Role of Tumor Necrosis Factor in Macrophage Activation and Tumoricidal Activity

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ABSTRACT

Tumor necrosis factor (TNF)-sensitive (LM) and -insensitive (P815) target cell lines were used to examine the role of TNF in both the activation and lytic phases of macrophage-mediated lysis. LM cells were lysed spontaneously by thioglycollate-elicited macrophages in an 18-h assay (media or activating agents added with targets) or 36-h assay (macrophages cultured with media or activating agents for 18 h, washed, and targets added for a subsequent 18 h). In contrast, P815 cells were lysed only in the 36-h assay by macrophages exposed to appropriate activation signals. Using antibody to murine TNF, it was shown that lysis of LM cells but not P815 cells was TNF mediated. The addition of lipopolysaccharide (LPS) to the 18-h assay resulted in augmented LM killing. This was probably due to the fact that LPS stimulates macrophages to produce TNF. Conversely, when macrophages were pretreated with LPS for 18 h, washed, and assessed for lytic activity during the subsequent 18 h, lysis of LM cells was reduced relative to the endogenous level.

Although macrophage lysis of P815 was not mediated by TNF, the addition of TNF to macrophage activation cultures facilitated LPS triggering of cytolytic activity against P815. Similarly, the addition of TNF to the activation cultures partially prevented the LPS-induced reduction in macrophage-mediated LM cell lysis. Taken together, these data suggest that TNF may act as an autocrine signal during macrophage activation, in addition to being directly lytic to a select number of sensitive target cell lines.

INTRODUCTION

The in vitro activation of macrophages, obtained from a variety of tissues, to become cytolytic or cytostatic for tumor cells or microorganisms has been recently reviewed (1). The molecular events which occur during the triggering and expression of macrophage-mediated cytotoxicity are receiving considerable attention (2). In several recent studies (3-8), it was reported that TNF (5) is elaborated by macrophages during the activation process and that this is the primary mechanism by which activated macrophages kill tumor target cells. The demonstration that TNF was involved in macrophage-mediated lysis was generally accomplished by blocking this reaction with antibody against TNF. However, these studies were all rather limited in scope since the target cell lines used were sensitive to direct lysis by TNF. In studies on the growth of established cell lines in vitro, TNF was shown to either not effect growth, inhibit growth (as observed with L-cells), or enhance growth (9). Therefore, the proposal that tumoricidal macrophage activity is due to direct TNF lysis is limited to situations involving TNF-sensitive tumor lines.

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4 The abbreviations used are: TNF, tumor necrosis factor; [3H]TdR, tritiated deoxythymidine; LAK, lymphokine-activated killer (cells); NK, natural killer (cell); rHL-TNF, recombinant human tumor necrosis factor; SN, supernatants; RCO, Bacillus Calmette-Guirin.

Numerous other reports have demonstrated divergent biological effects of TNF in a variety of experimental systems (10-12) and many investigators have reported that TNF can modulate various immune responses (3, 13-15). It was therefore reasonable to postulate that, under normal conditions, TNF functions as a regulatory cytokine (13). However, the relative physiological importance of the various biological effects induced by TNF has yet to be clarified.

The present studies were designed to evaluate what functions TNF may regulate during the activation and/or lytic phases of tumoricidal macrophage activity against both TNF-sensitive and -insensitive target cells. Because of the numerous other lytic factors produced by macrophages (e.g., reactive oxygen, neutral proteases, interleukin 1, complement, arginase, or factors inhibiting oxidative metabolism (16)), experimental conditions were chosen such that TNF production by macrophages could be dissociated from macrophage killing. The results of this study, which have been reported previously in preliminary form (17), confirm that TNF can serve as the lytic mediator in the tumoricidal macrophage response against TNF-sensitive targets; but, perhaps more importantly, it also serves as a regulatory molecule during the activation of tumoricidal macrophages. Furthermore, data are presented to support the postulate that tumor cell phenotype in part determines susceptibility to nonspecific effector cell lytic mechanisms.

MATERIALS AND METHODS

Mice. Specific pathogen-free female C57BL/6Cr mice were obtained from Simonsen Laboratories, Gilroy, CA, and were used for experiments at 8 to 12 weeks of age.

Culture Media. The medium used for all assays, macrophage incubations, and cell line maintenance was RPMI 1640 supplemented with 0.1 mg/ml of gentamicin (Gibco Laboratories, Grand Island, NY), 25 mm N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer, and 10% fetal calf serum (HyClone Laboratories, Logan, UT). For the generation of LAK cells this medium was further supplemented with 1 mm glutamine, 0.1 mm nonessential amino acids, 1 mm sodium pyruvate, and 50 mu g 2-mercaptoethanol (complete medium). Tissue culture media were endotoxin free (<0.05 ng/ml endotoxin), as determined by Limulus amebocyte lysate assay (M.A. Bioproducts, Walkersville, MD).

Reagents. rH-TNF was supplied by Asahi Chemical Industry, Tokyo, Japan and the activity units of the preparations had been defined in an L-cell killing assay by the method of Yamazaki et al. (18). Rabbit antiserum against murine TNF-α was a gift provided through the extramural research program of Genentech, Inc., San Francisco, CA. For LAK generation, semipurified rat interleukin 2 was purchased from Collaborative Research, Inc., Lexington, MA (Lot 861485). LPS (Escherichia coli 0111:B4) was purchased from Difco Laboratories, Detroit, MI; thioglycolate was from Becton Dickinson, Lincoln Park, NJ, and polymyxin B was from Sigma Chemical Co., St. Louis, MO. [3H]TdR (6.7 Ci/mol) was obtained from New England Nuclear, Boston, MA, and Na251CrO4 (250-500 μCi/μg chromium) was obtained from American, Arlington Heights, IL.

Cell Lines. L929 and its subline, LM, were obtained from American Type Culture Collection, Rockville, MD. A TNF-resistant line (L929R) was developed by culturing L929 cells in the presence of rat-h-TNF (50 units/ml) for 1 month. This line remained resistant after passage in vitro, without further exposure to TNF. Half-confluent monolayers of L-cells were harvested from culture flasks by gentle dislodgement by
were P815 mastocytoma (syngeneic to DBA/2J mice) and YAC-1 to 96-well flat-bottomed plates (Corning Co., Corning, NY) in 0.1 ml. balanced salt solution (Gibco). These cells (1.25 x 10^5/well) were added counted, and tested for cytolytic activity (4 h) at 100:1 effector:target ratio.

Isolation and Activation of Macrophages. Mice were given injections i.p. of 1 ml of 3% thioglycolate 5 days prior to sacrifice. Peritoneal exudate cells were harvested by lavage, using 7 ml of cold Hanks’ balanced salt solution (Gibco). These cells (1.25 x 10^5/well) were added to 96-well flat-bottomed plates (Corning Co., Corning, NY) in 0.1 ml. After a 2-h incubation at 37°C in 5% CO_2, nonadherent cells were removed by three washes and activation cultures were started immediately. The adherent population of cells was greater than 90% macrophages as determined by morphology and nonspecific esterase. Macrophages were cultured with or without agents (LK, LPS, TNF, polymyxin B) for an 18-h activation period, washed, and ^51Cr-labeled target cells (10^4/well) were added for a further 18-h incubation (36-h assay). A variation of this assay used the coaddition of agents and radiolabeled targets to adherent macrophages for 18 h (18-h assay).

NK and LAK Lytic Activities. Spleens were aseptically removed on day of sacrifice and single cell suspensions prepared by passage through coarse (50 mesh) and fine (200 mesh) stainless steel gauze. Viable cells, as determined by trypan blue exclusion, were counted on a hemacytometer. For cytoytic NK assays, spleen cells (10^6/ml) were plated in round bottomed microwell plates (Linbro, Flow Laboratories, McLean, VA) in a volume of 0.1 ml and 10^4 ^51Cr-labeled YAC-1 target cells were added in 0.1 ml. The plates were incubated for 4 h, centrifuged, and 0.1 ml of supernatant was removed from each well for determination of radioactivity. For LAK generation, spleen cells (5 x 10^6/ml) were combined with interleukin 2 (10 half-maximal units/well) and 1 µM indomethacin. LAK effector cells were harvested after 4 days of culture, counted, and tested for cytolytic activity (4 h) at 100:1 effector:target ratio (target cell number equaled 5 x 10^4/well). In each assay of NK and LAK effectors at least 3 other effectors:target ratios were used and similar results were obtained (data not shown).

Evaluation of Specific ^51Cr Release. After 4 h (NK, LAK) or 18 (macrophage) of incubation of effector cells with target cells, the microwell plates were centrifuged at 300 x g for 5 min and the amount of ^51Cr released into 100 µl of cell-free supernatant from each well was determined in a gamma counter. Percentage of specific ^51Cr release was calculated by the following formula:

\[
\text{% of specific } ^{51}\text{Cr release} = \frac{\text{Experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100
\]

Experimental release is the radioactivity released in wells containing activated effector cells and target cells; spontaneous release is the radioactivity released from target cells incubated in medium alone; total release is the radioactivity from target cells lysed with 1% (v/v) Triton X-100. The spontaneous release was <10% in 4-h assays and <28% in 18-h assays. Each test group was assayed in triplicate or quadruplicate and all experiments were repeated at least three times.

LK Preparation. Lymphokine-enriched culture supernatant of concanavalin A-stimulated mouse spleen cells was prepared as described (19). Briefly, spleen cells from untreated mice (7.5 x 10^6 viable cells/ml) were cultured for 24 h in complete medium containing 5 µg/ml concanavalin A (Pharmacia, Uppsala, Sweden). After the incubation, the supernatants were harvested, centrifuged, and treated with Sephadex G-10 (10 mg/ml) to remove residual concanavalin A from the LK. The LK was stored at 4°C and its interferon activity was determined to be 45 units/ml by a standard microtiter antiviral procedure (19).

Tumor Necrosis Serum Preparation. As a primary stimulus for tumor necrosis serum production, C57BL/6 mice were given injections i.v. of 2 mg of viable BCG (~6 x 10^7 viable organisms) from Kyowa Hakko Co., Tokyo, Japan. After 10 days, an i.v. injection of 50 µg LPS was administered to the BCG-treated mice. Blood was collected, by ventricle puncture without anticoagulants, 2 h after LPS administration and serum was stored at -70°C.

Statistical Methods. All experiments were repeated at least three times with similar results obtained each time. Data from single experiments are shown expressed as mean values ± SD. P values were calculated by Student's t test.

RESULTS

rH-TNF-mediated Cytolysis of Murine Tumor Cell Lines. Four tumor cell lines were assayed for their sensitivity to rH-TNF in an 18-h ^51Cr-release assay (Fig. 1). The L929 cell line, although sensitive to rH-TNF, was less sensitive than LM cells and therefore the LM cell was used in the subsequent studies. L929R, an L929 subline resistant to rH-TNF, and P815, the standard target cell line for assessing activated macrophage cytotoxicity, were resistant (in an 18-h assay) to rH-TNF, and P815, the concentrations tested (5 x 10^-4~50 units/ml). Using the rH-TNF and the four targets under the same conditions in a parallel 48-h postlabeling experiment (procedure as given in Fig. 3), similar results were obtained (data not shown).

Target Cell Cytolysis by Macrophages Pretreated with Different Activation Signals. Thioglycolate-elicited macrophages were exposed to various factors for 18 h, washed, and recultured with ^51Cr-labeled tumor target cells for an additional 18 h. Macrophages activated by LPS, LK plus LPS or LK plus TNF were significantly (P < 0.01) cytolytic toward TNF-insensitive P815 cells (Fig. 2). Macrophages cultured with medium alone were lytolytic against TNF-sensitive LM cells and this spontaneous lysis was not affected by LK or LK plus TNF and was augmented by TNF alone. In contrast, preexposure of macrophages to LPS or LK plus LPS significantly (P < 0.01) reduced subsequent lytic activity against LM cells relative to that seen with the untreated control. Under the conditions used, no cytolysis of L929R cells by macrophages was observed with any of the test groups. A similar pattern of lysis was observed with resident macrophages; however, the absolute levels were lower (data not shown).

![Fig. 1. Effect of rH-TNF on various murine tumor cell lines. LM, L929, P815, or L929R cells were labeled with ^51Cr. Tumor target cells (10^4/well) were combined with rH-TNF (0.1 ml) in an 18-h ^51Cr-release assay. The data shown represent the average of triplicate wells. SD was ±5% for all experimental values. * significant cytotoxicity (P < 0.001).](cancerres.aacrjournals.org)
Inhibition of TNF-dependent Tumor Cell Killing by Anti-TNF Antiserum. Antiserum raised in rabbits against murine TNF was used to block spontaneous macrophage killing of LM cells (Table 1). TNF-sensitive LM target cells were added to unstimulated thioglycolate-elicited macrophages in the presence or absence of antibody against murine TNF. In its absence, significant lytic activity was observed after 8 h and it doubled by 18 h. The anti-TNF-antiserum abrogated this activity at both time points but had no effect on macrophage-mediated lysis of P815 cells (data not shown). As a positive control for the specificity of this antiserum, it was also demonstrated that it abrogated the lytic activity (18 h) of tumor necrosis serum from BCG-primed, LPS-induced mice.

Time Dependence of Cytolysis by Macrophages. In a 36-h assay, LPS stimulated macrophages to be lytic for P815 cells but inhibited spontaneous lysis of LM cells (Fig. 2). In order to understand these conflicting results, experiments were performed to determine the levels of macrophage-mediated cytolysis during and after exposure to LPS. Macrophages were: (a) incubated with labeled target cells ± LPS (18-h assay); or (b) incubated ±LPS for 18 h (activation period), washed, and target cells were added for 18 h (36-h assay). In both cases, target cell lysis during an 18-h period was measured and is expressed as percentage of specific 51Cr release (Table 2). Without LPS exposure, macrophages were cytolytic to TNF-sensitive LM cells, in both 18- and 36-h assays. Macrophage-mediated LM cytolysis was enhanced by LPS addition to the 18-h assay.

Conversely, LM cytolysis mediated by macrophages in the 36-h assay was reduced when LPS, present during the activation period (the first 18 h), was removed prior to the addition of target cells. P815 target cells were not lysed in the 18-h assay with or without LPS or in the 36-h assay without LPS activation of the macrophage. During the 36-h assay, significant macrophage-mediated cytolyis of the TNF-resistant P815 target cells occurred if LPS was present during the initial 18-h activation period.
Reduced numbers of viable cells, as indicated by decreased [3H]dThd uptake, were observed with SN from LPS-treated macrophage cultures but not from cultures without LPS addition. The elaboration of soluble cytotoxic factors increased with increasing LPS concentrations.

Effect of Polymyxin B Addition to Macrophage Activation Cultures. In order to further establish the essential role of LPS in: (a) stimulating macrophage to lyse P815; and (b) reducing spontaneous LM cell lysis, experiments using polymyxin B to neutralize the LPS activity were carried out (Table 3). The addition of polymyxin B (10 µg/ml) blocked the LPS (1000 ng/ml) induction of P815 cell lytic activity and the LPS (10-1000 ng/ml) induced down modulation of LM lysis.

Effect of TNF Addition to Macrophage Activation Cultures with or without LPS. Previously, using similar experimental conditions, TNF in combination with low concentrations of LK (19) or recombinant murine γ-interferon (20) was shown to activate macrophages to kill TNF-resistant P815 cells. In order to determine if TNF would have similar effects in combination with LPS, rH-TNF at various concentrations (0.05-5000 units/ml) was added to macrophage 18-h activation cultures with or without LPS (10 ng/ml). The cells were then washed, and labeled target cells were added for the cytolytic assay. Significant activation of macrophage by TNF or LPS alone did not occur at the concentrations tested (Fig. 4). When the two agents were combined, however, a TNF concentration-dependent tu-

Table 3. Addition of polymyxin B to macrophage activation culture

<table>
<thead>
<tr>
<th>Tumor target</th>
<th>LPS * (ng/ml)</th>
<th>Control</th>
<th>+ polymyxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>P815</td>
<td>0</td>
<td>0 ± 0.6</td>
<td>0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0 ± 0.1</td>
<td>2.3 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0 ± 0.7</td>
<td>0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>24.0 ± 3.4</td>
<td>0 ± 1.7</td>
</tr>
<tr>
<td>LM</td>
<td>0</td>
<td>52.2 ± 1.1</td>
<td>52.4 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>41.3 ± 9.6</td>
<td>47.0 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.7 ± 1.9</td>
<td>43.1 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>3.5 ± 0.6</td>
<td>31.5 ± 3.8</td>
</tr>
</tbody>
</table>

* Adherent macrophages from thioglycollate-elicited peritoneal exudate cells (1.25 × 10^6/0.1 ml) were incubated with or without LPS for 18 h (activation culture) in the presence or absence of polymyxin B (10 µg/ml). The effector cells were then washed and radiolabeled target cells (P815 and LM) were added. The data presented represent the average percentage of specific 51Cr release after 18 h from triplicate wells ± SD. When the +LPS group was less than in -LPS group (P < 0.001) or when the +LPS group was significantly less than that in the +LPS control (+polymyxin B) group (P < 0.001). When the +LPS group was less than in -LPS group (P < 0.001).

Fig. 4. Effect of TNF on macrophage activation with LPS. Macrophages were cultured for 18 h ± TNF (0.05-5000 units/ml) with (A) or without (B) LPS (10 ng/ml). In a second 18-h incubation period, P815 target cells were added. Points, average percentage of specific 51Cr release of triplicate wells; bar, SD. *significant cytotoxicity (P < 0.01) of TNF + LPS versus TNF alone.

Fig. 5. Effect of TNF addition at various times to macrophage activation culture. Macrophages were cultured for 24 h with medium (M) or LPS (10 ng/ml). As indicated, certain cultures received TNF at various times, at 0, 8, or 18 h during this 24-h activation period or together with targets at the end of the period (24 h) after all the cultures were washed. Lytic activity was then assayed in an 18-h 51Cr-release assay. Columns, average percentage of specific 51Cr-release values of triplicate wells; bar, SD. **significant cytotoxicity (P < 0.01) compared to control. *significantly decreased cytotoxicity (P < 0.01) of +LPS control compared to -LPS control. ○, significant (P < 0.01) reversal of LPS-induced decrease of cytotoxicity.
were readily lysed by LAK cells. In fact, by testing target cell evidence for this and show that the evaluation of tumoricidal conditions differed for the two targets. Interestingly, the L929R TNF sensitivity and susceptibility to lysis by nonspecific effec
tor cells was with the LM cells. However, the activation of macrophages, it was shown that the only apparent correlation between the inherently TNF-resistant P815 cells could be lysed by activated macrophages. Both the TNF-sensitive LM cells and the target cells was at an effector/target ratio of 100:1. NK: fresh spleen cells were used as effector cells to assay NK activity at the effectortarget ratio of 100:1.

The present studies were designed to evaluate possible func
tionalities against infectious disease and neoplasia.

**Table 4 Differential target cell susceptibility to cell-mediated lysis**

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P815</td>
</tr>
<tr>
<td>Macrophage -LPS</td>
<td>1.1 ± 1.2*</td>
</tr>
<tr>
<td>+LPS</td>
<td>25.1 ± 1.3*</td>
</tr>
<tr>
<td>LAK -IL-2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>+IL-2</td>
<td>26.3 ± 1.6*</td>
</tr>
<tr>
<td>NK</td>
<td>2.5 ± 3.6</td>
</tr>
</tbody>
</table>

* The sensitivity of the different target cells (10^6/well) to lysis by three different nonspecific effector cell populations. Macrophage cytotoxicity: macrophages were incubated for 18 h ± LPS (1 μg/ml), washed, and 111C-labeled target cells were added. LAK: spleen cells were cultured for 4 days with 1 μg indomethacin and ± IL2 (10 half-maximal units/ml), washed, counted, and added to labeled target cells at an effectortarget ratio of 100:1. NK: fresh spleen cells were used as effector cells to assay NK activity at the effectortarget ratio of 100:1.

LM cells as shown previously (Table 2). With LPS-stimulated macrophages, significant lysis of P815, YAC-1, and LM tumor target cell lines was observed. The L929R cell line was resistant to macrophage lytic effectors under all these experimental conditions. When these same targets were assayed for suscepti
tibility to LAK-mediated lysis, a different pattern of activity was observed. The YAC-1 and LM target cells were lysed very effectively (>70% 111Cr release). Significant lysis although at lower levels occurred against P815 and even the macrophage-insensitive L929R cell line. Of the four lines tested for sensitivi
ty to NK-mediated lysis, only YAC-1, the standard NK target, was significantly lysed.

**DISCUSSION**

Although tumor necrosis factor was first identified and has been most extensively characterized in terms of its direct anti
tumor activity, the elaboration of TNF by macrophages can potentially result in other physiological changes in vivo. One of the potential functions of TNF may be related to immunomodulation and/or expression of host defense cytotoxic mechan
isms against infectious disease and neoplasia.

The present studies were designed to evaluate possible function(s) of TNF during the activation and lytic phases of the tumor
cidal macrophage response as assessed against both TNF-resistant and -sensitive target cell lines. Each of the 3 target cell lines used in this study displayed a different pheno
type in terms of sensitivity to direct lysis by TNF and by activated macrophages. Both the TNF-sensitive LM cells and the inherently TNF-resistant P815 cells could be lysed by appropriately activated macrophage. However, the activation conditions differed for the two targets. Interestingly, the L929R cell line which had an acquired resistance to TNF was not lysed in 18 h by any tested activated macrophage population. However, these cells were not simply resistant to lysis since they were readily lysed by LAK cells. In fact, by testing target cell susceptibility to lysis by NK and LAK in addition to macrophage, it was shown that the only apparent correlation between TNF sensitivity and susceptibility to lysis by nonspecific effec
tor cells was with the LM cells.

It has been shown repeatedly that macrophage activation is strictly dependent upon timing of exposure and concentration of in vitro signals (1). The results of this study provide further evidence for this and show that the evaluation of tumoricidal activation also depends upon the choice of target cell. Thus, the TNF-sensitive LM cells were lysed spontaneously in either the 18- or 36-h assay. This spontaneous lysis was TNF mediated since it could be completely abrogated by anti-murine-TNF antisera. Although the usual macrophage-activating signals were not necessary during the activation stage of a 36-h assay, LM lysis was augmented when macrophages were exposed to TNF before the lytic period. This is probably due to the fact that TNF acts as an autocrine signal for macrophage to release more TNF (3) and/or to the binding of the exogenous TNF to receptors on the macrophage plasma membrane (21). LPS had differing effects on macrophage-mediated LM lysis depending on timing of exposure. LM lysis was augmented by LPS in the 18-h assay but was significantly reduced in the 36-h assay (LPS present for 18 h, then washed out before lytic phase). The augmenting effect of LPS in the short term assay correlates with the finding reported by others (4, 22) and confirmed in this report that soluble factors with TNF activity can be elabo
rated by macrophages following LPS stimulation. The loss of TNF-mediated LM lytic activity in the 36-h assay following LPS exposure represents a down modulation of TNF expres
sion. The removal of the positive stimulus supplied by LPS at the end of the 18-h activation period may result in the down regulation of macrophage membrane-bound TNF (23), as well as result in a decrease in soluble TNF production (22). In addition to a loss of the positive stimulus supplied by LPS, a negative regulatory mechanism such as that mediated by prostaglandins may be involved. The addition of exogenous prosta
glandin E₂ has been shown to suppress TNF production by LPS-stimulated macrophages (24). Furthermore, LPS is known to be a potent signal for the elaboration of prostaglandin E₂ by macrophages (25).

When P815 was used as a target cell, an entirely different picture emerged in terms of the conditions required for macrophage activation. P815 cells were only lysed in the 36-h assay and only by macrophages which had been exposed to appropriate activation signals (LPS alone, LK plus LPS, LK plus TNF). Under the conditions studied there was no evidence of LPS
duced down regulation of this P815 lysis. The tumoricidal activation of macrophages by LK plus LPS or LPS alone has been observed in numerous studies (1, 26). The activation of murine macrophages by LK plus TNF (19) or γ-interferon plus TNF (20) has been recently reported by this laboratory.

It was clear from these studies that when LPS is used as a macrophage-activating signal and then removed, it is possible to demonstrate that macrophage tumoricidal activity through a TNF-mediated mechanism (LM) can be dissociated from that exerted through a TNF-independent mechanism (P815). The fact that LPS is essential for these effects to occur was further confirmed by the observation that they could be blocked by the coadministration of polymyxin B to the macrophage cultures.

Since it had been demonstrated that TNF, in cooperation with other agents, acts as a macrophage activation signal (19, 20), studies were initiated to determine if TNF would have a similar positive effect on macrophage activation by LPS. The addition of TNF to macrophage activation cultures facilitated LPS triggering of TNF-independent cytolytic activity (P815). These data further support the suggestion that TNF acts as an autocrine activation signal during macrophage activation. Previous studies, using polymyxin B, anti-TNF antiserum, and heat treatment had excluded the possibility that LPS contami
nation of the TNF preparation could be responsible for the observed activation (19, 20). Talmadge et al. (27), using B16 melanoma cells as targets, recently reported a similar TNF
duced activation of macrophages. Our study was different in...
that unlike the B16 melanoma, P815 is not sensitive to direct lysis by TNF.

Any one or more of the various stages in a lytic mechanism such as recognition, binding, the production and/or secretion of lytic moieties by effector cells, or target cell sensitivity to these lytic factors may be involved in determining a target cell response or lack of response to an effector. These phenomena are reminiscent of the complex interactions which are known to occur between target cell determinants and cytotoxic anticancer drugs (28). The recognition of these complex interactions together with the fact that tumor cells within a single tumor mass have been demonstrated to be heterogeneous in terms of drug cytotoxicity (29) are fundamental to the concept of multidrug combination therapy. If this concept can be applied to immunotherapy, it can be suggested that a coordinated activation of multiple effector cell populations with different lytic mechanisms will be required.

In summary, macrophages can be activated in vitro to a tumoricidal state and the lytic profile of a population of activated macrophages is clearly dependent upon the activation signals used, the timing of exposure to various activation signals, and the target cells used for assessing tumoricidal activity. This report indicates that both TNF-dependent and -independent lytic mechanisms occur within one, in vitro-activated, macrophage population and that it is possible to dissociate the two. TNF-dependent macrophage lytic activity in this and previous studies (3-8, 27) is limited to target cell lines directly sensitive to TNF. This report indicates that both TNF-dependent and -independent lytic mechanisms occur within one, in vitro-activated, macrophage population and that it is possible to dissociate the two. TNF-dependent macrophage lytic activity in this and previous studies (3-8, 27) is limited to target cell lines directly sensitive to TNF. This report indicates that both TNF-dependent and -independent lytic mechanisms occur within one, in vitro-activated, macrophage population and that it is possible to dissociate the two.

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