Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8417–8421, November 1989
Cell Biology

Tumor necrosis factor induces phosphorylation of a 28-kDa mRNA cap-binding protein in human cervical carcinoma cells

(monokine/antitumor factor/initiation factor/cachexia/cancer therapy)

MICHAEL W. MARINO*, LAWRENCE M. PFEFFER†, PETER T. GUIDON, JR.*, AND DAVID B. DONNER*‡

*The Memorial Sloan–Kettering Cancer Center and the Sloan–Kettering Division of the Cornell University Graduate School of Medical Sciences, 1275 York Avenue, New York, NY 10021; and †The Rockefeller University, 1230 York Avenue, New York, NY 10021

Communicated by Lloyd J. Old, July 24, 1989 (received for review May 11, 1989)

ABSTRACT **Tumor necrosis factor α (TNF-α) stimulated the phosphorylation of a 28-kDa protein (p28) in the ME-180 line of human cervical carcinoma cells. The effect of TNF-α on the phosphorylation state of p28 was rapid (4-fold increase within 15 min) and persistent, remaining above the basal level for at least 2 hr. The specific binding of ^{125}I-labeled TNF-α to cell-surface binding sites, the stimulation of p28 phosphorylation by TNF-α, and the inhibition of cell proliferation by TNF-α occurred with nearly identical dose–response relationships. Two-dimensional SDS/PAGE resolved p28 into two isoforms having pI values of 6.2 and 6.1. A phosphorylated cap-binding protein was substantially enriched from lysates of control or TNF-α-treated ME-180 cells by affinity chromatography with 7-methylguanosine 5′-triphosphate–Sepharose. The phosphoprotein recovered from this procedure was the substrate for TNF-α-promoted phosphorylation, p28. Thus, TNF-α stimulates the phosphorylation of this mRNA cap-binding protein, which may be involved in the transduction of TNF-α–receptor binding into cellular responses.**

Tumor necrosis factor α (TNF-α), or cachectin, is produced by macrophages in response to infections and cancer (1, 2). TNF-α alters cellular growth, differentiation, and metabolism (3–7). Of special interest are its cytostatic and cytotoxic actions on tumor cell lines in culture (1, 3–5, 8) and its ability to cause hemorrhagic necrosis and regression of tumors in experimental animals (1, 8).

The first step in TNF-α action is binding to specific, high-affinity cell-surface receptors, which is necessary, but not sufficient, to elicit cellular responses (5, 9). The events that occur subsequent to TNF-α–receptor binding and that mediate the cellular responses are not clearly understood. The actions of many hormones, growth factors, and other cytokines involve the selective phosphorylation–dephosphorylation of specific enzymes or other target proteins (10, 11). We, therefore, investigated the effects of TNF-α on the phosphorylation of proteins in the well-characterized human ME-180 cervical carcinoma cell line, to which TNF-α is cytotoxic (5, 12, 13).

We report here that TNF-α increases the phosphorylation of a 28-kDa protein (p28) in ME-180 cells. We have partially purified p28 by affinity chromatography on 7-methylguanosine 5′-triphosphate (m^{7}GTP)–Sepharose, which identifies p28 as a cap-binding protein. The demonstration that TNF-α acts upon a component of the eukaryotic initiation factor complex, an mRNA cap-binding protein, is important in identifying a possible pathway through which the TNF-α–receptor interaction may be transduced into cellular responses.

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.

MATERIALS AND METHODS

Recombinant human TNF-α (5×10^7 units/mg) was provided to L. Old by M. Palladino (Genentech). Other materials were as follows: carrier-free ^{32}P (New England Nuclear); carrier-free ^{125}I (Amersham); Nonidet P-40, soybean trypsin inhibitor, and bacitracin (Sigma); gentamicin (Schering–Plough); micrococcal nuclease, deoxyribonuclease 1, and ribonuclease A (Worthington); Ampholine carrier ampholytes (LKB); and molecular weight and pI standards (Bio-Rad).

Cell Culture. The human cervical carcinoma ME-180 cell line obtained from L. Old of the Memorial Sloan–Kettering Cancer Center was grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, nonessential amino acids, L-glutamine, penicillin (500 units/ml), and streptomycin (100 units/ml).

Labeling of ME-180 Cells with ^{32}P. ME-180 cells were washed twice with phosphate-free Krebs–Ringer bicarbonate medium supplemented with bovine serum albumin (10 mg/ml), bacitracin (0.8 mg/ml), and gentamicin (50 μg/ml) (buffer A). Cells were equilibrated in buffer A containing 15 mM glucose and carrier-free [^{32}P](ortho)phosphate (0.05–0.33 mCi/ml; 1 Ci = 37 GBq) for 45 min at 37°C and exposed to TNF-α (10^{-13}–10^{-7} M) as indicated.

Gel Electrophoresis and Autoradiography. One- and two-dimensional gel electrophoresis were conducted according to established procedures. After electrophoresis, gels were stained, destained, dried, and exposed to Kodak XAR-5 film for 3–7 days at −80°C in cassettes that contained DuPont Cronex Lightning Plus enhancing screens. Standards used to estimate the molecular weights of phosphoproteins were myosin (M_r, 200,000), β-galactosidase (M_r, 116,250), phosphorylase B (M_r, 92,500), bovine serum albumin (M_r, 66,200), ovalbumin (M_r, 45,000), carbonic anhydrase (M_r, 31,000), and soybean trypsin inhibitor (M_r, 21,000).

Preparation and Characterization of ^{125}I-Labeled TNF-α. ^{125}I was coupled to TNF-α in an Iodo-Gen-catalyzed reaction (9). ^{125}I-labeled TNF-α (specific activity, ≈60 Ci/g) was separated from free ^{125}I by chromatography on Sephadex G-75. We have found that ^{125}I-labeled TNF-α retains >80% of the antiproliferative activity of unlabeled TNF-α on ME-180 cells.

Binding of ^{125}I-Labeled TNF-α to Cells. Subconfluent ME-180 cells in 6-well plates were incubated for 4 hr at 15°C in Hanks' balanced salt solution (HBSS)/1% bovine serum albumin/1 mM phenylmethylsulfonyl fluoride/10^{-11}–10^{-9} M ^{125}I-labeled TNF-α. Cells were washed twice with ice-cold HBSS, solubilized into 1 M NaOH/1% SDS, and assayed for specific binding, which is defined as the difference in radio-

Abbreviations: TNF-α, tumor necrosis factor α; m^{7}GTP, 7-methylguanosine 5′-triphosphate.

‡To whom reprint requests should be addressed at: Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

active uptake without or with 200-fold excess of unlabeled TNF-α.

Inhibition of Cell Proliferation. ME-180 cells (1×10^5 cells per 25-cm^2 flask) were incubated with various concentrations (1×10^{-12}–3×10^{-8} M) of TNF-α for 3 days. Monolayers were washed twice with calcium- and magnesium-free phosphate-buffered saline and trypsinized. Cell counts were performed with a Coulter counter. Cell viability was verified by trypan blue exclusion using a hemocytometer. Data points are the average of duplicate determinations, and results from at least two experiments are illustrated ± SD. Results are expressed as 100 × (1 − cell number in TNF-α-treated flasks/cell number in control flasks).

Affinity Chromatography of Phosphoproteins on m^{7}GTP–Sepharose. Phosphoproteins from control or TNF-α-treated cells were purified by affinity chromatography on m^{7}GTP–Sepharose according to the procedure of Duncan *et al.* (14) with modifications. ^{32}P-labeled cells (2×10^6 cells/100-mm^2 plate) were solubilized into 1 ml of KHEGM buffer [100 mM KCl/20 mM Hepes, pH 7.5/0.2 mM EDTA/10% (vol/vol) glycerol/7 mM 2-mercaptoethanol/2 mM sodium vanadate/0.5% Triton X-100]. Nuclei and cellular debris were pelleted by centrifugation (10,000 × g × 5 min). The recovered supernatant (S10) was incubated for 1 hr at 4°C with 300 μl of a slurry of m^{7}GTP–Sepharose in a 1.5-ml centrifuge tube and then centrifuged (12,000 × g × 10 sec at 4°C). The supernatant was aspirated, and the pelleted m^{7}GTP–Sepharose was washed repeatedly with KHEGM (1 ml) until the supernatants were essentially free of phosphoproteins (five to eight washes). After nonspecifically adsorbed proteins had been dissociated from the m^{7}GTP–Sepharose by incubation with 1 mM GTP (1 hr at 4°C), specifically bound proteins were eluted by incubation with 75 μM m^{7}GTP (1 ml, 1 hr at 4°C). At each step, phosphoproteins in the eluate were precipitated with 4 vol of acetone at −20°C and analyzed by one-dimensional SDS/PAGE and autoradiography.

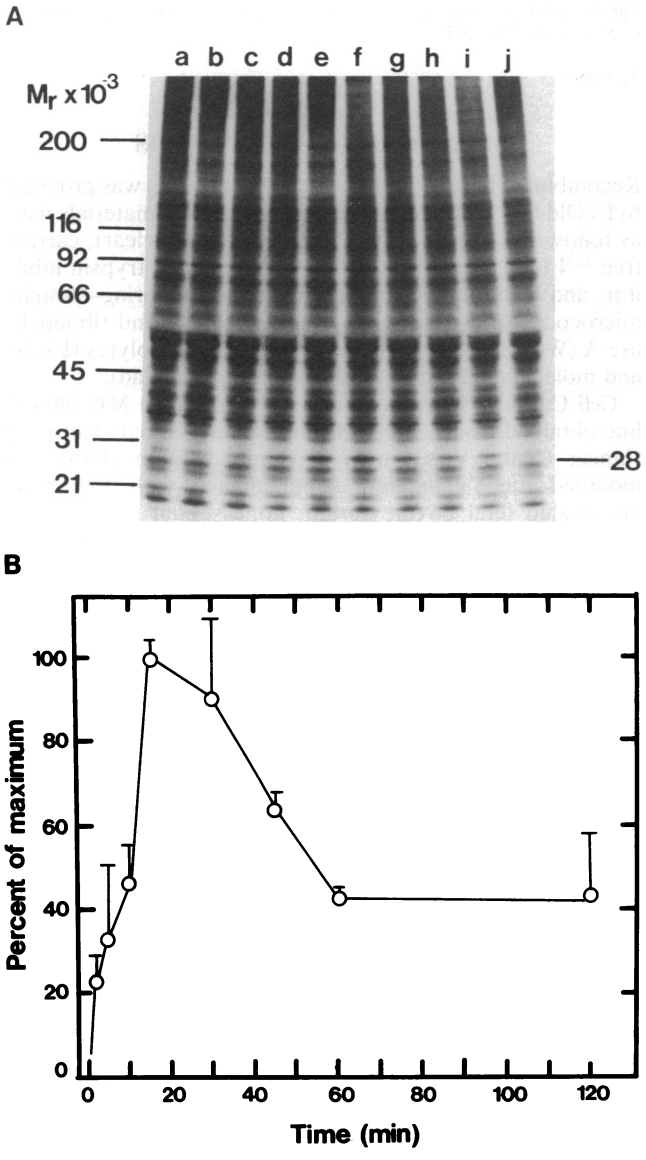

FIG. 1. TNF-α-induced phosphorylation in ME-180 cervical carcinoma cells. (*A*) After equilibration with ^{32}P, cells were incubated with TNF-α (1 nM) at 37°C for 0 time control (lanes a and j); 1 min (lane b), 5 min (lane c), 10 min (lane d), 15 min (lane e), 30 min (lane f), 45 min (lane g), 60 min (lane h), and 120 min (lane i). Reactions were terminated by aspirating the medium and freezing the monolayers on dry ice/isopropanol. Frozen monolayers were thawed and solubilized into 3% SDS/10% (vol/vol) glycerol/10 mM P_i/2% 2-mercaptoethanol/0.01% bromophenol. Phosphoproteins were fractionated on 1.5-mm linear gradient gels of 5–15% acrylamide (acrylamide/bisacrylamide ratio of 37.5:1) by using the discontinuous buffer system described by Laemmli (15). Phosphoproteins were detected after SDS/PAGE and autoradiography. (*B*) Each lane in the autoradiogram shown in *A* and in a replicate experiment was scanned with a Helena Laboratories Quick-Scan Jr. densitometer to quantitate protein phosphorylation. The fraction of maximal phosphorylation of the M_r 28-kDa phosphoprotein is plotted against time. Data points are the average of duplicate experiments ± SD.

RESULTS

Characterization of TNF-α-Induced Phosphorylation in ME-180 Cells. Phosphoproteins from control and TNF-α-treated ME-180 cells were characterized by SDS/PAGE and autoradiography. TNF-α enhanced the phosphorylation of a 28-kDa protein within minutes (Fig. 1*A*). Maximal stimulation was induced after 15 min (Fig. 1*B*). Subsequently, phosphorylation of p28 diminished but remained above the basal level 2 hr after TNF-α addition.

Extent of the TNF-α-induced phosphorylation of p28 depended on the TNF-α concentration to which cells were exposed (Fig. 2). Each lane in the autoradiograph of Fig. 2 and

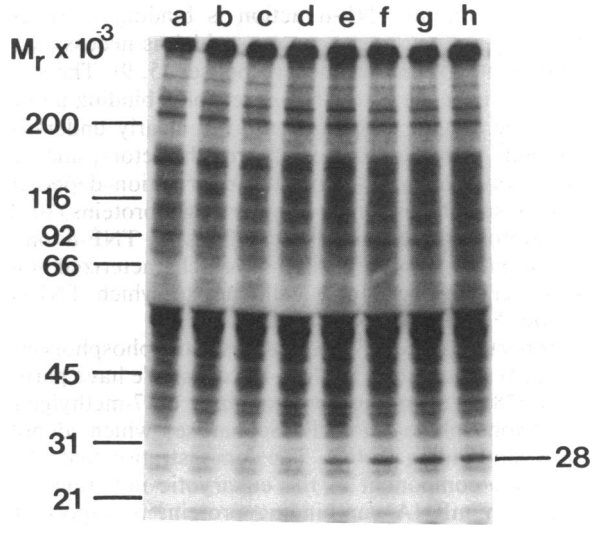

FIG. 2. Dose–response of TNF-α-induced phosphorylation. Cells were equilibrated with ^{32}P and then incubated at 37°C for 15 min with no TNF-α (control) (lane a), 0.1 pM TNF-α (lane b), 1 pM TNF-α (lane c), 0.01 nM TNF-α (lane d), 0.1 nM TNF-α (lane e), 1 nM TNF-α (lane f), 10 nM TNF-α (lane g), and 100 nM TNF-α (lane h). Phosphoproteins were fractionated by SDS/PAGE as described in the legend to Fig. 1.

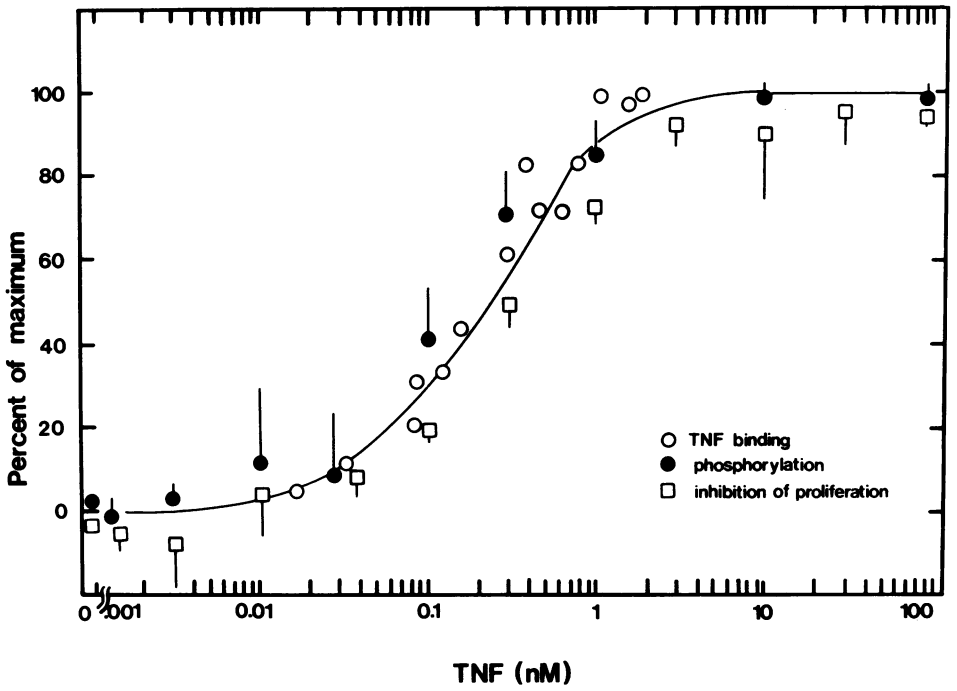

FIG. 3. Relationship between TNF-α concentration and receptor occupancy, phosphorylation, and inhibition of cell proliferation. The fraction of maximal binding, maximal phosphorylation, and maximal inhibition of cell proliferation is plotted against TNF-α concentration. Data points are the average of duplicate experiments ± SD.

in five replicate experiments was scanned with a densitometer to quantitate protein phosphorylation. Maximal stimulation in ME-180 cells (4.4 ± 0.8, n = 6) was elicited by 15-min incubation with 1 nM TNF-α (Fig. 2). The effect was half-maximal with 0.1 nM TNF-α. Interleukin 1 and interferon γ did not increase the phosphorylation of p28 (data not shown), indicating that the response of ME-180 cells to TNF-α was specific.

We next defined the relationships between the TNF-α concentration and (*i*) TNF-α binding to cell surface receptors; (*ii*) phosphorylation of p28; and (*iii*) inhibition of ME-180 cell proliferation. The binding of ^{125}I-labeled TNF-α to ME-180 cells was saturated by 1 nM ^{125}I-labeled TNF-α. Transformation of the binding data according to the method of Scatchard (16) yielded a linear plot (data not shown), indicating that ME-180 cells contain a single class of binding sites (2900 receptors per cell; K_d = 0.13 nM). Maximal inhibition of cell growth (77 ± 14% inhibition) was generally seen after incubation of ME-180 cells with 1–3 nM TNF-α (Fig. 3). Half-maximal inhibition was seen after treatment with 0.1–0.3 nM TNF-α. Fig. 3 illustrates that the extent of receptor occupancy relates to the inhibition of cell proliferation and to the phosphorylation of p28.

Two-Dimensional Gel Electrophoresis of Phosphoproteins from Control and TNF-α-Treated Cells. Fractionation of phosphoproteins from control (Fig. 4*A*) or TNF-α-treated (Fig. 4*B*) ME-180 cells by two-dimensional gel electrophoresis confirmed that p28 was the predominant substrate for TNF-α-induced phosphorylation. Two variants of p28 (pI 6.2 and 6.1) were resolved by isoelectric focusing in the first

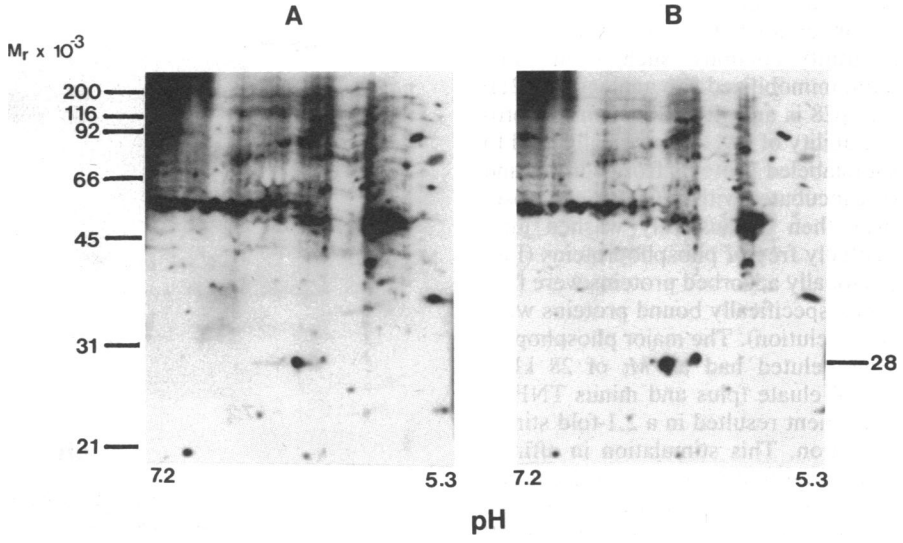

FIG. 4. Two-dimensional gel electrophoresis of phosphoproteins. Control (*A*) or TNF-α-treated (*B*) ME-180 cells were solubilized and fractionated by two-dimensional gel electrophoresis according to O'Farrell (17) with modifications (18). The approximate pH range of the first dimension was 7.2 (left)–5.3 (right).

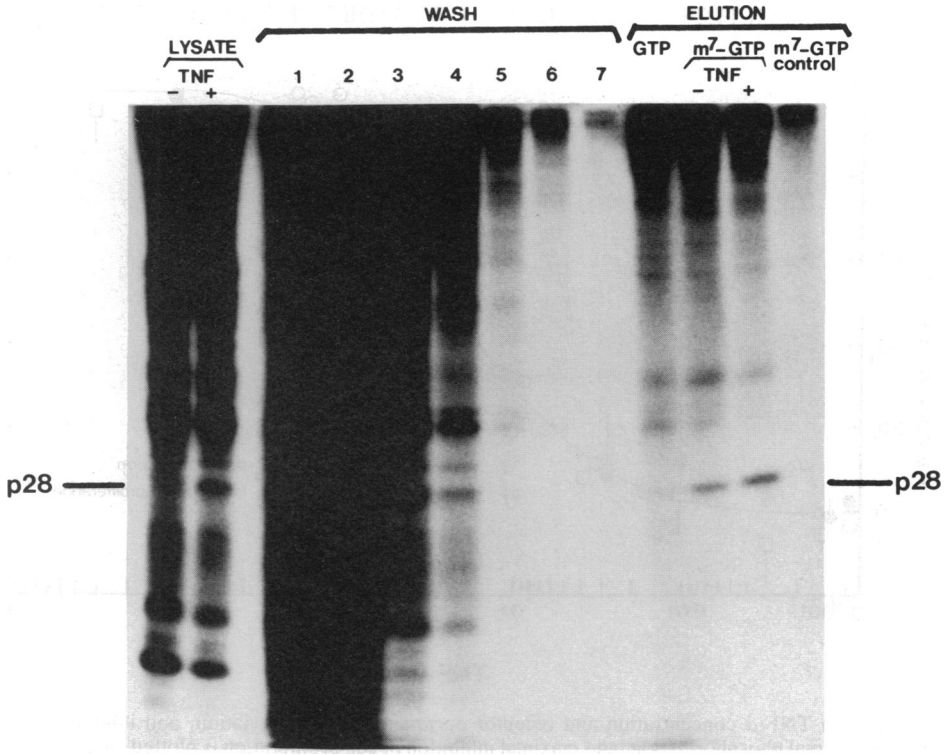

FIG. 5. Binding and elution of p28 from m^{7}GTP–Sepharose. Diluted lysates of control or TNF-α-treated cells were fractionated by SDS/PAGE to illustrate the effect of TNF-α on p28 phosphorylation (LYSATE − or + TNF-α). Undiluted lysates were incubated with m^{7}GTP–Sepharose, which was sequentially washed with KHEGM buffer until supernatants were essentially free of phosphoproteins (WASH 1–7). (Because nonadsorbed phosphoproteins were the same for control or TNF-α-treated cells, wash supernatants of only the latter are shown.) Elution from the affinity matrix was accomplished by incubation with GTP and then with m^{7}GTP, as described (ELUTION). Preincubation of lysates with m^{7}GTP (m^7-GTP control) tested the specificity of adsorption to the affinity matrix by competition for binding. Phosphoproteins were fractionated by SDS/PAGE (Fig. 1, legend) on a linear gel of 10% acrylamide.

dimension; these may be mono- and diphosphorylated isoforms of p28.

Identification of p28 as an mRNA Cap-Binding Protein. The M_r and pI of p28, the substrate for TNF-α action seen here, are similar to those of eIF-4E (14, 19), an mRNA cap-binding protein the phosphorylation state of which has been found altered during heat shock in HeLa cells (14). *In vivo*, such cap-binding proteins bind specifically to the 7-methylguanosine cap found on the 5′ end of eukaryotic mRNAs. *In vitro*, cap-binding proteins are defined on the basis of (*i*) crosslinking to the 5′ cap of mRNAs or (*ii*) binding and specific elution from affinity columns, such as m^{7}GTP–Sepharose, which contain immobilized cap analogs (20, 21).

To determine whether p28 is an mRNA cap-binding protein, we investigated the ability of p28 to specifically bind to m^{7}GTP–Sepharose. Radiolabeled proteins from control and TNF-α-treated cells were incubated with m^{7}GTP–Sepharose. The affinity matrix was then exhaustively washed until supernatants were essentially free of phosphoproteins (Fig. 5, washes 1–7). Nonspecifically adsorbed proteins were first eluted with GTP, and then specifically bound proteins were eluted with m^{7}GTP (Fig. 5, elution). The major phosphoprotein that was specifically eluted had an M_r of 28 kDa. Comparison of the m^{7}GTP eluate (plus and minus TNF-α) indicates that TNF-α treatment resulted in a 2.1-fold stimulation of p28 phosphorylation. This stimulation in affinity eluates as compared with the 4.4-fold stimulation in crude cell lysates may reflect the action of phosphatases during the affinity purification. Preincubation of lysates with m^{7}GTP inhibited p28 binding to m^{7}GTP–Sepharose, confirming the specificity of the molecular interaction underlying the affinity chromatography procedure (Fig. 5, m^{7}GTP elution control).

Two-Dimensional Gel Electrophoresis of Phosphoproteins Recovered After Affinity Chromatography. Phosphoproteins purified from lysates of TNF-α-treated cells by elution from m^{7}GTP–Sepharose with m^{7}GTP were fractionated by two-

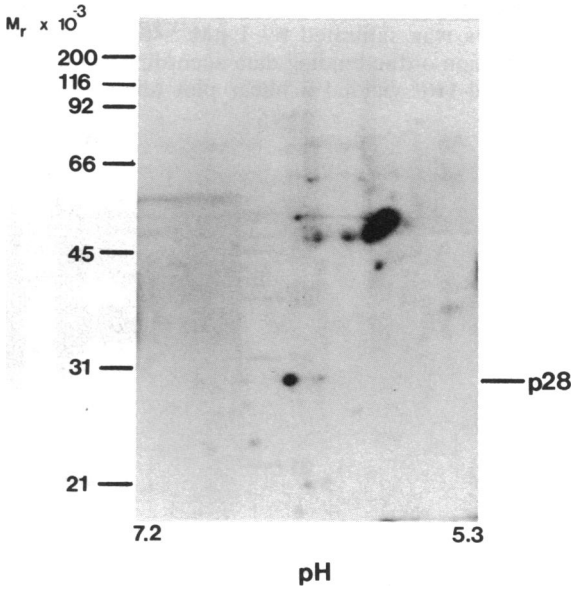

FIG. 6. Two-dimensional gel electrophoresis of phosphoproteins eluted from m^{7}GTP–Sepharose. ^{32}P-equilibrated ME-180 cells were incubated with TNF-α (1 nM, 15 min at 37°C), lysed, and then incubated with m^{7}GTP–Sepharose. Phosphoproteins eluted from the affinity matrix by m^{7}GTP were fractionated by two-dimensional SDS/PAGE.

dimensional SDS/PAGE (Fig. 6). This analysis confirms that p28, the substrate for TNF-α-induced phosphorylation, is the 28-kDa protein specifically eluted from m^{7}GTP–Sepharose. Furthermore, comparison of Figs. 4 and 6 shows that p28 was substantially enriched by affinity chromatography.

DISCUSSION

Previous studies have shown that TNF-α stimulates the phosphorylation of a 26-kDa protein in U937 cells (22) and a 27-kDa protein in human dermal fibroblasts (23), HeLa cells, and ME-180 cells (24). However, TNF-α-stimulated phosphorylation of a 26- to 28-kDa phosphoprotein is not detected in murine L strain cells sensitive to TNF-α (24).

The present study characterizes TNF-α-promoted phosphorylation in the ME-180 human cervical carcinoma cell line and identifies a substrate upon which TNF-α acts as a cap-binding protein. One- and two-dimensional gel electrophoresis of proteins solubilized from control or TNF-α-treated ME-180 cells indicate that a 28-kDa protein is the predominant substrate for TNF-α-stimulated phosphorylation. Phosphorylation of the 28-kDa protein occurs within minutes and persists even after 2 hr. Increased phosphorylation depends on the concentration of TNF-α to which cells are exposed, reaching a 4-fold increase after 15-min incubation with 1 nM TNF-α. The extent of occupancy of TNF-α receptors is directly related to TNF-α-stimulated phosphorylation of p28 and the inhibitory action of TNF-α on cell proliferation (Fig. 3).

Affinity chromatography using the specific interaction between m^{7}GTP–Sepharose and cap-binding proteins has allowed purification of such proteins from crude cytosolic cell fractions (14, 19–21). We have partially purified a phosphorylated cap-binding protein from lysates of control and TNF-α-treated ME-180 cells by affinity chromatography. TNF-α stimulated the phosphorylation of the 28-kDa protein, which was enriched by affinity chromatography. The specificity of adsorption to and elution from m^{7}GTP–Sepharose identifies p28 as an mRNA cap-binding protein. The M_r and the pI of the isoforms of p28 resemble those of another cap-binding protein of the initiation factor complex eIF-4E (14, 19). However, definitive identification of the particular cap-binding protein upon which TNF-α acts must await complete purification of this nonabundant cellular protein.

Initiation factor complexes are necessary for the efficient translation of capped mRNA (20). Cap-binding proteins in initiation factor complexes mediate the interaction of mRNAs with polyribosomes (for review, see ref. 20). The effect of phosphorylation upon the activities of cap-binding proteins is poorly understood—possibly due to limitations of current *in vitro* assay systems (14). However, phosphorylation of cap-binding proteins is decreased by heat shock (14) and at metaphase (25), which correlates with inhibition of protein synthesis. Thus, mRNA cap-binding proteins may be involved in the translational control of cellular protein synthesis.

TNF-α is thought to be one of the host-derived factors that leads to the mobilization of energy stores during the acute-phase response (26) to tissue injury, infection, or chronic disease states, including cancer (27, 28). TNF-α is also important in the immune response and hematopoiesis, as it stimulates the synthesis of other cytokines, such as interleukin 1 (29), interleukin 6 (30), and granulocyte–macrophage colony-stimulating factor (31). Our observations suggest that transduction of TNF-α–receptor binding into various cellular responses may, in part, be effected by TNF-α action on an mRNA cap-binding protein, which may then be important in regulating protein synthesis.

We thank Drs. Lloyd Old, Igor Tamm, Karen Manchester, Hyung-Mee Han, and Warren Heston for critical evaluation of this manuscript. This work was supported by grants from the National Institutes of Health (AM30788, GM36716) and the National Cancer Institute (CA44747) and by a core grant from the National Cancer Institute (CA08748) to the Memorial Sloan–Kettering Cancer Center. D.B.D. is grateful for a special grant from the New York City Division of the American Cancer Society (BC620). L.M.P. is a Scholar of the Leukemia Society of America.

1. Old, L. J. (1985) *Science* **230,** 630–632.
2. Beutler, B. & Cerami, A. (1986) *Nature (London)* **320,** 584–588.
3. Helson, L., Green, S., Carswell, E. A. & Old, L. J. (1975) *Nature (London)* **258,** 731–732.
4. Williamson, B. D., Carswell, E. A., Rubin, B. Y., Prendergast, J. S. & Old, L. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5937–5941.
5. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. S., Palladino, M. A., Jr., & Shepard, H. M. (1985) *Science* **230,** 943–945.
6. Torti, F. M., Dieckmann, B., Beutler, B., Cerami, A. & Ringold, G. M. (1985) *Science* **229,** 867–869.
7. Vilcek, J., Palombella, V. J., Henriksen-DeStefano, D., Swenson, C., Feinman, R., Hirai, M. & Tsujimoto, M. (1986) *J. Exp. Med.* **163,** 632–643.
8. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
9. Kull, F. C., Jr., Jacobs, S. & Cuatrecasas, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5756–5760.
10. Rosen, O. M. & Krebs, E. G. (1981) *Cold Spring Harbor Conf. Cell Proliferation* **8,** 715–808.
11. Greengard, P. (1978) *Science* **199,** 146–152.
12. Aggarwal, B. B. & Eessalu, T. E. (1987) *J. Biol. Chem.* **262,** 10000–10007.
13. Aggarwal, B. B., Traquina, P. R. & Eessalu, T. E. (1986) *J. Biol. Chem.* **261,** 13652–13656.
14. Duncan, R., Milburn, S. C. & Hershey, J. W. B. (1987) *J. Biol. Chem.* **262,** 380–388.
15. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
16. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51,** 660–675.
17. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
18. Yamada, K., Lipson, K. E., Marino, M. W. & Donner, D. B. (1987) *Biochemistry* **26,** 715–721.
19. Buckley, B. & Ehrenfeld, E. (1986) *Virology* **152,** 497–501.
20. Sonenberg, N. (1988) *Prog. Nucleic Acid Res. Mol. Biol.* **35,** 173–207.
21. Webb, N. R., Chari, R. V. J., DePillis, G., Kozarich, J. W. & Rhoads, R. E. (1984) *Biochemistry* **23,** 177–181.
22. Schutze, S., Scheurich, P., Pfizenmaier, K. & Kronke, M. (1989) *J. Biol. Chem.* **264,** 3562–3567.
23. Kaur, P. & Saklatvala, J. (1988) *FEBS Lett.* **241,** 6–10.
24. Hepburn, A., Demolle, D., Boeynaems, J.-M., Fiers, W. & Dumont, J. E. (1988) *FEBS Lett.* **227,** 175–178.
25. Bonneau, A.-M. & Sonenberg, N. (1987) *J. Biol. Chem.* **262,** 11134–11139.
26. Gordon, A. H. & Koj, A. (1985) in *The Acute Phase Response to Injury and Infection* (Elsevier, Amsterdam).
27. Darlington, G. J., Wilson, D. R. & Lachman, L. B. (1986) *J. Cell Biol.* **103,** 787–793.
28. Mackiewicz, A., Ganapathi, M. K., Schultz, D., Samols, D., Reese, J. & Kushner, I. (1988) *Biochem. J.* **253,** 851–857.
29. Bachwich, P. R., Chensue, S. W., Larrick, J. W. & Kunkel, S. W. (1986) *Biochem. Biophys. Res. Commun.* **136,** 94–101.
30. Kohase, M., Henriksen-DeStefano, D., May, L. T., Vilcek, J. & Sehgal, P. B. (1986) *Cell* **45,** 659–666.
31. Munker, R., Gasson, J., Ogawa, M. & Koeffler, H. P. (1986) *Nature (London)* **323,** 79–82.