Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5603–5607, August 1990
Biochemistry

Tumor necrosis factor α modifies agonist-dependent responses in human neutrophils by inducing the synthesis and myristoylation of a specific protein kinase C substrate

(*N*-myristoylglycine/signal transduction)

MARCUS THELEN, ANTONY ROSEN, ANGUS C. NAIRN, AND ALAN ADEREM

The Rockefeller University, 1230 York Avenue, New York, NY 10021

Communicated by Zanvil A. Cohn, May 18, 1990

ABSTRACT **Tumor necrosis factor α (TNF-α) and bacterial lipopolysaccharide (LPS) induce the synthesis and cotranslational myristoylation of an 82-kDa specific protein kinase C substrate in human neutrophils. The myristic acid is covalently bound via a hydroxylamine-resistant amide linkage to the N-terminal glycine of the protein. The isoelectric point of the protein is at pH 4.6. The protein is rapidly phosphorylated when neutrophils are stimulated with chemotactic agonists or with phorbol 12-myristate 13-acetate, an activator of protein kinase C, and displays two characteristic phosphopeptides in one- and two-dimensional separation systems. Identical phosphopeptides were detected when the 82-kDa protein was phosphorylated *in vitro* with purified kinase C. The 82-kDa protein was immunoprecipitated by a polyclonal antiserum raised against the 87-kDa specific protein kinase C substrate from bovine brain. From these biochemical and immunological criteria it is concluded that the 82-kDa protein is the human neutrophil homolog of MARCKS, the *m*yristoylated, *a*lanine-*r*ich *C k*inase *s*ubstrate previously described in bovine and rat brain and in murine fibroblasts and macrophages. TNF-α and LPS prime human neutrophils for potentiated protein kinase C-dependent responses such as the respiratory burst and exocytosis. Consistent with this, these mediators do not induce the phosphorylation of MARCKS but prime the neutrophils for enhanced phosphorylation of this protein when the cells subsequently encounter activators of protein kinase C. This increase in MARCKS phosphorylation can be explained by the elevated levels of the protein observed in TNF-α- or LPS-treated neutrophils. Indeed, MARCKS constitutes 90% of all proteins synthesized in response to TNF-α or LPS. These data strongly suggest that MARCKS acts as a critical effector molecule in the transduction pathway of these important inflammatory mediators.**

Neutrophils regulate host defense, in part, by secreting a number of inflammatory mediators in response to agonists such as chemotactic peptides and bioactive lipids. The signal-transduction pathways mediating these events often involve protein kinase C (1–3). In addition, a number of cytokines or bacterial lipopolysaccharide (LPS) can prime neutrophils for potentiated agonist-dependent responses (4, 5). Among the cytokines that have been shown to prime neutrophils are tumor necrosis factor α (TNF-α) (6, 7), granulocyte/macrophage-colony-stimulating factor (GM-CSF) (8, 9), and interferon γ (IFN-γ) (10). The biochemical events that mediate cytokine- and LPS-dependent priming of neutrophils are poorly understood.

LPS also primes murine macrophages for potentiated protein kinase C-dependent responses such as the release of arachidonic acid metabolites (11). This priming has been shown to correlate with the expression of a 68-kDa myristoylated specific protein kinase C substrate (12, 13). This protein is similar to the 87-kDa and 80-kDa specific protein kinase C substrates described in bovine and rat brain and in murine fibroblasts (14–18). The cDNAs encoding the bovine brain, chicken brain, and murine macrophage proteins have been cloned and they encode acidic, alanine-rich proteins with molecular masses of 32 kDa, 28 kDa, and 29 kDa, respectively (refs. 19 and 20; J. Seykora, A.A., and J. Ravetch, unpublished data). Because of the discrepancy between the actual and apparent molecular masses, it was proposed that the protein be referred to by the acronym MARCKS (for *m*yristoylated, *a*lanine-*r*ich *C k*inase *s*ubstrate; ref. 19).

We report here that TNF-α and LPS, factors that prime human neutrophils for enhanced protein kinase C-dependent signal transduction, also induce the synthesis and myristoylation of an 82-kDa MARCKS protein. In addition, the 82-kDa MARCKS is the major protein synthesized *de novo* in TNF-α- and LPS-treated neutrophils.

MATERIALS AND METHODS

Mediators. Smooth LPS from *Salmonella abortus equi* was a gift from C. Galanos (Max Planck Institute, Freiburg, F.R.G.); recombinant human TNF-α (4×10^7 units/ml) was obtained from A. Cerami (Rockefeller University); and recombinant human IFN-γ (Ro 41-3503, 10^8 units/mg) was provided by G. Garotta (Hoffmann–La Roche, Basel). Recombinant human GM-CSF (2.5×10^5 units/ml) was purchased from Amgen Biologicals. All cytokines contained <0.1 ng of LPS per ml as determined by the *Limulus* assay system (Whittaker Bioproducts). Recombinant human GM-CSF purchased from an alternative vendor induced the synthesis and myristoylation of MARCKS but was found to be contaminated with low levels of LPS.

Neutrophils. Neutrophils were prepared from fresh donor blood by dextran sedimentation and were purified over Ficoll/Hypaque (21). Cells were resuspended at 3×10^6 cells per ml of RPMI-1640 (GIBCO), added (3.9×10^5 cells per cm^2) to tissue culture dishes (Corning) pretreated with 25% fetal bovine serum in RPMI-1640 (22), and incubated for 30 min at 37°C prior to labeling or stimulation.

Cell Labeling. *[^{3}H]Myristic acid.* [^{3}H]Myristic acid (40 Ci/mmol, New England Nuclear; 1 Ci = 37 GBq) was dried under a stream of nitrogen and sonicated into a small volume of RPMI-1640 supplemented with ovalbumin at 100 μg/ml. Forty microcuries of [^{3}H]myristic acid (1 nmol) was added

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: GM-CSF, granulocyte/macrophage-colony-stimulating factor; IFN-γ, interferon γ; LPS, lipopolysaccharide; MARCKS, myristoylated, alanine-rich C kinase substrate; PMA, phorbol 12-myristate 13-acetate; TCA, trichloroacetic acid; TNF-α, tumor necrosis factor α.

per ml of medium and the cells were incubated for 4 hr (if not stated otherwise) in the absence or presence of cytokines or LPS. At the end of the incubation, >95% of the cells excluded trypan blue. Incubations were terminated by placing the cell culture dishes on ice and adding 2 mM EGTA. Neutrophils were scraped off with a rubber policeman, the dishes were washed with phosphate-buffered saline containing 1 mM EDTA, and the cells were pelleted at 4°C in a microcentrifuge for 5 min.

[^{3}H]Lysine. Neutrophils were incubated in lysine-free RPMI-1640 supplemented with lysine (100 Ci/mmol, New England Nuclear) at 20 μCi/ml for 4 hr in the presence or absence of activator and, where indicated, cycloheximide (5 μg/ml). Cells were recovered as described above.

[^{32}P]Phosphate. Neutrophils were added to culture dishes as described above and were incubated in the presence or absence of TNF-α for 2.5 hr in phosphate-free RPMI-1640. Two millicuries of [^{32}P]orthophosphoric acid (9 Ci/μmol, New England Nuclear) was then added per ml of medium and the incubation continued for 90 min. The cells were stimulated with phorbol 12-myristate 13-acetate (PMA, 100 nM) for 2 min or f-Nle-Leu-Phe (1 μM) for 45 sec. Incubations were stopped with ice-cold trichloroacetic acid (TCA; final concentration, 10%) and the dishes were rapidly cooled on ice. After 20 min, precipitated material was recovered by centrifugation.

Immunoprecipitation. Cell pellets were resuspended in 0.5 ml of lysis buffer [10 mM Tris/15 mM EDTA/1% (wt/vol) Nonidet P-40/50 mM NaH_2PO_4/50 mM KF/10 mM sodium pyrophosphate, pH 7.4] containing the protease inhibitors leupeptin (1 mM), phenylmethylsulfonyl fluoride (1 mM), aprotinin (0.24 trypsin inhibitor unit), and diisopropyl fluorophosphate (3 mM). After removal of nuclei, cell extracts were precleared with 20% (vol/vol) protein A-Sepharose and the 82-kDa myristoylated protein was immunoprecipitated with a rabbit antiserum directed against bovine brain MARCKS (13).

Immunoprecipitation from TCA-precipitated proteins was performed as follows. TCA (final concentration, 10%) was added to the neutrophils and after 20 min on ice the lysates were transferred to microcentrifuge tubes. Precipitated material was washed twice with acetone and boiled in 40 μl of 1% $NaDodSO_4$/20 mM Tris, pH 8.0. The samples were then diluted 10-fold with 1% (wt/vol) Triton X-100/1% (wt/vol) sodium deoxycholate/150 mM NaCl/1 mM EDTA/20 mM Tris, pH 8.0, and the now-soluble 82-kDa protein was immunoprecipitated as described above.

***In Vitro* Phosphorylation and Phosphopeptide Analysis.** These were performed as described (15, 23). In brief, 7.5×10^6 cells were resuspended in lysis buffer containing 1% Nonidet P-40 and the postnuclear supernatant was phosphorylated with purified mouse brain protein kinase C (15, 23). One-dimensional phosphopeptide mapping of immunoprecipitated MARCKS was performed after limited proteolysis with *Staphylococcus aureus* V8 protease (Boehringer Mannheim) (13, 24). Two-dimensional thin-layer phosphopeptide mapping was performed as described (23). In brief, gel pieces containing ^{32}P-labeled MARCKS were digested with thermolysin (Boehringer Mannheim) and the released peptides were separated on cellulose thin-layer sheets (Eastman Kodak) by electrophoresis (first dimension) followed by chromatography (second dimension). Phosphopeptides were visualized by autoradiography.

Electrophoresis. Cell pellets were resuspended in ice-cold water and TCA was added to a final concentration of 10%. The precipitated protein was washed twice with acetone, air-dried, and resuspended in sample buffer prepared for isoelectric focusing (25) or $NaDodSO_4$/PAGE (26).

Analysis of the Fatty Acyl Group. Hydroxylamine sensitivity of ^{3}H-labeled proteins was determined as described (12). TCA-precipitated and acetone-washed cell pellets (7.5×10^6 cells) were incubated for 4 hr at room temperature with 1 ml of 1 M hydroxylamine (pH 10) or 1 ml of 1 M Tris·HCl (pH 7). Proteins were then precipitated with TCA and separated by $NaDodSO_4$/PAGE.

Identification of the protein-bound fatty acyl group was performed by analysis of immunoprecipitated 82-kDa protein (13, 27). In brief, [^{3}H]myristic acid-labeled MARCKS was eluted from immunoprecipitates with 0.1% trifluoroacetic acid and extracted three times with petroleum ether. The aqueous phase was lyophilized, resuspended in 20 mM glycine (pH 10.2), and incubated for 36 hr with proteinase K from *Tritirachium album* (Sigma). The pH was then adjusted to pH 7.5 with 500 mM potassium phosphate (27) and the incubation continued for 36 hr in the presence of Pronase E from *Streptomyces griseus* (Sigma). *N*-Myristoylglycine was extracted and identified by reverse-phase HPLC (27). For fatty acid analysis, the proteinase digest was extracted and the organic phase was subjected to acid methanolysis. Fatty acids and methyl esters were identified by reverse-phase HPLC (27).

RESULTS

TNF-α and LPS Induce the Myristoylation of an 82-kDa Protein. Incubation of neutrophils for 4 hr in the presence of [^{3}H]myristic acid resulted in the prominent incorporation of the label into three proteins (18, 38, and 40 kDa). When the cells were treated with TNF-α or with LPS, the myristoylation of a distinct protein with an apparent molecular mass of 82 kDa was strongly induced (Fig. 1*A*). The TNF-α- and LPS-induced, acylated 82-kDa protein was specifically and completely immunoprecipitated with a rabbit serum raised against the bovine brain 87-kDa MARCKS protein (Fig. 1*A*) (13, 15). Two-dimensional gel electrophoresis of lysates from neutrophils previously treated with TNF-α revealed that the 82-kDa [^{3}H]myristic acid-labeled protein had an isoelectric point at pH 4.6 (Fig. 1*B*), which is similar to that of murine and bovine MARCKS (13, 15). In the presence of cycloheximide (5 μg/ml) the acylation of the 82-kDa protein was completely inhibited, suggesting that the attachment of myristic acid occurred cotranslationally (Fig. 1*B*). Under these conditions, an acylated 48-kDa protein with an acidic isoelectric point of pH 5.4 was observed (the detailed characterization of this protein will be presented elsewhere; P. G. Heyworth, M.T., A.R., A.A., and J. T. Curnutte, unpublished data). The acylation of the 82-kDa protein was resistant to hydroxylamine treatment at pH 10, indicating that the myristic acid was linked to the protein by an amide bond rather than through an alkaline-sensitive ester linkage. Complete degradation of the protein with proteinase K and Pronase E (27) showed that myristic acid was exclusively recovered as *N*-myristoylglycine (data not shown). Following acid methanolysis of the protein (12), all the radiolabel was coeluted from a C_{18} reverse-phase HPLC column with the methyl myristate or myristic acid standards (data not shown). From these results it is concluded that the acylated 82-kDa protein in neutrophils represents human MARCKS.

Myristoylation of MARCKS: Temporal Response and Concentration Dependence of Activators. The effect of TNF-α, LPS, IFN-γ, and GM-CSF on the myristoylation of MARCKS was examined in [^{3}H]myristic acid-labeled neutrophils (Fig. 2). Proteins were electrophoretically separated and the resulting fluorographs were analyzed by laser densitometry. TNF-α induced half-maximal myristoylation at 10 units/ml, and 100 units/ml was required for maximal MARCKS expression. A similar maximal level of MARCKS myristoylation was observed with LPS. The response to LPS was more sensitive, in that no protein acylation was induced by LPS at 0.5 ng/ml whereas maximal levels were obtained with 5 ng/ml. By

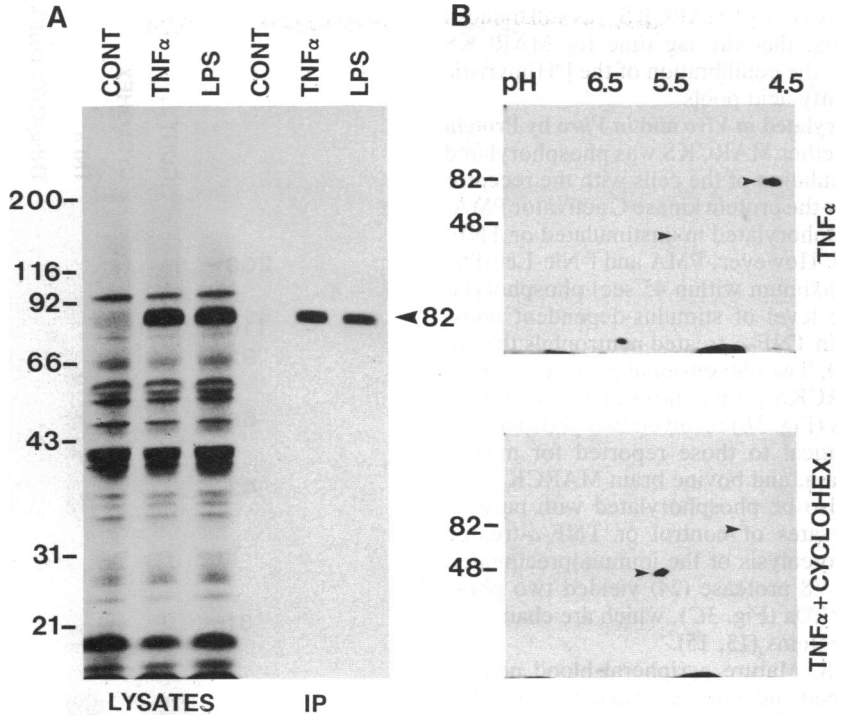

FIG. 1. TNF-α- and LPS-induced protein myristoylation in human neutrophils. (*A*) Neutrophils (7.5×10^6 cells) were labeled for 4 hr with [^{3}H]myristic acid (40 μCi/ml) in the absence (control, CONT) or presence of TNF-α (100 units/ml) or LPS (100 ng/ml). The cells were then lysed, and the 82-kDa protein was immunoprecipitated (IP) with a rabbit serum directed against bovine MARCKS (13). A second aliquot of the same lysate was loaded directly for $NaDodSO_4$/PAGE (lysates). (*B*) Two-dimensional electrophoresis (first dimension, isoelectric focusing) of [^{3}H]myristic acid-labeled neutrophils (3×10^6) treated for 3 hr with TNF-α (100 units/ml) in the absence (*Upper*) or presence (*Lower*) of cycloheximide (5 μg/ml). All panels show fluorographs of dried $NaDodSO_4$/polyacrylamide gels exposed for 5 days on Kodak XAR-5 film. Molecular size markers are in kilodaltons.

contrast, treatment with IFN-γ or GM-CSF at 0.1–1000 units/ml did not induce the acylation of MARCKS (Fig. 2*A*), even though all the mediators caused distinct morphological changes in the cells at 1–10 units/ml (data not shown). TNF-α- and LPS-dependent myristoylation of MARCKS were first apparent after 80 min of incubation and continued to increase for at least 4 hr (Fig. 2*B*). IFN-γ and GM-CSF did not induce the acylation of MARCKS over the same time period. In some experiments neutrophils were preequilibrated with [^{3}H]myristic acid for 1 hr before treatment with TNF-α or LPS. Under

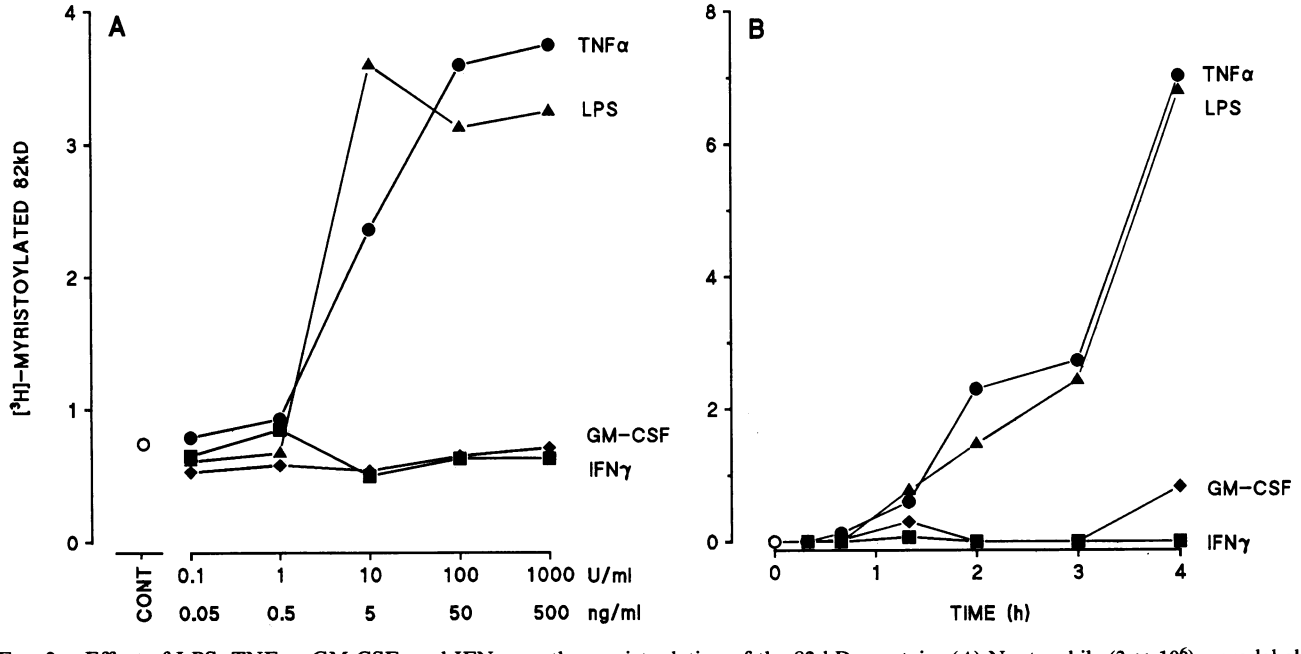

FIG. 2. Effect of LPS, TNF-α, GM-CSF, and IFN-γ on the myristoylation of the 82-kDa protein. (*A*) Neutrophils (3×10^6) were labeled with [^{3}H]myristic acid for 3 hr in the presence of TNF-α (●), GM-CSF (◆), or IFN-γ (■) at 0.1–1000 units (U)/ml or LPS (▲) at 0.05–500 ng/ml. CONT, control. (*B*) Neutrophils (3×10^6) were labeled with [^{3}H]myristic acid for the indicated times in the presence of TNF-α (●) at 100 units/ml, LPS (▲) at 100 ng/ml, GM-CSF (◆) at 100 units/ml, or IFN-γ (■) at 100 units/ml. Fluorographs were quantitated by laser-densitometry (arbitrary absorbance units). In *B*, the absorbance was corrected for the corresponding untreated cells.

these conditions the expression of MARCKS was still induced after ≈80 min, indicating that the lag time for MARCKS synthesis was not due to the equilibration of the [^{3}H]myristic acid with endogenous fatty acid pools.

MARCKS Is Phosphorylated *in Vivo* and *in Vitro* by Protein Kinase C. We tested whether MARCKS was phosphorylated in neutrophils after stimulation of the cells with the receptor agonist f-Nle-Leu-Phe or the protein kinase C activator PMA. MARCKS was not phosphorylated in unstimulated or TNF-α-treated cells (Fig. 3*A*). However, PMA and f-Nle-Leu-Phe stimulated the rapid (maximum within 45 sec) phosphorylation of MARCKS. The level of stimulus-dependent phosphorylation was higher in TNF-α-treated neutrophils than in untreated cells (Fig. 3*A*). Two-dimensional peptide mapping of phosphorylated MARCKS from control (data not shown) and TNF-α-treated cells (Fig. 3*B*) resolved two phosphopeptides, which were identical to those reported for murine macrophage, murine brain, and bovine brain MARCKS (23, 28). MARCKS could also be phosphorylated with purified protein kinase C in lysates of control or TNF-α-treated neutrophils. Limited proteolysis of the immunoprecipitated protein with *S. aureus* V8 protease (24) yielded two phosphopeptides of 13 and 9 kDa (Fig. 3*C*), which are characteristic of all MARCKS proteins (13, 15).

Synthesis of MARCKS. Mature peripheral blood neutrophils have a short life-span and a limited capacity to synthesize proteins (29). The profile of proteins synthesized by neutrophils in 3 hr (Fig. 4) showed that 30–40 proteins incorporated [^{3}H]lysine, with the major proteins having apparent molecular masses of 55, 42, and 30 kDa. Translation was completely inhibited by cycloheximide. TNF-α induced the prominent synthesis of a protein of 82 kDa (90% of all TNF-α-induced protein synthesis) and the modest synthesis of a protein of 57 kDa (Fig. 4). The 82-kDa protein was identified as MARCKS by immunoprecipitation (Fig. 4) and by two-dimensional electrophoresis (data not shown). In addition, MARCKS represented a major component of all the proteins synthesized by the TNF-α-treated neutrophils. Similar results were obtained when the cells were treated with LPS (data not shown).

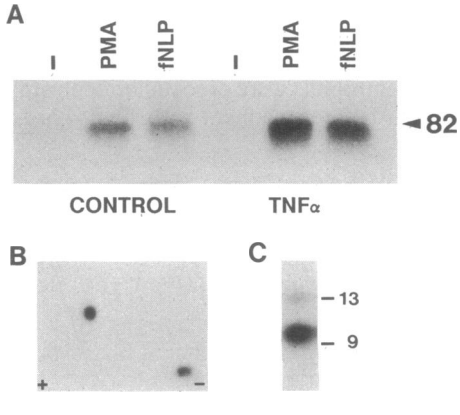

FIG. 3. Phosphorylation of the 82-kDa protein. (*A*) Autoradiogram showing *in vivo* phosphorylation of the 82-kDa protein. Control and TNF-α-treated (100 units/ml) neutrophils were labeled with [^{32}P]orthophosphate and then stimulated for 2 min with 100 nM PMA or for 45 sec with 1 μM f-Nle-Leu-Phe (fNLP). Unstimulated (−) and stimulated cells were TCA-precipitated, and following solubilization, the 82-kDa protein was immunoprecipitated and analyzed by NaDodSO$_4$/PAGE. (*B*) Two-dimensional phosphopeptide mapping of the 82-kDa protein. The immunoprecipitates shown in *A* were excised from the gel, digested with thermolysin, and separated on thin-layer cellulose sheets. Electrophoresis was used in the first dimension (+/−) and ascending chromatography in the second dimension. Phosphopeptides were visualized by autoradiography. Identical peptide maps were obtained for all immunoprecipitates shown in *A*. (*C*) Limited proteolysis of *in vitro* phosphorylated 82-kDa protein. Neutrophil lysates were phosphorylated with purified protein kinase C, after which the 82-kDa protein was immunoprecipitated and resolved by NaDodSO$_4$/PAGE. ^{32}P-labeled 82-kDa protein was excised and subjected to limited digestion with *S. aureus* V8 protease during electrophoresis. Phosphopeptides (9 and 13 kDa) were visualized by autoradiography.

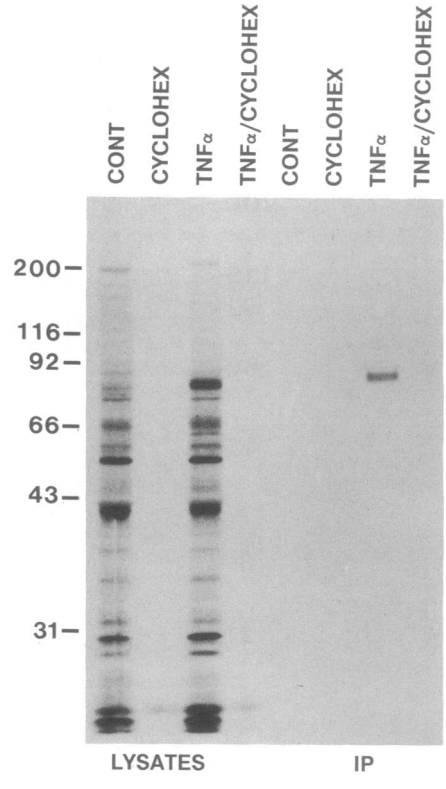

FIG. 4. Expression of the 82-kDa protein in human neutrophils. Neutrophils (7.5 × 10^6) were labeled for 3 hr with [^{3}H]lysine (20 μCi/ml) in the absence (control, CONT) or presence of TNF-α (100 units/ml). Where indicated, cycloheximide (5 μg/ml) was also included. The cells were then solubilized in lysis buffer and an aliquot of the lysate was resolved by NaDodSO$_4$/PAGE and fluorography (lysates). The 82-kDa protein was immunoprecipitated (IP) from a second aliquot of the lysate.

DISCUSSION

TNF-α is a key mediator of the inflammatory response. It is released from macrophages and exerts a profound modulatory effect on a number of cell types, including neutrophils, which it primes for enhanced phagocytosis, respiratory burst, and secretion (4, 6). The precise mechanism by which TNF-α primes neutrophils is unknown, but a clue to the underlying pathway is provided by the observation that protein kinase C is involved in all these processes (2, 30). Since protein kinase C must act by phosphorylating effector substrates, we have focused on the effects of TNF-α on protein kinase C substrates in neutrophils. LPS also primes macrophages and neutrophils for enhanced protein kinase C-dependent responses such as the secretion of arachidonic acid metabolites and increased oxidative metabolism (5, 11). Priming of macrophages with LPS is accompanied by the synthesis and myristoylation of a specific protein kinase C substrate, MARCKS (13).

Treatment of human neutrophils with TNF-α or LPS induces the synthesis and cotranslational myristoylation of an 82-kDa protein kinase C substrate, which was identified as the human homolog of MARCKS. Since MARCKS represents >90% of all newly synthesized proteins induced by either TNF-α or LPS, it is likely that this protein plays a central role

in the altered physiology of neutrophils promoted by these two priming agents. The enhanced level of MARCKS observed in TNF-α- and LPS-primed neutrophils was paralleled by its increased phosphorylation when the cells were stimulated with the chemotactic peptide f-Nle-Leu-Phe or with PMA. The phosphorylation of MARCKS (maximum within 45 sec of f-Nle-Leu-Phe addition) occurs within the time frame of neutrophil responses such as cytoskeletal alteration, activation of the respiratory burst, and exocytosis (31).

Chemotactic peptides such as f-Met-Leu-Phe have been shown to mobilize diacylglycerol and Ca^{2+} via a signal-transduction pathway mediated by the receptor and a guanine nucleotide-binding protein (reviewed in ref. 32). These two second messengers have the capacity to activate protein kinase C, which has, by inference, been implicated as a transduction intermediate. A role for protein kinase C in chemotactic peptide-dependent signal–response coupling has also been implied by a number of pharmacologic studies (2, 3, 33). Our observation that f-Nle-Leu-Phe induces the rapid phosphorylation of a specific protein kinase C substrate (MARCKS) provides direct evidence for the activation of protein kinase C following receptor agonist stimulation.

Priming of neutrophils and macrophages with TNF-α and LPS refers to the capacity of these agents to potentiate preexisting signaling pathways such as those leading to the activation of the oxygen burst and to the secretion of arachidonic acid metabolites (5, 11, 34). In line with this, MARCKS is phosphorylated rapidly in response to chemotactic peptides and phorbol esters in unprimed neutrophils and the levels of phsophorylated MARCKS are increased 4- to 5-fold in TNF-α- or LPS-primed cells. Peptide mapping revealed that MARCKS is phosphorylated on the same sites in unprimed and in TNF-α- and LPS-primed neutrophils, suggesting that the increased phosphorylation observed in primed cells is due to elevated levels of the substrate. This conclusion is supported by the observation that TNF-α and LPS induce the synthesis of MARCKS.

The specific function of MARCKS remains obscure. It is known to bind calmodulin and is rapidly phosphorylated during neurosecretion, during growth factor-dependent mitogenesis, and during secretion and phagocytosis in macrophages and neutrophils (17, 23, 35, 36). All these events require profound cytoskeletal rearrangement. For example, disassembly of the cortical actin meshwork in neutrophils appears to allow the secretory vesicle access to the plasma membrane. Consistent with such a model, actin-destabilizing agents such as cytochalasin B profoundly potentiate agonist-dependent secretion in neutrophils (37). Data to be reported elsewhere show that MARCKS has a role in regulating the association of actin with the plasma membrane of macrophages (A.R., J. Hartwig, K. F. Keenan, A.C.N., and A.A., unpublished data). The highly motile neutrophil dynamically regulates its actin cytoskeleton and is therefore an ideal model system in which to investigate the function of MARCKS. In addition, these studies will provide insights into the mechanism of action of inflammatory mediators such as TNF-α and LPS.

We thank Drs. Zanvil A. Cohn and Paul Greengard for advice and support. This work was supported in part by National Institutes of Health Grant AI25032. M.T. is a Fellow of the Deutsche Forschungsgemeinschaft. A.R. is a recipient of a Cancer Research Institute/Richard C. and Susan B. Ernst Fellowship. A.A. is a Pew Scholar in the Biomedical Sciences.

1. Berridge, M. J. (1986) *Biol. Chem. Hoppe-Seyler* **367,** 447–456.
2. Dewald, B., Thelen, M. & Baggiolini, M. (1988) *J. Biol. Chem.* **263,** 16179–16184.
3. Dewald, B., Thelen, M., Wymann, M. P. & Baggiolini, M. (1989) *Biochem. J.* **264,** 879–884.
4. Steinbeck, M. J. & Roth, J. A. (1989) *Rev. Infect. Dis.* **11,** 549–568.
5. Guthrie, L. A., McPhail, L. C., Henson, P. M. & Johnston, R. B., Jr. (1984) *J. Exp. Med.* **160,** 1656–1671.
6. Shalaby, M. R., Aggarwal, B. B., Rinderknecht, E., Svedersky, L. P., Finkle, B. S. & Palladino, M. A., Jr. (1985) *J. Immunol.* **135,** 2069–2073.
7. Cybulsky, M. I., McComb, D. J. & Movat, H. Z. (1989) *Am. J. Pathol.* **135,** 227–237.
8. Weisbart, R. H., Golde, D. W., Clark, S. C., Wong, G. G. & Gasson, J. C. (1985) *Nature (London)* **314,** 361–363.
9. Wirthmueller, U., De Weck, A. L. & Dahinden, C. A. (1989) *J. Immunol.* **142,** 3213–3218.
10. Berton, G., Zeni, L., Cassatella, M. A. & Rossi, F. (1986) *Biochem. Biophys. Res. Commun.* **138,** 1276–1282.
11. Aderem, A. A., Cohen, D. S., Wright, S. D. & Cohn, Z. A. (1986) *J. Exp. Med.* **164,** 165–179.
12. Aderem, A. A., Keum, M. M., Pure, E. & Cohn, Z. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5817–5821.
13. Aderem, A. A., Albert, K. A., Keum, M. M., Wang, J. K. T., Greengard, P. & Cohn, Z. A. (1988) *Nature (London)* **332,** 362–364.
14. Albert, K. A., Walaas, S. I., Wang, J. K. T. & Greengard, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2822–2826.
15. Albert, K. A., Nairn, A. C. & Greengard, P. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7046–7050.
16. Patel, J. & Kligman, D. (1987) *J. Biol. Chem.* **262,** 16686–16691.
17. Blackshear, P. J., Wen, L., Glynn, B. P. & Witters, L. A. (1986) *J. Biol. Chem.* **261,** 1459–1469.
18. Rodriguez-Pena, A. & Rozengurt, E. (1986) *EMBO J.* **5,** 77–83.
19. Stumpo, D. J., Graff, J. M., Albert, K. A., Greengard, P. & Blackshear, P. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4012–4016.
20. Graff, J. M., Stumpo, D. J. & Blackshear, P. J. (1989) *Mol. Endocrinol.* **3,** 1903–1906.
21. Curnutte, J. T., Kuver, R. & Scott, P. J. (1987) *J. Biol. Chem.* **262,** 5563–5569.
22. Nathan, C. F. (1987) *J. Clin. Invest.* **80,** 1550–1560.
23. Rosen, A., Nairn, A. C., Greengard, P., Cohn, Z. A. & Aderem, A. A. (1989) *J. Biol. Chem.* **264,** 9118–9121.
24. Huttner, W. B. & Greengard, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5402–5406.
25. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
26. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
27. Towler, D. & Glaser, L. (1986) *Biochemistry* **25,** 878–884.
28. Albert, K. A., Wu, W. S., Nairn, A. C. & Greengard, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3622–3625.
29. Granelli-Piperno, A., Vassalli, J.-D. & Reich, E. (1979) *J. Exp. Med.* **149,** 284–289.
30. Wright, S. D. & Meyer, B. C. (1986) *J. Immunol.* **136,** 1759–1764.
31. Thelen, M., Peveri, P., Kernen, P., von Tscharner, V., Walz, A. & Baggiolini, M. (1988) *FASEB J.* **2,** 2702–2706.
32. Snyderman, R. & Verghese, M. W. (1987) *Rev. Infect. Dis.* **9,** 562–569.
33. Wilson, E., Olcott, M. C., Bell, R. M., Merrill, A. H., Jr., & Lambeth, J. D. (1986) *J. Biol. Chem.* **261,** 12616–12623.
34. Berkow, R. L., Wang, D., Larrick, J. W., Dodson, R. W. & Howard, T. H. (1987) *J. Immunol.* **139,** 3783–3791.
35. Graff, J. M., Young, T. M., Johnson, J. D. & Blackshear, P. J. (1989) *J. Biol. Chem.* **264,** 21818–21823.
36. Wang, J. K. T., Walaas, S. I. & Greengard, P. (1988) *J. Neurosci.* **8,** 281–288.
37. Dewald, B. & Baggiolini, M. (1986) *Biochim. Biophys. Acta* **888,** 42–48.