The Role of Tyrosine Phosphorylation in Lipopolysaccharide- and Zymosan-Induced Procoagulant Activity and Tissue Factor Expression in Macrophages


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The expression of surface procoagonulants by exudative macrophages represents an important mechanism underlying local fibrin deposition at sites of extravascular inflammation. The present studies investigated the contribution of tyrosine phosphorylation to the generation of macrophage procoagulant activity (PCA) and tissue factor expression in response to proinflammatory stimuli. Both lipopolysaccharide (LPS) and zymosan rapidly stimulated tyrosine phosphorylation in elicited murine peritoneal macrophages. This effect was prevented by the tyrosine kinase inhibitors genistein and herbimycin and augmented by the addition of the phosphotyrosine phosphatase inhibitor vanadate. The vanadate-mediated rise in phosphotyrosine accumulation was abrogated by the use of diphenylene iodonium, an inhibitor of the respiratory burst oxidase, suggesting a role for peroxides of vanadate as contributors to the tyrosine phosphorylation. This notion was supported by the finding that vanadyl hydroperoxide markedly increased the accumulation of phosphotyrosine residues. To define the role of tyrosine phosphorylation in the induction of macrophage PCA by LPS, the effects of tyrosine kinase inhibition by genistein and herbimycin were investigated. Both agents inhibited the expression of macrophage PCA. Further, Northern blot analysis with the cDNA probe for murine tissue factor indicated that the inhibition occurred at the mRNA level or earlier. Since vanadate augmented phosphotyrosine accumulation, it was hypothesized that it might enhance generation of macrophage products. However, vanadate reduced induction of PCA in response to LPS. By contrast, vanadate augmented basal prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) release and stimulated PGE\textsubscript{2} release by macrophages. Indomethacin prevented the increase in PGE\textsubscript{2} but only partially restored normal levels of PCA. The effect of vanadate on tissue factor expression appeared to be posttranscriptional. These studies thus demonstrate, by functional Western blotting and Northern blotting techniques, that tyrosine phosphorylation plays a role in the regulation of macrophage PCA and tissue factor expression in response to proinflammatory stimuli.

Fibrin deposition is a ubiquitous response to inflammation. Studies have implicated a role for macrophage-mediated coagulation (i.e., procoagulant activity [PCA]) in the pathogenesis of a variety of pathological processes. For example, the accumulation of fibrin in delayed-type hypersensitivity (DTH) responses is responsible for the induration observed at these sites (14, 15). Fibrin deposition in these lesions is mediated by the influx of macrophages and subsequent expression of the surface procoagulant, tissue factor (TF). Antibodies inhibiting the interaction between TF and factor VII/VIIa prevent the development of the DTH response by precluding the initiation of the coagulation cascade (35). Extravascular coagulation also appears to contribute to the development of intra-abdominal abscesses containing a mixed bacterial flora. Reduction in local fibrin deposition effected by the use of anticoagulants or fibrinolytics or, alternatively, by systemic defibrinogenation abrogates abscess formation in part by augmenting bacterial clearance by host phagocytic cells (12, 25, 54, 65). While gram-negative enteric bacteria are known to induce macrophage PCA, both in vivo and in vitro, (1, 2, 61) the cellular mechanisms leading to the expression of PCA remain incompletely defined.

Lipopolysaccharide (LPS) is an integral component of the cell wall of gram-negative enteric bacilli. It is a potent stimulus for the expression of macrophage PCA as well as for the release of other macrophage-derived mediators such as tumor necrosis factor (TNF), both in vitro and in vivo (4, 55). LPS binds to the CD14 receptor on the surface of macrophages after associating with the LPS-binding protein (LBP) (69, 82) or the septin complex (81). The contribution of the various components of the signalling pathway to the ultimate effector functions of macrophages has been studied extensively. Studies with pertussis toxin have implicated a role for guanine nucleotide-binding proteins in mediating LPS-stimulated cell activation (19, 36, 83). Inhibitors of Ca\textsuperscript{2+} and calmodulin cause variable effects on the transcription of mRNAs for a number of cytokines (39, 59), while agents which impair phospholipase A\textsubscript{2} activity have been shown to block the induction of TNF transcripts (56). Initial studies examining the ability of LPS to induce protein phosphorylation suggested that this event was mediated via protein kinase C (PKC) (60, 80). However, subsequent studies have indicated no significant increase in PKC activity following cell stimulation with LPS (22). Further, inhibitors of PKC have varied effects on the ability of LPS to stimulate the production of mediator molecules by macrophages or to effect macrophage priming (10, 11, 39, 40, 62), suggesting the existence of PKC-independent signalling pathways in these cells.

Recent studies have implicated a role for tyrosine phosphory-
ylation in the generation of mediator molecules by macrophages in response to a variety of stimuli (3, 5, 21, 22, 27, 66, 67, 76, 77). For example, tyrosine phosphorylation is required for induction of several cytokines including TNF, interleukin-1, and interleukin-6 following stimulation of monocytes by LPS, as evidenced by the ability of tyrosine kinase inhibitors to abrogate release of these products (3, 27). The early signalling mechanisms contributing to LPS-induced tyrosine phosphorylation have, in part, been characterized. Although CD14, the glycosyl-phosphatidylinositol-linked receptor for the LPS–LPS-binding protein complex, has no intrinsic tyrosine kinase activity (33), there is physical coupling and activation of one member of the src family of tyrosine kinases, lyn, and activation of two others, hck and c-fgr, following LPS treatment (70). Studies have also shown that tyrosine kinase inhibitors prevent activation of the transcription factor NF-κB by LPS (31).

Induction of PCA in LPS-treated murine peritoneal macrophages is due to increased expression of TF, a 47-kDa surface expressed glycoprotein (50). This process is regulated both transcriptionally and posttranscriptionally (7, 28). The promoter region of the murine TF gene has been shown to contain consensus binding sequences for Sp1, egr-1, AP-1, and NF-κB. Functional analysis of the promoter region indicates that ligation of both the AP-1 and the NF-κB binding sequences is required for optimal gene transcription. Since tyrosine kinase inhibitors have been shown to impair NF-κB activation, it was hypothesized that these agents might impair induction of macrophage PCA and TF expression by reducing LPS-induced transcription of the TF gene.

MATERIALS AND METHODS

Animals. Six to 8-week-old female Swiss Webster mice were obtained from Charles River Laboratories. Following delivery to our animal facility, the animals were allowed to acclimate for 2 to 4 days prior to use in these studies. Animals were then maintained in colonies of no more than five animals per cage and fed standard mouse chow and water ad libitum.

Materials. Fetal bovine serum, Hank's balanced salt solution (HBSS, Ca<sup>2+</sup> and Mg<sup>2+</sup> free), glutamine, penicillin or streptomycin, gentamicin, and heparin A were from Gibco. RPMI 1640 medium, zymosan, molecular mass standards, Coomassie blue, Nonidet P-40, HEPES, rabbit brain thromboplastin, and dimethyl sulfoxide (DMSO) were from Sigma. Bovine thrombosthenin (BT) was purchased from Calbiochem. Staurosporine was from Biomol. Sodium orthovanadate was from Aldrich Chemicals, and hydrogen peroxide was from Fluka. Cytokines. The monoclonal antiphosphotyrosine antibody PY20 immunoglobulin G2B (IgG2B) was obtained from ICN. The polyclonal antiphosphotyrosine antibody for rat brain thromboplastin standard in which 36 mg (dry weight) per ml was assigned a value of 100,000 mU of PCA. Results of previous studies have demonstrated that LPS-induced mouse macrophage PCA is only slightly less effective in shortening the clotting time of human plasma than in shortening the clotting time of mouse plasma (43). The induction of PCA from a baseline of 300 mU/10<sup>6</sup> macrophages to 2,800 mU/10<sup>6</sup> macrophages in cells stimulated by LPS alone represented a shortening of the clotting time from 70 to 52 s. The assay was used over the range of 10 to 10,000 mU of PCA, this range being linear with a normal plasma substrate. Previous studies have shown that PCA induced with E. coli LPS has TF-like activities, making comparison with a thromboplastin standard valid (64).

TF mRNA induction. TF mRNA induction was assessed by Northern blot analysis with the CDNA probe for murine TF (63) kindly provided by Michael Getz, Mayo Clinic, Rochester, Minnesota. Briefly, 10<sup>6</sup> to 10<sup>7</sup> cells were pelleted, and total RNA was extracted by the method described by Chomczynski and Sacchi (13). After electrophoresis, RNA was transferred to GeneScreen and hybridized with a 32P random-labelled cDNA probe for murine TF. The loading of comparable amounts of RNA between lanes was assured by probing with a cDNA probe for rat B tubulin (44) or ribosomal 18S.

Macrophage chemiluminescence. LPS- and zymosan-stimulated chemiluminescence was monitored during a 240-min period with a luminometer (Automat model LB 95; EG & G Berthold, Bad Wildbad, Germany). Chemiluminescence was integrated during this interval with software provided by the manufacturer whereby the area under the curve correlates with the release of reactive oxygen species.

Measurement of PG<sub>E2</sub>. PG<sub>E2</sub> in cell supernatants was measured by using a PG<sub>E2</sub>-labelled radiomunnoassay system from Amersham International, Markham, Ontario, Canada.

Endotoxin contamination. RPMI 1640, HBSS, fetal calf serum, sterile water, sterile saline, and all other reagents were tested for endotoxin contamination by the standard Limulus amoebocyte lysate assay (Association of Cape Cod, Woods Hole, Mass.) and were found to contain <0.1 ng of endotoxin per ml, which constituted the lower limit of the test.

Statistics. Statistics were calculated by one-way analysis of variance and Newman–Keuls for comparison between groups. Data are expressed as the means and standard errors for n observations.

RESULTS

Tyrosine phosphorylation in stimulated macrophages. Stimulation of cells with LPS (10 μg/ml) caused a time-dependent increase in phosphotyrosine accumulation in peritoneal macrophages (Fig. 1). The specificity of the PY20 IgG2B monoclonal antibody for tyrosine-phosphorylated polypeptides had been previously established by competition experiments from the blots by phosphotyrosine but not by phosphoserine or phosphothreonine (29). Tyrosine phosphorylation occurred rapidly (as early as 2 min) and persisted for up to 4 h. The most prominent bands had molecular masses of approximately 38, 41, 47, 55, 61, 73, 80, and 102 kDa. The rapid induction of
tyrosine phosphorylated proteins, particularly in the molecular mass range of 38 to 47 kDa, has previously been reported (31, 77, 78). Pretreatment of cells for 1 h with the tyrosine kinase inhibitor genistein (10 μg/ml) reduced the accumulation of phosphotyrosine (Fig. 2). Genistein also reduces baseline phosphotyrosine activation induced by thioglycolate-stimulated migration of cells into the peritoneal cavity.

Incubation of peritoneal macrophages with the particulate stimulus zymosan similarly resulted in a time- and dose-dependent increase in tyrosine phosphorylation of several proteins (Fig. 3). Tyrosine phosphorylation increased as early as 10 min and peaked at 60 to 120 min, with an optimal concentration of zymosan (100 to 500 μg/ml). The major bands had molecular masses of approximately 39, 51, 71, and 94 kDa. Several other minor bands were observed, particularly at the higher molecular masses. Preincubation of cells with genistein impaired phosphotyrosine accumulation (data not shown).

The level of phosphotyrosine accumulation represents a balance between tyrosine kinase activity and the opposing tyrosine phosphatase activity. Vanadate, an inhibitor of tyrosine phosphatase activity, was tested for its effect on tyrosine phosphorylation induced by zymosan and LPS (Fig. 4A and B, respectively). While vanadate treatment alone caused little effect on phosphotyrosine accumulation (data not shown), its addition to zymosan resulted in a marked augmentation (Fig. 4A). Similarly, vanadate stimulated phosphotyrosine accumulation in LPS-treated cells, albeit to a lesser extent (Fig. 4B). Peroxides of vanadate were recently shown to be potent inhibitors of tyrosine phosphatases (74). Since the reaction of vanadate with superoxide and hydrogen peroxide leads to the generation of peroxovanadyl and vanadyl hydroperoxide, respectively (48, 49), and macrophages are known to release these reactive oxygen species in response to various stimuli (75), studies were performed to discern whether peroxides of vanadate may be contributing to the accumulation of tyrosine-phosphorylated proteins in macrophages exposed to vanadate. The effect of diphenylene iodonium (DPI), an inhibitor of the respiratory burst oxidase (24), on phosphotyrosine accumulation in stimulated cells exposed to vanadate was studied. Figure 4C illustrates the chemiluminescent response of macrophages as a measure of the respiratory burst. Stimulation was observed with both agents but was more profound with zymosan than with LPS. DPI reduced stimulated chemiluminescence levels to below control levels. DPI also caused a slight reduction in the zymosan- and LPS-induced phosphotyrosine accumulations in the absence of vanadate. The addition of DPI abrogated the vanadate-induced increase in tyrosine phosphorylation in cells exposed to both zymosan and LPS (Fig. 4A and B, respectively). Control levels of phosphotyrosine accumulation were again below those observed in zymosan- or LPS-treated cells. In addition, vanadyl hydroperoxide, prepared by mixing equimolar concentrations of sodium orthovanadate and hydrogen peroxide, markedly enhanced phosphotyrosine accumulation (Fig. 4A). DPI had no effect on phosphotyrosine accumulation induced by vanadyl hydroperoxide, indicating that its inhibition of tyrosine phosphorylation by zymosan in the presence of vanadate was unlikely to be artifactual.

Role of tyrosine phosphorylation in induction of PCA. Inhibitors of tyrosine kinase activity were used to determine whether LPS-induced tyrosine phosphorylation participated in the signalling pathways for the generation of macrophage PCA. At concentrations shown to impair LPS-induced phosphotyrosine accumulation, both genistein (10 μg/ml with a 1-h
pretreatment (Fig. 5A) and herbimycin A (1 μg/ml with a 2-hour pretreatment (Fig. 5B)) reduced PCA expression by macrophages following stimulation with LPS. Similar inhibition was observed when PCA was stimulated by the addition of zymosan (data not shown). Inhibition was not due to the use of DMSO (0.1%) as a vehicle or to cell toxicity since viability remained greater than 84% as determined by trypan blue exclusion.

PCA induced by LPS treatment of monocytes/macrophages has been shown to be due to increased surface expression of the surface glycoprotein TF. TF gene transcription has been shown to regulate the presence of AP-1 and NF-κB binding sites in the promoter region. Since tyrosine kinase inhibitors have been shown to prevent NF-κB translocation in LPS-stimulated cells (31), it was hypothesized that their effect on PCA expression might be due to reduced TF gene transcription. To examine this possibility, Northern blot analysis of TF mRNA in LPS-treated cells was performed. Both genistein and herbimycin reduced the level of TF mRNA transcripts observed in LPS-treated cells to control levels (Fig. 6A and B, respectively).

Since vanadate augmented phosphotyrosine accumulation in stimulated cells, it was postulated that it might increase production of macrophage PCA in response to LPS. As shown in Fig. 7, vanadate (100 μM) unexpectedly reduced PCA expression by macrophages stimulated with LPS. This result was not secondary to a nonspecific effect of vanadate itself in the clotting assay as the direct addition of vanadate to LPS-stimulated cells during the clotting assay had no effect (data not shown). The mechanism underlying the vanadate-induced inhibition of LPS-stimulated PCA production was examined in further detail. Recent studies have reported a correlation between arachidonic acid release and tyrosine phosphorylation in LPS-stimulated macrophages (76). Since several products of arachidonic acid metabolism, including those of the PGE series, have been shown to impair PCA in response to LPS (17), it was hypothesized that vanadate effected inhibition by augmenting PGE2 production.

Vanadate increased basal PGE2 release by cells (54 ± 6 pg/ml for control versus 136 ± 34 pg/ml for vanadate; n = 6; P < 0.05 [Fig. 8A]). Vanadate also increased LPS-induced PGE2 release by 89% (range, 8 to 273%; n = 6, P < 0.05). To determine whether this rise in PGE2 could account for the reduction in PCA, cells were simultaneously treated with indomethacin (3 μM). This agent completely prevented the release of PGE2 by LPS-stimulated cells. Consistent with our previous reports (40), indomethacin had no effect on LPS-stimulated PCA (Fig. 8B). In the presence of vanadate, indomethacin caused a partial restoration (~38%) of LPS-stimulated PCA (Fig. 8B). This effect was observed in all six experiments performed. To further examine...
The present studies investigated the role of tyrosine phosphorylation in the induction of macrophage PCA following exposure to proinflammatory stimuli. Both LPS and zymosan induced phosphotyrosine accumulation and a rise in PCA. The functional relevance of the tyrosine phosphorylation was demonstrated by the ability of two distinct tyrosine kinase inhibitors, genistein and herbimycin, to inhibit PCA induction in murine peritoneal macrophages. Northern blot analysis of TF mRNA expression revealed that the effect of tyrosine phosphorylation occurred at the mRNA level or earlier. Considered together, these functional, Western immunoblotting and Northern blotting data support the conclusion that tyrosine phosphorylation is an important component of the signalling pathway leading to the expression of PCA and TF in stimulated murine peritoneal macrophages.

Several studies have suggested a role for PKC-dependent activation. Similarly, another PKC inhibitor, staurosporine, caused only partial inhibition of PCA induction (Fig. 9A). Thus, inhibition of LPS-induced PCA correlated more closely with a reduction in tyrosine phosphorylation than it did with the inhibition of PKC activation.

FIG. 5. (A) Effect of genistein on induction of PCA activity by LPS. Macrophages (M6) (10^6/ml) were stimulated with LPS (10 μg/ml) in the presence (□) or absence (■) of genistein (10 μg/ml) for 4 h. Cells were preincubated in genistein for 1 h prior to stimulation. At 4 h, cells were pelleted, resuspended in RPMI, and frozen at −70°C for later assay of PCA activity. The data represent the means and standard errors of the means for three separate experiments, each performed in duplicate. *, P of <0.05 versus control; **, P of <0.05 versus untreated for each group. (B) Effect of herbimycin on induction of PCA activity by LPS. Macrophages (M6) (10^6/ml) were stimulated with LPS (10 μg/ml) in the presence of herbimycin (1 μg/ml) or DMSO (0.1%) for 4 h. Cells were preincubated in herbimycin for 2 h prior to stimulation. At 4 h, cells were pelleted, resuspended in RPMI, and frozen at −70°C for later assay of PCA activity. The data are the means and standard errors of the means for four separate experiments, each performed in duplicate. □, untreated macrophages; ■, herbimycin-treated macrophages; ■, macrophages treated with 0.1% DMSO; *, P of <0.05 versus control; **, P of <0.05 versus untreated for each group.

**DISCUSSION**

The role of PKC activation in LPS-induced PCA. Previous studies have reported an important role for PKC stimulation in the induction of monocyte/macrophage PCA by LPS (10, 73). The PKC signalling pathway may overlap with that defined for tyrosine phosphorylation in several ways. First, tyrosine phosphorylation of the γ-1 isoform of phospholipase C may lead to phosphatidylinositol hydrolysis and subsequent activation of PKC (32). Second, treatment of cells with phorbol esters may induce phosphotyrosine accumulation (30, 68). After the determination that tyrosine phosphorylation participated in the induction of PCA and TF by LPS, the relative contribution of PKC to the signalling pathway was evaluated with inhibitors of PKC activation. The specific PKC inhibitor BIM (2.5 μM) was tested for its ability to inhibit LPS-induced PCA. BIM did not inhibit PCA induction by LPS (Fig. 9A), despite its ability to totally abrogate phorbol ester-stimulated chemiluminescence (data not shown), thereby confirming its ability to inhibit PKC

![Diagram](http://iai.asm.org/)

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signalling in the stimulation of monocyte/macrophage PCA by LPS. These conclusions were based on the ability of PKC agonists to stimulate PCA (10, 37, 38, 51, 52, 73) as well as the observation that various PKC inhibitors were able to abrogate LPS-stimulated PCA (10, 73). However, these findings have not been uniformly reported. Brozna and Carson (8) demonstrated that phorbol myristate acetate, at concentrations sufficient to translocate PKC activity from the cytosolic to the particulate fraction in adherent human monocytes, blocked adherence-induced PCA expression and suppressed PCA expression in cells activated by adherence or LPS treatment. Our laboratory has previously shown that PMA alone had little direct effect on PCA in thioglycollate-elicited murine peritoneal macrophages, although it primed cells for increased PCA expression in response to LPS and live bacteria (40, 41). The variability observed with respect to the role of PKC in LPS-induced PCA in monocytes/macrophages may be related to the source of the cell studied as well as the degree of cellular activation. In the present studies, thioglycollate-elicited murine peritoneal macrophages were used. These cells are considered to be in a state of heightened activation, by virtue of the fact that they are induced to migrate into the peritoneal cavity by the initiation of sterile inflammation. Presumably, these cells are representative of those which might be recruited into the peritoneal cavity during intraabdominal infection. In these cells, two tyrosine kinase inhibitors, genistein and herbimycin, completely prevented the stimulation of PCA by LPS, while BIM, a PKC inhibitor which completely abrogated phorbol ester-stimulated chemiluminescence, had no effect on LPS-induced PCA. These findings are consistent with those recently reported by Han and colleagues (31). These investigators reported that herbimycin A, but not the PKC inhibitor GF 109203X, prevented the induction of tyrosine phosphorylation and activation of NF-κB by LPS. Given that LPS-stimulated TF gene expression requires NF-κB activation (9, 53), these data support the notion that signalling may occur through a pathway requiring tyrosine phosphorylation but one independent of PKC activation. Another PKC inhibitor, staurosporine, caused partial inhibition of PCA. This agent is known to have tyrosine kinase inhibitor activity, and thus, its partial inhibitory effect may be related to this action (34). The inhibition of LPS-induced PCA reported for other PKC inhibitors, such as H7, might be explained on a similar basis (10, 73). Alternatively, under the experimental conditions used in those studies, PKC-dependent signalling pathways, possibly via induction of tyrosine phosphorylation, may have participated in PCA induction (66).

Vanadate, a tyrosine phosphatase inhibitor, augmented phosphotyrosine accumulation in stimulated cells. Recent studies performed with permeabilized HL60 cells that differentiated along granulocytic lines demonstrated that vanadate-induced tyrosine phosphorylation was mediated in part by the reaction products of reduced oxygen metabolites and vanadate (i.e., vanadyl hydroperoxide or peroxovanadate) (74). The present studies demonstrate that a similar mechanism is active in intact macrophages. Three lines of evidence support this conclusion. First, DPI reduced the vanadate-induced increase
in phosphotyrosine accumulation in response to both zymosan and LPS, at a concentration which abrogated the respiratory burst. Secondly, vanadyl hydroperoxide, generated by the co-incubation of hydrogen peroxide and sodium orthovanadate, mimicked the effect of vanadate seen in stimulated cells. The addition of DPI had no effect on this response, indicating that its ability to reduce vanadate-induced phosphotyrosine accumulation was not artifactual. Finally, the increase in phosphotyrosine accumulation seen with the addition of vanadate correlated well with the magnitude of the respiratory burst induced in the cells. Specifically, it was relatively small in LPS-treated cells and comparatively large following exposure to zymosan. The precise mechanism whereby peroxides of vanadate mediate their effect is not clear, although recent studies performed with neutrophils suggest that the redox state of the cell may directly modulate accumulation of tyrosine phosphoproteins (23, 26).

Despite increasing phosphotyrosine accumulation, vanadate unexpectedly caused an inhibition of LPS-stimulated PCA production. Cell viability, as determined by trypan blue exclusion, remained high in LPS-treated cells incubated with vanadate (>84%). More importantly, another cell product, PGE2, was released in greater amounts in vanadate-treated cells, thus making it unlikely that cell toxicity played a significant role in the observed reduction in PCA production. Stimulation of PGE2 release by vanadate represents one possible mechanism contributing to this effect, since this and other prostaglandins have been shown to inhibit the generation of macrophage products (18, 42). The present studies showed a rise in PGE2 release by vanadate in both stimulated and unstimulated cells. To determine the possible contribution of prostaglandins, including PGE2, to the inhibition of LPS-induced PCA by vanadate, the effects of indomethacin on the vanadate-induced increase in PGE2 and reduction in PCA were examined. Although indomethacin abolished PGE2 release, PCA was only partially restored to levels observed in the absence of vanadate. These data suggest that while PGE2 may contribute to the depression in PCA generation, other inhibitory influences appear to be induced by the vanadate-mediated phosphotyrosine accumulation. The Northern blot analysis evaluating the effect of vanadate on levels of TF mRNA induced by LPS supports this conclusion. While previous studies have shown that prostanoids generally inhibit TF expression at the level of transcription (18), the present studies demonstrate that vanadate acts posttranscriptionally.

Vanadate has been shown to increase arachidonic acid metabolism in response to various stimuli in rat hepatocytes, dog kidney cells, and bovine aorta smooth muscle cells (46) and, in the present studies, augmented PGE2 release in elicited peritoneal macrophages both in the presence and in the absence of a second stimulus LPS. The precise mechanism underlying this effect has not been defined. A recent report has documented the ability of p42 mitogen-activated protein (MAP) kinase to phosphorylate phospholipase A2 and induce its activation (57). In this regard, stimulation of macrophages with LPS has recently been shown to induce tyrosine phosphorylation and activation of several different MAP kinases (21, 31, 78). LPS-induced tyrosine phosphorylation of proteins with molecular masses similar to those of proteins previously identified as MAP kinases (38 and 41 kDa) was also observed in the present studies. Thus, the ability of vanadate to augment phosphorylation of these kinases may represent one possible mechanism leading to increased arachidonic acid release and synthesis of PGE2.

In summary, the present studies demonstrate a primary role for tyrosine phosphorylation in the induction of macrophage PCA and TF by proinflammatory stimuli. A recent report has documented that tyrosine kinase inhibition resulted in decreased human monocyte TF mRNA expression in response to LPS and phorbol myristate acetate (72). The present report identifies induction of tyrosine phosphoproteins in this process and the relatively greater role of tyrosine kinase signal transduction in contrast to signalling processes mediated via PKC. However, investigators have begun to identify further potential for cross talk between these two pathways previously believed to be exclusive. This has been described as, in effect, a “team-blue-sees-red” (6) phenomenon whereby a protein tyrosine kinase acts to relay messages from PKC and cytoplasmic calcium to Shc, (a Grb-2 adaptor protein), thus activating Ras-mediated regulation (45). The relative importance of these relay kinases in other systems remains to be examined. A recent report has documented the ability of tyrosine kinase inhibition to abrogate lethality in a murine endotoxemia model (58). Since previous studies have demonstrated that an anti-TF antibody as well as TF pathway inhibitor reduced mortality in an experimental E. coli septic shock model (16, 71), data presented herein suggest the possibility that the beneficial effect of tyrosine kinase inhibition may be attributed in part to inhibition of endotoxin-induced TF expression.

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