant IL-2 when plating 1.25–5.00 × 10^4 lymphocytes/ml. Data are presented from one of two representative experiments.

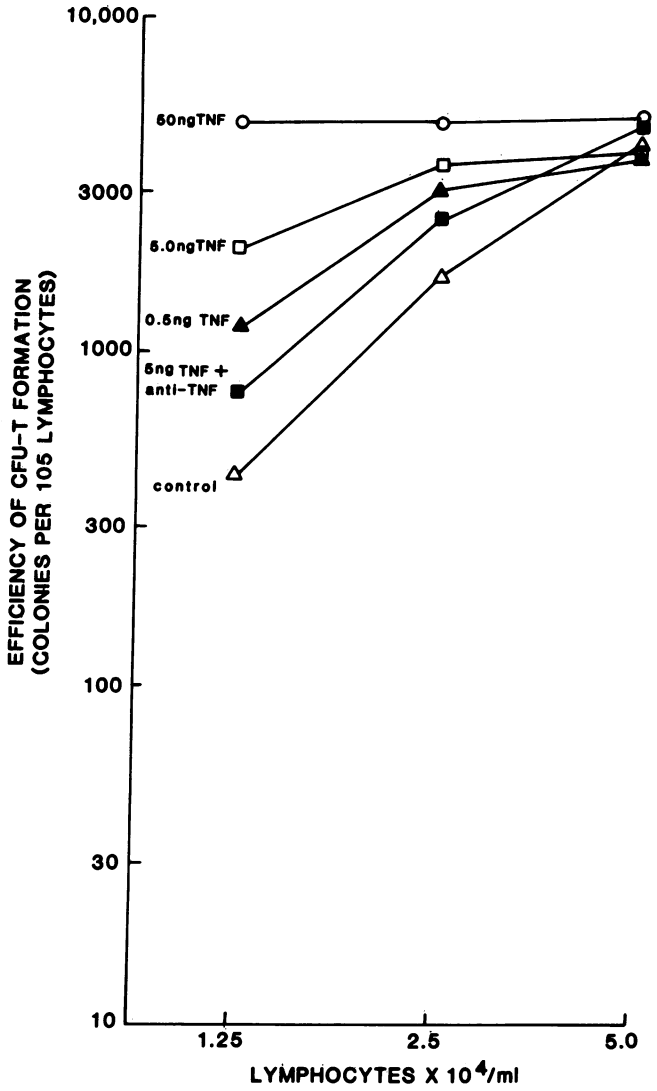

Figure 4. Influence of increasing concentrations of rTNF on the efficiency of T cell colony formation in the absence of added IL-2 when plating 1.25–5.00 × 10^4 lymphocytes/ml. Data are presented from one of three representative experiments. $P < 0.001$ when comparing control vs. 0.5–50 ng TNFα at 1.25 × 10^4 lymphocytes/ml. $P < 0.001$ when comparing 5 ng TNFα + anti-TNFα vs. 5 ng TNFα at 1.25 × 10^4 lymphocytes/ml.

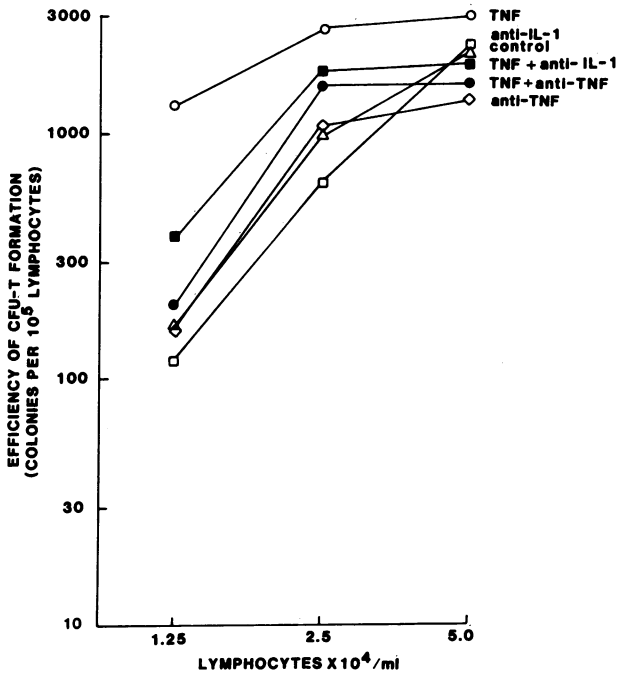

Figure 5. Effect of anti-TNFα and anti-IL-1 on TNFα-induced increase in T cell colony formation efficiency between the plating concentration of 1.25 and 5.00 × 10^4 lymphocytes/ml. Data are presented from one of three representative experiments. $P < 0.001$ when comparing TNFα + anti-IL-1 or TNFα + anti-TNFα vs. TNFα at 1.25 × 10^4 lymphocytes/ml.

MCM was incubated with antibody to IL-1, antibody to TNFα, or antibody to both IL-1 and TNFα before assaying for T cell colony formation. Fig. 6 indicates that while incubation of MCM with either anti-TNFα or anti-IL-1 alone suppressed T cell colony formation, incubation of MCM with both anti-IL-1 and anti-TNFα together gave the greatest suppression of T cell colony formation, suggesting that TNFα may exert its stimulatory influence independent of IL-1.

Discussion

The mechanisms regulating T cell colony formation in agarose from peripheral blood lymphocytes are poorly understood. Using excess exogenous IL-2 and performing cell dose-response curves, the number of T lymphocyte colony-forming cells per 10^5 lymphocytes plated can be determined. This number reflects the proportion of T lymphocyte colony-forming cells present among peripheral blood lymphocytes and should result in a flat-line dose-response curve as seen in Fig. 1. Plating cell dose-response curves in the absence of exogenous IL-2 requires not only the presence of mitogen, but also of IL-1, and results in the release of IL-2. This occurs as a result of cooperation among a number of different cell types within the colony culture system (9, 10, 22, 23). These cell types include both helper and suppressor T cells and monocytes and/or macrophages. There have been contradictory reports concerning the role of monocytes in T cell colony formation. Some workers support a positive influence on T cell colony growth (8, 10, 23), while others (7, 24, 25) have implicated a suppressive role possibly due to their release of prostaglandins, which may inhibit colony growth. We have shown that the net regulatory role of macrophages (stimulatory or inhibitory) in T cell colony formation is dependent upon their concentration in culture (26).

In the present study, we investigated the role of two known activated monocyte products (IL-1 and TNFα) on T lymphocyte colony formation. MCM produced a dose-dependent increase in T cell colony formation that could be partially suppressed by either anti-TNFα or anti-IL-1. Greater suppression occurred when both antibodies were used together, suggesting that MCM contained both IL-1 and TNFα and that both of these materials were capable of stimulating T cell colony formation independent of each other. Whether TNFα directly stimulates the T colony-forming cell or whether it acts similarly to or via IL-1 to stimulate helper T cells to produce IL-2, which then results in T colony formation, is not known at present.

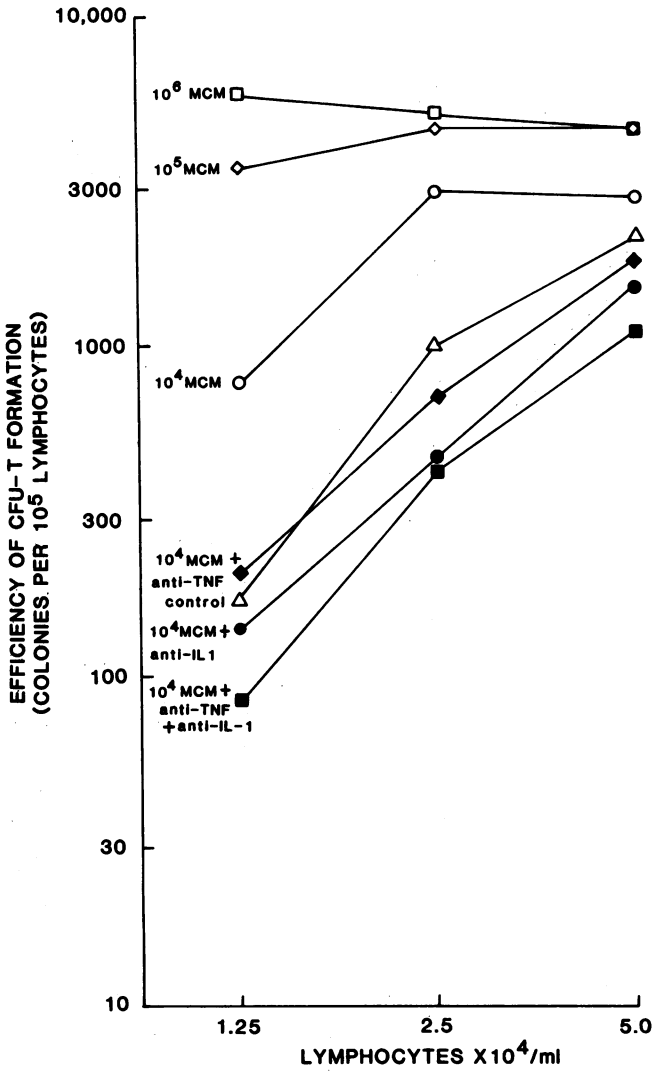

Figure 6. Influence of MCM obtained from cultures of increasing concentrations of monocytes on the efficiency of T cell colony formation between the plating concentration of 1.25 and 5.00 × 10^4 lymphocytes/ml. The effect of prior incubation of MCM with anti-TNFα, anti-IL-1, or both anti-TNFα and anti-IL-1 on the efficiency of T cell colony formation. Data are presented from one of two representative experiments. $P < 0.001$ when comparing control, 10^4 MCM + anti-IL-1 or 10^4 MCM + anti-TNFα vs. 10^4 MCM at 1.25 or 2.5 × 10^4 lymphocytes/ml.

In addition, both recombinant IL-1 and rTNF increased T cell colony formation in a dose-dependent manner when added to the culture system in the absence of exogenous IL-2. Their positive influence was suppressed when incubated with their respective antibodies before addition to the colony assay. TNFα-stimulated T cell colony formation was inhibited by anti-IL-1, suggesting that TNFα may stimulate IL-1 production by cells present in the T cell colony assay system. This effect of anti-IL-1 on TNFα stimulation was not due to crossreactivity by anti-IL-1, since no effect of anti-IL-1 was seen in the TNFα assay that used actinomycin D-treated L-929 cells (Table I). However, suppression of TNFα-stimulated T cell colony formation by anti-IL-1 may also be due to the presence of endogenous IL-1 in the culture system. In any event the stimulation of IL-1 production by TNFα is not without precedent, since TNFα has been reported to stimulate IL-1 production by both monocytes (11, 27, 28) and endothelial cells (29, 30). In addition, large granular lymphocytes (31) and B lymphocytes (32, 33) have also been reported to be capable of producing IL-1, and these cell populations are present in the nonadherent peripheral blood mononuclear cells used in the present study.

TNFα has also been reported to be associated with both stimulatory and inhibitory effects on in vitro hematopoietic progenitor cells (34–37). TNFα was shown to stimulate the production of granulocyte–macrophage colony-stimulating factor by fibroblasts and endothelial cells, thus displaying a positive influence on myeloid colony formation (34–36). In the present report, a positive influence of TNFα was also shown for T lymphocyte colony formation. TNFα has been reported to demonstrate a suppressive influence on myeloid progenitor cell proliferation (37). However, in this study, no suppressive influence by TNFα on IL-2–stimulated T cell colony formation was seen (Fig. 3). Therefore, we conclude that activated monocytes produce at least two products, IL-1 and TNFα, which are capable of stimulating T cell colony formation. The exact action of TNFα is unknown at present. TNFα may exert its effect through the stimulation of IL-1 by accessory cells present in the T cell colony assay system. It may also act independently of IL-1 and stimulate IL-2 production by helper T cells or it may directly stimulate the T colony-forming cell. These possibilities are currently under investigation.

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