Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6341–6345, October 1983
Immunology

Macrophage-mediated cytotoxicity: Role of a soluble macrophage cytotoxic factor similar to lymphotoxin and tumor necrosis factor

(antilymphotoxin/calcium ionophore A23187)

CHARLES M. ZACHARCHUK, BETH-ELLEN DRYSDALE, MANFRED M. MAYER, AND HYUN S. SHIN

Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Contributed by Manfred M. Mayer, July 5, 1983

ABSTRACT **Guinea pig peritoneal macrophages, when activated for cytotoxicity by the calcium ionophore A23187 or lipopolysaccharide, produce a cytotoxic factor [macrophage cytotoxic factor (Mϕ-CF)] that is not blocked by catalase or protease inhibitors. Fractionation of culture supernates containing Mϕ-CF by gel filtration revealed one peak of cytotoxic activity of $M_r \approx$ 45,000, the same as guinea pig lymphotoxin (LT). Antiserum prepared against purified guinea pig LT completely neutralized the cytotoxic activity of Mϕ-CF. In addition, the cytotoxic factor in guinea pig tumor necrosis serum was found to have a M_r of 45,000 and was neutralized by anti-LT. Thus, Mϕ-CF is physicochemically and immunochemically similar to LT and tumor necrosis factor, if not identical. To investigate the role of Mϕ-CF in macrophage-mediated cytotoxicity, anti-LT was added to A23187- or lipopolysaccharide-activated macrophages before addition of L-929 target cells. In 10 of 16 experiments, the inhibition of macrophage-mediated cytotoxicity was 100%. In the others, cytotoxicity was blocked partially, the lowest inhibition being 49%. The effectiveness of inhibition appeared to be inversely related to the intensity of macrophage activation. These results indicate that Mϕ-CF plays a significant role in macrophage-mediated cytotoxicity but involvement of another mechanism cannot be excluded.**

Extracellular cytotoxic reactions mediated by macrophages have been attributed to a number of substances from these cells, such as metabolites of oxygen, the enzymes arginase and neutral protease, complement fragment C3a, a membrane-associated toxic substance, and the cytotoxic factor in tumor necrosis serum (TNS) (1–6). The relevance of some of these substances appears to depend on the mode of activation and on the design of the cytotoxic system (7–9). As reviewed in detail in the *Discussion*, a number of investigators have studied soluble factors from lymphocytes and macrophages that appear similar to lymphotoxin (LT). Also, their possible relationship to tumor necrosis factor (TNF) has been noted. In previous studies, we have shown that mouse macrophages, when activated for tumor cytotoxicity by A23187 or lipopolysaccharide (LPS), produce a cytotoxic factor [macrophage cytotoxic factor (Mϕ-CF)] of $M_r \approx 45{,}000$, which has properties resembling those of LT (10), a product of stimulated lymphoid cells (11).

We have extended these studies by investigating the relationship of Mϕ-CF to LT and other cytotoxins and also by examining the role of Mϕ-CF in macrophage-mediated cytotoxicity. Experiments were done with guinea pigs because of the availability of an antiserum directed against purified guinea pig LT. In the present paper, we demonstrate that guinea pig macrophages produce a Mϕ-CF similar to the mouse Mϕ-CF and that the cytotoxic activity of guinea pig Mϕ-CF can be completely neutralized by anti-LT. Similarly, the cytotoxic factor present in guinea pig TNS, referred to as TNF, is also neutralized by anti-LT. Finally, the cytotoxicity of guinea pig macrophages is inhibited by anti-LT, which indicates that Mϕ-CF plays a major role in the cytotoxicity of these cells.

MATERIALS AND METHODS

Reagents. LPS was prepared as described (12). Stock solutions of the calcium ionophore A23187 (Sigma or Calbiochem) were prepared by dissolving it in dimethyl sulfoxide at a concentration of 10 mM; solvent alone had no activating effect on macrophages. At the concentrations used, all A23187 solutions were free of detectable LPS, as measured in *Limulus* amebocyte lysate assays (Difco). Catalase was obtained as a sterile aqueous solution from Calbiochem. Aprotinin, bovine pancreatic trypsin inhibitor, and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone were purchased from Sigma.

Macrophage-Mediated Cytotoxicity Assay. The tissue culture medium (TCM) and the cytotoxicity assay are the same as previously described (12), except for the following modifications. Guinea pig peritoneal exudate cells, 3×10^5 in TCM, were added to microtiter wells. Subsequently, nonadherent cells were removed. The incubation period with target cells was extended to 42 hr to insure completion of the cytotoxic reaction. The L-929 cell line, which is sensitive to Mϕ-CF, LT, and TNF, was used as the target cell.

LT, Mϕ-CF, and TNS Production. LT was prepared from guinea pig lymph node cell suspensions stimulated with 10 μg of concanavalin A per ml for 24 hr as described (13).

For the production of Mϕ-CF, starch-elicited guinea pig peritoneal exudate cells were allowed to adhere to a plastic dish for 2.5 hr in TCM (10^6 cells per cm^2). The dish was washed to remove nonadherent cells and cultured with activators for various times at 37°C. For kinetic experiments of Mϕ-CF production, the macrophage monolayers were cultured in the presence of activating agents for 4 hr and were washed with TCM. Supernates collected at various times were then assayed for Mϕ-CF activity. For antibody neutralization experiments, ionophore-induced Mϕ-CF was generated by incubating macrophage monolayers for 4 hr with 20 μM A23187 in TCM, washing with TCM, and culturing in TCM for 4 hr. LPS-induced Mϕ-CF was produced by culturing macrophage monolayers for 6 hr with 10 μg of LPS per ml in TCM. The Mϕ-CF used in chromatography studies was prepared by culturing macrophages continuously with 10 μM A23187 and 10 μg of LPS per ml in RPMI 1640 with antibiotics for 18 hr.

TNS was produced essentially as described by Carswell *et al.* (14). Hartley guinea pigs were injected intravenously with 4 ×

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.

Abbreviations: LT, lymphotoxin; LPS, lipopolysaccharide; Mϕ-CF, macrophage cytotoxic factor; NRS, normal rabbit serum; TNF, tumor necrosis factor; TNS, tumor necrosis serum.

10^7 colony-forming units of bacillus Calmette–Guerin, Phipps substrain TMC 1029 (Trudeau Institute, Saranac Lake, NY), and were challenged intravenously 14 days later with 20 μg of LPS. Two hours after LPS injection, blood was collected by cardiac puncture and used to prepare the TNS.

Cytotoxicity Assay of Mϕ-CF, LT, and TNF. Killing of the L-929 cell line was used to measure the cytotoxic activity of soluble factors, essentially as described by Ruff and Gifford (15). Serial 1:2 or 1:3 dilutions (final volume, 0.1 ml) of test samples were made in 96-well microtiter plates (Costar, Cambridge, MA) with a multi-channel pipette (Flow Laboratories). L-929 cells (5×10^4 cells per 0.1 ml) were then added to the microtiter plates in the presence of actinomycin D (Cosmegan, Merck Sharpe and Dohme), giving a final concentration of 1 μg/ml. The cells were incubated at 37°C for 18 hr, the supernates were discarded, and the remaining viable adherent cells were stained by immersing the microtiter plates in a beaker of crystal violet (0.2% in 2% ethanol) for 10 min. Microtiter plates were rinsed with tap water and 0.1 ml of 1% sodium lauryl sulfate was added to each well to solubilize the stained cells. The absorbance of each well was read at 470 nm with a model MR 580 Microelisa Autoreader (Dynatech, Alexandria, VA). Percent cytotoxicity is calculated by the following formula, in which A_{sample} is absorbance of the sample at 570 nm and $A_{control}$ is that of the well containing TCM only: % cytotoxicity = $(1 - A_{sample}/A_{control}) \times 100$. Cytotoxic activity was quantitated by plotting % cytotoxicity versus log sample dilution to linearize the dose–response curve. A least squares analysis of the data points allowed interpolation of the reciprocal dilution of sample at 50% cytotoxicity, which yielded the sample activity in units/ml. A unit of cytotoxicity is arbitrarily defined as the reciprocal dilution of sample required to reduce the absorbance by 50%.

HPLC. Mϕ-CF samples were fractionated on a Bio-Sil TSK-125 gel filtration column (Bio-Rad) by using a Waters HPLC system (Waters Associates) consisting of a 6000 A pump, a model 660 solvent programer, a model 450 variable-wavelength absorption detector, and a model 730 data module. The column was equilibrated with 10 mM sodium phosphate buffer (pH 6.8) containing 0.2 M Na_2SO_4. Chromatography conditions are given in the figure legends. For calibration, thyroglobulin, ovalbumin, myoglobin, and phenylalanine were used.

Neutralization of Mϕ-CF, LT, and TNF with Anti-LT. Rabbit anti-guinea pig LT was raised against a purified LT preparation as described by Gately *et al.* (16). Briefly, culture fluids from ovalbumin-immune guinea pig lymph node cells stimulated with ovalbumin were concentrated by ultrafiltration and fractionated by Pevikon block electrophoresis, DEAE-cellulose chromatography, and polyacrylamide disc gel electrophoresis. Only one stainable protein band was observed that coeluted with LT activity. Purification of control supernates also yielded a similar protein band, indicating that the purified LT preparation contained at least one major contaminant. Rabbits were immunized subcutaneously with purified LT in complete Freund adjuvant at 2-wk intervals. The animals were bled 10 days after each immunization and the sera were tested for the ability to neutralize LT activity. Gately *et al.* (16) reported that antibody raised in this manner did not neutralize the action of migration-inhibitory factor or macrophage-activating factor nor did it interfere with the production of these factors by lymphocytes. For the neutralization experiments, equal volumes of LT, TNF, or Mϕ-CF were mixed with various dilutions of normal rabbit serum (NRS) or anti-LT. Samples were incubated for 20 hr at 4°C and then assayed in duplicate for residual cytotoxic activity. This antiserum did not crossreact with mouse LT, Mϕ-CF, or TNS.

RESULTS

Activation of Guinea Pig Macrophages for Cytotoxicity and for Mϕ-CF Production. We have shown that mouse macrophages can be activated by LPS or the calcium ionophore A23187 for tumor cell killing and for production of a soluble cytotoxic factor, Mϕ-CF (10). These experiments also indicated functional and physicochemical similarities between Mϕ-CF and LT. Due to the availability of an antiserum against purified guinea pig LT, we were able to investigate the role of Mϕ-CF in guinea pig macrophage-mediated cytotoxicity.

As in the mouse system, A23187 activates guinea pig macrophages for L-929 cell killing in the same micromolar concentration range used for mouse macrophages (results not shown). Fig. 1 shows the kinetics and dose–response of Mϕ-CF production. A23187 induced significant Mϕ-CF production, but LPS did not, which correlates with its inability to activate guinea pig macrophages for cytotoxicity during a 4-hr incubation. However, LPS potentiated Mϕ-CF production by A23187. This synergistic effect was also observed in the mouse system (10). It is not clear why the guinea pig macrophages responded poorly to LPS compared to the mouse. As will be discussed below, we have seen significant activation of Hartley guinea pig macrophages under different experimental conditions in which LPS is present throughout the cytotoxicity assay (see Table 1).

Functional Properties of Mϕ-CF. As shown in Fig. 2, three protease inhibitors, bovine pancreatic trypsin inhibitor, aprotinin, and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, that have been reported to inhibit a cytotoxic neutral protease from mac-

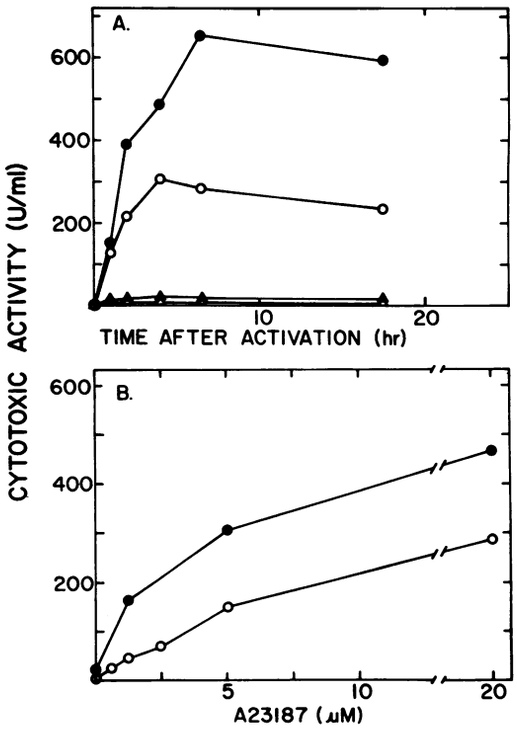

FIG. 1. Kinetics and dose–response curve for Mϕ-CF production. (*A*) Macrophage monolayers were incubated for 4 hr with TCM (△), 10 μg of LPS per ml (▲), 10 μM A23187 (○), or 10 μg of LPS per ml with 10 μM A23187 (●) and then were washed and cultured in TCM. Supernates were collected at the times indicated and assayed for cytotoxic activity. (*B*) Macrophage monolayers were incubated for 4 hr with various doses of A23187 in TCM (○) or in the presence of 10 μg of LPS per ml (●) and then were washed and cultured in TCM. Supernates were collected 4 hr after the activation period and were assayed for cytotoxic activity.

Table 1. The effect of NRS or anti-LT on the cytotoxic activity of LPS-induced Mϕ-CF

Sample	Cytotoxic activity, units/ml
Mϕ-CF	
+ TCM	133
+ NRS*	137
+ Anti-LT*	13

See text for details of the experiment.
* 5 μl of NRS or anti-LT was added per 0.2 ml of reaction mixture.

rophages (3), failed to block the action of Mϕ-CF on the L-929 cells. Further, catalase, which detoxifies H_2O_2, did not inactivate Mϕ-CF.

Gel Filtration of TNS and Mϕ-CF Supernates. Similarities between Mϕ-CF, TNF, and LT led us to investigate the size distribution of cytotoxic activity in TNS- and Mϕ-CF-containing culture fluids. HPLC gel filtration was performed on undialyzed and unconcentrated guinea pig macrophage culture supernates. As expected, a complex pattern of absorbance peaks was seen, but only one peak of cytotoxic activity was observed with an apparent M_r of 45,000 (Fig. 3A). Interestingly, the reported M_r of guinea pig LT is 45,000 (17). Similarly, HPLC gel filtration of guinea pig TNS revealed one peak of cytotoxic activity of the same molecular weight (Fig. 3B).

Neutralization of Mϕ-CF and TNF Activity with Anti-LT. We next investigated whether anti-LT could block the cytotoxic activity of Mϕ-CF and TNF. Fig. 4A shows that LT and Mϕ-CF could be completely neutralized by anti-LT. NRS added at the highest dose had no effect on LT or Mϕ-CF cytotoxic activity. The cytotoxic activity of TNF was also completely neutralized by anti-LT (Fig. 4B). Thus, Mϕ-CF appears antigenically and physicochemically related to LT and TNF. Mϕ-CF and TNF were neutralized slightly more efficiently than LT. Presumably, this is due to a difference in the relative proportion of active and inactive cytotoxic molecules.

Inhibition of Macrophage-Mediated Cytotoxicity by Anti-LT. In the results presented so far, we have shown that A23187-activated macrophages kill L-929 cell targets and that they produce a cytotoxic factor that can be neutralized by anti-LT. To test whether Mϕ-CF is involved in macrophage-mediated cytotoxicity, macrophages were activated with A23187, washed with TCM, and treated with anti-LT 30 min before the addition of target cells. Fig. 5 shows that anti-LT, but not NRS, markedly inhibited killing by macrophages. Hyperimmune rabbit antiovalbumin serum did not block the cytotoxic reaction (data not shown).

Specifically, in a series of six experiments, macrophages were activated by a low dose of A23187 (*ca.* 2.5 μM). At the highest feasible concentration of antiserum, 2 μl per well, 100% inhibition of macrophage-mediated cytotoxicity was observed. In another seven experiments, macrophages were activated with a high dose of A23187 (*ca.* 10 μM). In two of these, inhibition

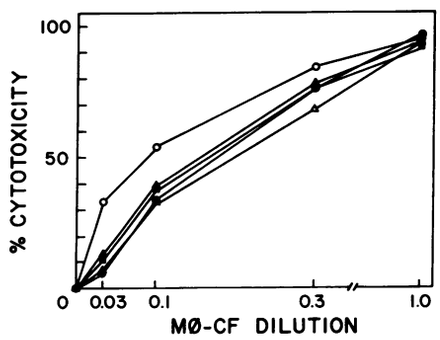

FIG. 2. The effect of catalase and protease inhibitors on Mϕ-CF activity. Various dilutions of Mϕ-CF were mixed with TCM (■), 500 units of catalase (○), 135 kallikrein inhibitor units of aprotinin (△) or bovine pancreatic trypsin inhibitor (▲), or 0.1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone (●). The mixtures were then assayed for Mϕ-CF activity. Neither catalase nor the protease inhibitors alone were toxic for the L-929 cells. The highest dose of Mϕ-CF designated as 1 corresponded to 5 units/ml.

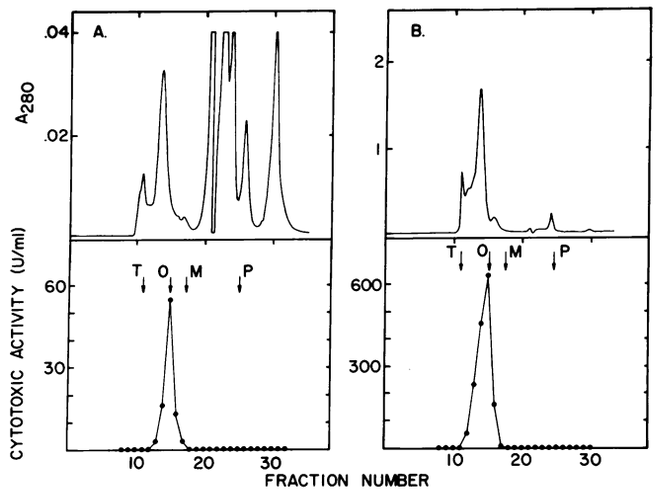

FIG. 3. HPLC gel filtration of Mϕ-CF and TNS. (*A*) Unconcentrated and undialyzed Mϕ-CF-containing supernate (0.2 ml); (*B*) TNS (0.05 ml). Both were fractionated by gel filtration with a Waters HPLC system at a flow rate of 0.5 ml/min. Fractions of 0.5 ml were collected and assayed for cytotoxic activity. The molecular weight markers used were thyroglobulin (T), ovalbumin (O), myoglobin (M), and phenylalanine (P).

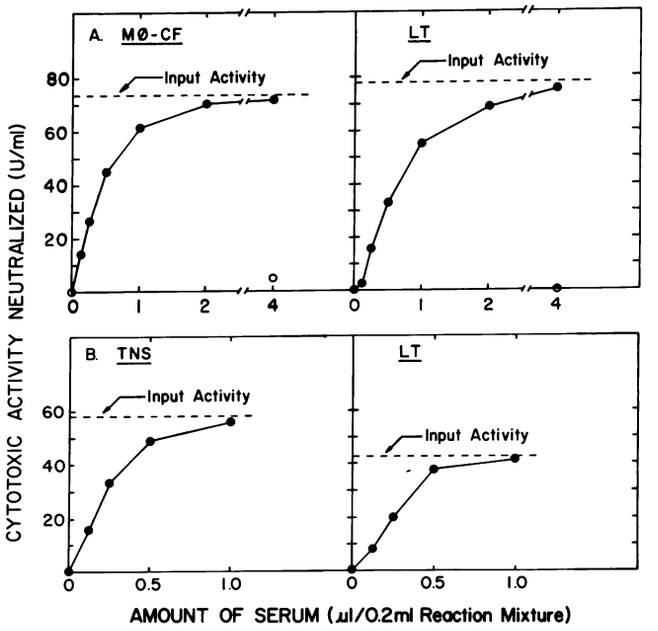

FIG. 4. The effect of varying the dose of serum, NRS or anti-LT, on the cytotoxic activity of Mϕ-CF and LT or TNS and LT. Various doses of anti-LT (●) were mixed with a fixed amount of Mϕ-CF or LT. NRS (○) was also used at the highest dose. Samples were incubated at 4°C for 20 hr and residual cytotoxic activity was assayed in duplicate. Control Mϕ-CF and LT activities (no serum added) are indicated by the dashed lines. *A* (Mϕ-CF and LT) and *B* (TNS and LT) are separate experiments.

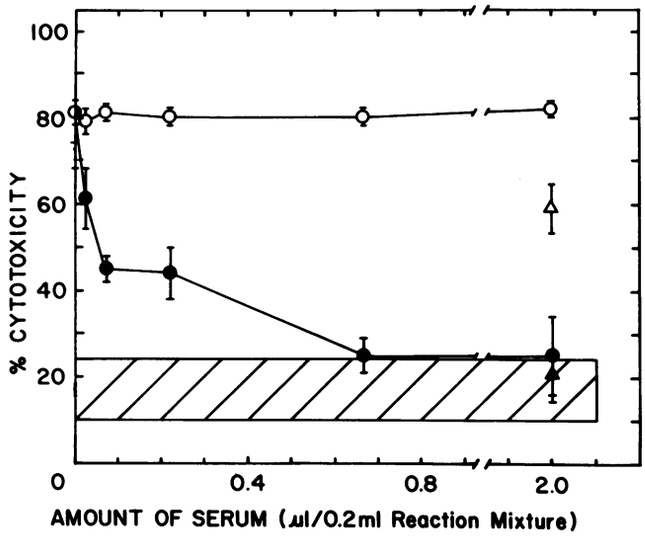

FIG. 5. The effect of NRS or anti-LT on the cytotoxicity of A23187- or LPS-activated macrophages for L-929 cells. In one experimental series macrophage monolayers were incubated with 10 μM A23187 for 4 hr, washed with TCM, and various doses of NRS (○) or anti-LT (●) in TCM were added. After a 30-min incubation L-929 target cells were added and macrophage-mediated cytotoxicity was measured. In another experiment (only with the highest NRS or anti-LT dose), macrophages were cultured in the presence of 10 μg of LPS per ml. NRS (△) or anti-LT (▲) was added to macrophage monolayers 4 hr after addition of LPS. L-929 target cells were added 30 min later and macrophage cytotoxicity was measured. The percent killing (mean ± SD) of target cells by LPS-activated macrophages in TCM without NRS or anti-LT in this experiment was 72% ± 2%. Points indicate the mean of triplicate determinations and error bars represent ± 1 SD. Background cytotoxicity (no A23187 or LPS) is indicated by the hatched bar.

by anti-LT was 100%; in the other five, there was partial blocking, the lowest inhibition being 49%. Attempts to achieve complete inhibition with concentrations of antiserum higher than 2 μl per well were unsuccessful because of a nonspecific effect of serum on target cell growth or macrophage activation (or both).

During the customary 4-hr incubation period, LPS was incapable of activating Hartley guinea pig macrophages to a significant degree for cytotoxicity or for Mϕ-CF production. However, L-929 cell killing could be obtained when LPS (1 μg/ml) was present throughout the 42-hr period. Under these conditions, Mϕ-CF, neutralizable by anti-LT, was also produced; see Table 1. Importantly, LPS-activated macrophage-mediated cytotoxicity was inhibited by anti-LT (Fig. 5, triangles). Specifically, in two experiments, antiserum completely blocked macrophage cytotoxicity; in the third experiment the inhibition was 56%.

Because killing of L-929 cells by LPS- and A23187-activated macrophages can be blocked by anti-LT, we believe that Mϕ-CF plays a major role in macrophage-mediated cytotoxicity. However, the involvement of factor(s) other than Mϕ-CF cannot be excluded. Whether Mϕ-CF participates in macrophage-mediated cytotoxicity must be investigated by using different macrophage populations and various target cells.

DISCUSSION

In the guinea pig system, we have found that LPS and the calcium inophore A23187 activate macrophages for cytotoxicity and for the production of a cytotoxic factor, as in the case of the mouse system. In the series of studies presented, we investigated the relationship of Mϕ-CF to other cytotoxins and the role of Mϕ-CF in target cell killing by activated macrophages.

We showed that Mϕ-CF is different from cytotoxic neutral protease (3) or H_2O_2 (1). The physicochemical similarity between Mϕ-CF, LT, and TNF then led us to focus our studies on their possible relationship. We observed that guinea pig Mϕ-CF and mouse Mϕ-CF have M_rs of ≈45,000. This value corresponds to the reported molecular weight of one species of LT. Ross *et al.* (18) described several classes of LT molecules from a variety of species, including the guinea pig, that differ in size and stability. Sawada *et al.* (19) and Gately and Mayer (17) observed only one peak of LT activity in the guinea pig of M_r 50,000 and M_r 45,000, respectively. Mϕ-CF is also of similar size as one of the cytotoxic factors in TNS, referred to as TNF. In 1975 Carswell *et al.* (14) described the production of TNF, which caused acute hemorrhagic necrosis in transplanted tumors. TNF was toxic *in vitro* for L-929 cells but not for mouse embryo fibroblasts and they suggested that TNF was produced by macrophages. Kull and Cuatrecasas (20) reported three peaks of cytotoxic activity in mouse TNS of M_rs 50,000, 160,000, and 225,000. Other investigators have found only one peak of cytotoxic activity in both mouse and rabbit TNS with a M_r of 40,000–60,000 (11, 15, 21). To investigate the relationship of Mϕ-CF to LT and TNF, we used rabbit antiserum against guinea pig LT purified from antigen-stimulated lymph node culture fluids by Pevikon block electrophoresis, DEAE-cellulose chromatography, and polyacrylamide gel electrophoresis (16). As shown in Fig. 3, anti-LT completely neutralized Mϕ-CF and TNF activity. These results indicate that Mϕ-CF, LT, and TNF are physicochemically and immunochemically related and perhaps are identical molecules.

Our observations are in general agreement with those of several groups of investigators who have studied soluble cytotoxic factors from lymphocytes and macrophages that appear similar to LT. Heise and Weiser (23) found that the cytotoxic factors produced by antigen-stimulated guinea pig lymph node cells and activated lung macrophages were cytotoxic for mouse L cells and could not be distinguished by gel filtration and heat sensitivity. An antiserum produced against the cytotoxic activity found in the excluded volume of Sephadex G-100-purified lymph node cytotoxin neutralized the cytotoxic activities of both the lymphocyte and macrophage supernates. Kramer and Granger (24) observed two peaks of cytotoxic activity of M_r 150,000 and M_r 47,000 in the culture fluids of adherence-purified peritoneal exudate cells from tumor-immune mice. Antiserum raised against phytohemagglutinin-stimulated mouse LT fractionated by sequential ammonium sulfate precipitation was able to neutralize the cytotoxic activity of macrophage supernates. Reed and Lucas (25) reported that human blood mononuclear cells produced a cytotoxic factor that had one peak of cytotoxic activity of M_r 45,000 after gel filtration. Antiserum that could neutralize the M_r 80,000 form of human LT had no effect on the cytotoxic activity of either the macrophage cytotoxin or the smaller M_r 45,000 LT.

With regard to Mϕ-CF and TNF, Matthews (26) found that culture fluids from rabbit mononuclear cells were cytotoxic for L cells and that the toxic factor (M_r 30,000–50,000) was closely related biochemically to rabbit TNF. Männel *et al.* (27) extended these studies in the mouse system. Bacillus Calmette–Guerin-stimulated peritoneal exudate macrophages, *in vitro* propagated peritoneal macrophages, and a macrophage-like tumor line all produced a cytotoxic molecule when stimulated by LPS that was similar, by several biochemical criteria, to the serum-derived mouse TNF (M_r 50,000–60,000). Antiserum raised against TNF that was fractionated by gel filtration and ion-exchange chromatography neutralized these macrophage cytotoxins. The similarity between LT and TNF was also noted (28). Thus, it seems likely that both lymphocytes and macro-

phages produce a cytotoxic molecule of $M_r \approx 45,000$. LT is normally generated by lymphocyte-enriched cell populations when stimulated by specific antigen or T-cell mitogens. Mϕ-CF is produced by adherence-purified macrophage cell populations spontaneously or after induction with nonspecific agents such as LPS. TNF is released in the serum of bacillus Calmette–Guerin-immune animals that have been challenged with LPS. Taken together, previous work and our own biochemical and immunochemical studies indicate that macrophages and lymphocytes from a variety of species, when appropriately stimulated, can produce a cytotoxic molecule that is referred to as Mϕ-CF, LT, or TNF.

In our study, Mϕ-CF release correlates with macrophage activation, in that agents that induce macrophage-mediated cytotoxicity also lead to Mϕ-CF production. In addition, combinations of agents that are synergistic for macrophage-mediated cytotoxicity are synergistic for Mϕ-CF production. These correlations are consistent with, but do not establish, the notion that Mϕ-CF plays a role in macrophage-mediated cytotoxicity. To determine more directly whether Mϕ-CF participates in macrophage-mediated cytotoxicity we made use of anti-LT, which was shown to completely inactivate Mϕ-CF. We found that anti-LT completely blocked cytotoxicity mediated by moderately activated macrophages. However, inhibition varied between 49% and 100% with vigorously activated macrophages. These results indicate that Mϕ-CF plays an important role in macrophage-mediated cytotoxicity, but the involvement of other factor(s) cannot be excluded. Our observations are in general agreement with those of other investigators who have reported the use of antiserum raised against LT to inhibit lymphocyte-mediated cytotoxicity (29–34). However, more closely related to our study, Männel *et al.* (6) raised an antiserum against mouse TNF fractionated by gel filtration and DEAE chromatography. This antiserum partially inhibited the cytotoxicity of LPS-stimulated peritoneal macrophages from bacillus Calmette–Guerin-immune mice. Similarly, Matthews (35) noted that antiserum raised against a human monocyte cytotoxic factor fractionated by polyacrylamide gel electrophoresis or ion-exchange chromatography inhibited monocyte-mediated cytotoxicity.

In summary, we have demonstrated that Mϕ-CF, a cytotoxic molecule biochemically and antigenically similar to LT and TNF, can play a major role in macrophage-mediated cytotoxicity. Further work is required to establish the importance and generality of Mϕ-CF in macrophage-mediated cytotoxicity. If Mϕ-CF can be shown to cause tumor necrosis in animal models, an important link would be established between a defined cytotoxic protein, target cell cytotoxicity *in vitro*, and tumor destruction *in vivo*.

We thank Dr. G. Hart for the use of the HPLC system and Dr. R. Yolken for the use of the microelisa autoreader. We thank Mr. S. Duker and Mrs. D. Berry for their expert assistance in the preparation of this manuscript. This work was supported by Grants 5 R01 CA 24441, 5 R01 CA 14113, and GM 07309 from the National Institutes of Health.

1. Nathan, C. F., Silverstein, S. C., Brukner, L. H. & Cohn, Z. A. (1979) *J. Exp. Med.* **149,** 100–113.
2. Currie, G. A. (1978) *Nature (London)* **273,** 758–759.
3. Adams, D. O., Kao, K.-J., Farb, R. & Pizzo, S. V. (1980) *J. Immunol.* **124,** 293–300.
4. Ferluga, J., Schorlemmer, H. U., Baptista, L. C. & Allison, A. C. (1978) *Clin. Immunol.* **31,** 512–517.
5. Zacharchuk, C., Drysdale, B.-E., Shin, M. & Shin, H. S. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 1343 (abstr.).
6. Männel, D. N., Falk, W. & Meltzer, M. S. (1981) *Infect. Immun.* **33,** 156–164.
7. Sorrell, T. C., Lehrer, R. I. & Cline, M. J. (1978) *J. Immunol.* **120,** 347–352.
8. Fishman, M. (1980) *Cell. Immunol.* **55,** 174–184.
9. Goodman, M. G., Weigle, W. O. & Hugli, T. E. (1980) *Nature (London)* **283,** 78–80.
10. Drysdale, B.-E., Zacharchuk, C. M. & Shin, H. S. (1983) *J. Immunol.* **131,** 1–6.
11. Rosenau, W. & Tsoukos, C. D. (1976) *Am. J. Pathol.* **84,** 580–596.
12. Drysdale, B.-E. & Shin, H. S. (1981) *J. Immunol.* **127,** 760–765.
13. Okamoto, M. & Mayer, M. M. (1978) *J. Immunol.* **120,** 272–278.
14. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
15. Ruff, M. R. & Gifford, G. E. (1980) *J. Immunol.* **125,** 1671–1677.
16. Gately, M. K., Gately, C. L., Henney, C. S. & Mayer, M. M. (1975) *J. Immunol.* **115,** 817–826.
17. Gately, M. K. & Mayer, M. M. (1974) *J. Immunol.* **112,** 168–177.
18. Ross, M. W., Tiangco, G. J., Horn, P., Hiserodt, J. C. & Granger, G. A. (1979) *J. Immunol.* **123,** 325–331.
19. Sawada, J.-I., Shioiri-Nakano, K. & Osawa, T. (1975) *Transplantation* **19,** 335–342.
20. Kull, F. C., Jr., & Cuatrecasas, P. (1981) *J. Immunol.* **126,** 1279–1283.
21. Männel, D. A., Meltzer, M. S. & Mergenhagen, S. E. (1980) *Infect. Immun.* **28,** 204–211.
22. Matthews, N. & Watkins, J. F. (1978) *Br. J. Cancer* **38,** 302–309.
23. Heise, E. R. & Weiser, R. S. (1969) *J. Immunol.* **103,** 570–576.
24. Kramer, J. J. & Granger, G. A. (1972) *Cell. Immunol.* **3,** 88–100.
25. Reed, W. P. & Lucas, Z. J. (1975) *J. Immunol.* **115,** 395–404.
26. Matthews, N. (1978) *Br. J. Cancer* **38,** 310–315.
27. Männel, D. N., Moore, R. N. & Mergenhagen, S. E. (1980) *Infect. Immun.* **30,** 523–530.
28. Kull, F. C., Jr., & Cuatrecasas, P. (1981) *Cancer Res.* **41,** 4885–4890.
29. Gately, M. K., Mayer, M. M. & Henney, C. S. (1976) *Cell. Immunol.* **27,** 82–93.
30. Walker, S. M. & Lucas, Z. J. (1973) *Transplant. Proc.* **5,** 137–140.
31. Hiserodt, J. C. & Granger, G. A. (1977) *J. Immunol.* **119,** 374–380.
32. Ware, C. F. & Granger, G. A. (1981) *J. Immunol.* **126,** 1919–1926.
33. Sawada, J. I. & Osawa, T. (1978) *Transplantation* **26,** 319–324.
34. Leopardi, E. & Rosenau, W. (1982) *Cell. Immunol.* **70,** 148–159.
35. Matthews, N. (1983) *Immunology* **48,** 321–327.