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Lipopolysaccharide Induces Formyl Peptide Receptor 1 Gene Expression in Macrophages and Neutrophils via Transcriptional and Posttranscriptional Mechanisms

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Bacterial infection promotes the infiltration of inflammatory leukocytes mediated in part by receptors for formyl-methionine-terminated peptides. In this study, we show that LPS can markedly enhance the expression of the formyl peptide receptor gene (FPR1) in mouse macrophages and neutrophils by enhancing transcription and by stabilization of the mRNA. In untreated cells, FPR1 mRNA exhibits a half-life of ~90 min and this is markedly increased (to >6 h) following stimulation with LPS. Although FPR1 mRNA levels remained elevated over baseline for >20 h after stimulation, the half-life of the message is prolonged only transiently. LPS-induced FPR1 mRNA expression is mediated in part by the intermediate production of secreted factors. First, the response to LPS is partially blocked by the translational inhibitor cycloheximide. Second, a heat-labile but polymyxin B-insensitive factor present in supernatants from LPS-treated cells stimulates enhanced expression of FPR1 mRNA and, like LPS, promotes stabilization of FPR1 mRNA. Furthermore, supernatants from LPS-treated wild-type macrophages can stimulate FPR1 mRNA expression in LPS-insensitive macrophages from TLR4-mutant mice. Elevated FPR1 mRNA expression is also induced in response to ligands for TLR2 and TLR3. TNF-α but not IL-1, IL-6, IFN-β, and IFN-γ can mimic the effects of LPS although other factors apparently also contribute. Collectively, these findings define a distinct molecular pattern of response to TLR stimulation in inflammatory phagocytes and demonstrate that regulation of FPR1 expression is achieved through both transcriptional and posttranscriptional mechanisms. The Journal of Immunology, 2005, 175: 6085–6091.

The infiltration of tissue sites by leukocytes is an important determinative function of the magnitude and character of inflammatory response to infection and injury. This process is regulated in part via the action of a broad spectrum of chemoattractants and their cognate receptors (1–3). During bacterial infections, the early accumulation of neutrophils and monocytes is partially dependent on detection of peptides containing an N-terminal-formylated methionine residue that acts via a family of seven-transmembrane domain G protein-coupled receptors termed the formyl peptide receptors (FPRs). FPRs are commonly found in phagocytic leukocytes such as neutrophils and monocytes (3, 4).

In phagocytes, occupancy of FPRs by the ligand N-formylated methionyl-leucyl-phenylalanine (fMLP) activates diverse biological processes, including chemotaxis, superoxide anion production, and degranulation (5–8). Stimulation through the FPRs is pertussis toxin-sensitive, indicating that they most likely couple with the Gi subset of G proteins. In humans, the prototype FPR is activated by low concentrations of fMLP whereas a variant, FPR-like 1, is activated only at a higher concentration of fMLP and is defined as a low-affinity FPR. FPR1 and FPR2, the mouse counterparts of human FPR and FPR-like 1 genes, respectively, have been shown to interact with fMLP in a similar pattern as human receptors (5, 6).

The function of chemoattractant/chemoattractant receptor pairs is regulated in part by modulating the expression of the genes encoding the receptor proteins. Several reports have demonstrated that LPS, another molecular signature of bacterial invasion, is a potent stimulus capable of promoting enhanced expression of both FPR1 and FPR2 gene products (9–11). Mounting evidence indicates that selective modulation of the rates of specific mRNA decay is an important determinant of the expression of multiple genes exhibiting altered expression during inflammatory responses (12–14). Indeed, several chemoattractant receptors have been shown to be controlled through posttranscriptional mechanisms (15–17). Moreover, it has been reported that the FPR1 mRNA exhibits constitutive instability and decays with a half-life of ~90 min (18, 19). This latter observation suggests that modulation of FPR1 gene expression might be regulated at a posttranscriptional level. In the present study, we demonstrate that LPS induces the elevation in FPR1 mRNA levels by promoting both enhanced transcription and mRNA stability. In addition, this effect can be mediated by the intermediate production of secreted stimuli and their secondary action to enhance accumulation of FPR1 mRNA.

Materials and Methods

Reagents

RPMI 1640 and Dulbecco’s PBS were obtained from the Media Laboratory of the Lerner Research Institute at the Cleveland Clinic Foundation. Antibiotics, agarose, and Tris were purchased from Invitrogen Life Technologies. Formamide, dextran sulfate, salmon sperm DNA, actinomycin D (ActD), cycloheximide (CHX), MOPS, nuclear isolation kits, poly(IC), and LPS (prepared from the Escherichia coli serotype 0111:B4) were purchased from Sigma-Aldrich. RNase-free DNase and RNasin were obtained from Promega. Brewer’s thioglycolate (TG) broth was obtained from Difco Laboratories. Pam3Cys-Ser-(lys)4 hydrochloride was purchased from CALBIOCHEM. FBS was purchased from BioWhittaker. All cell culture reagents were specified to be endotoxin free. Guanidine thiocyanate, sarcosyl, and cesium chloride were purchased from Fisher Biotech. Random priming kits were purchased from Stratagene. Nylon transfer membrane...
was purchased from Micron Separation. Recombinant mouse IL-1β, IFN-γ, TNF-α and mouse anti-TNF-α Ab were purchased from R&D Systems. A goat polyclonal Ab to mouse FPR1 was obtained from Santa Cruz Biotechnology and mAb specific for GAPDH was obtained from Chemicon International. DuPont NEN Research Products is the source of [γ-32P]dCTP and [γ-32P]UTP. Restriction enzymes and BSA were purchased from Boehringer Mannheim.

Preparation of peritoneal exudates macrophages and neutrophils
Specific pathogen-free, female C57BL/6 and C3H/HeN mice, 6–8 wk of age, were purchased from Charles River Laboratories. Female C3H/HeJ mice (6–8 wk) were obtained from The Jackson Laboratory. All animals were housed in microisolation cages with autoclaved food and bedding to minimize exposure to viral and microbial pathogens and all procedures were approved by the Institutional Animal Care and Use Committee. Resident and TG-elicited peritoneal macrophages and neutrophils were prepared as described previously (20) and cultured in RPMI 1640 medium containing L-glutamine, penicillin, streptomycin, and 5% FBS. The macrophages were cultured overnight in RPMI 1640 at 37°C in an atmosphere of 5% CO2 and then treated with stimuli for the indicated times as described in the text. Mouse neutrophils were harvested 6 h after the injection of 3% TG broth as above and were washed four times with 10 ml of Dulbecco’s PBS before use.

Preparation of plasmids
The plasmids encoding FPR1, GAPDH, and KC cDNA fragments were as described previously (18, 20). Plasmids were prepared using kits from Qiagen according to the manufacturer’s instructions.

Preparation of RNA and Northern blot hybridization analysis
Total cellular RNA was extracted from primary peritoneal exudate macrophages and from neutrophils by the guanidine thiocyanate-cesium chloride method (21). Equal amounts of RNA (20 μg) were analyzed by Northern blot hybridization followed by autoradiography. The results are presented as the ratio of values for FPR1 and GAPDH. Similar results were obtained in two separate experiments.

Western blot analysis
Preparation of whole cell lysates and Western blot analysis were described previously (22). Abs specific for FPR1 and GAPDH were used to quantify the cellular content of each protein.

Preparation of nuclei and nuclear run-on assay
Intact nuclei were isolated from mouse peritoneal macrophages (4 × 10^6) using a nuclear isolation kit (Sigma-Aldrich). All steps were performed at

FIGURE 1. LPS induces FPR1 mRNA in macrophages (Mφ) and neutrophils. A, Resident and TG-elicited macrophages or neutrophils were prepared as described in Materials and Methods and plated at 1 × 10^6 cells in 100-mm diameter petri dishes before stimulation with LPS (100 ng/ml) for 4 h. Total RNA was prepared and used to determine levels of FPR1 and GAPDH mRNA by Northern blot hybridization followed by autoradiography. B, TG-elicited macrophages were plated at 1 × 10^7 cells in 100-mm petri dishes and stimulated with LPS (100 ng/ml) for the indicated times before harvest, and analysis of FPR1 and GAPDH mRNA levels as described in A. C, TG-elicited macrophages (5 × 10^6) in 60-mm diameter petri dishes were untreated or stimulated with LPS (100 ng/ml) for 8 or 20 h. Total cell extracts were prepared and analyzed for FPR1 and GAPDH protein by Western blot analysis. The results are presented as the ratio of values for FPR1 and GAPDH. Similar results were obtained in two separate experiments.

FIGURE 2. LPS treatment stimulates FPR1 gene transcription. TG-elicited macrophages were plated at 5 × 10^7 cells in 150-mm petri dishes and stimulated with nothing (NT) or LPS (100 ng/ml) for 2 or 4 h. Nuclei were harvested and used to assess transcriptional initiation frequency on the CXCL1, FPR1, and GAPDH genes in vivo by nuclear run-on analysis as described in Materials and Methods. The autoradiographs shown were quantified using the NIH Image software and the ratio of either FPR1 or KC to GAPDH are presented graphically. Similar results were obtained in two separate experiments.
4°C. Cells were washed twice with 10 ml of ice-cold Dulbecco’s PBS. Ten milliliters of ice-cold Nuclei EZ lysis buffer was added to each dish, and the cell lysate was transferred to a 15-ml centrifuge tube, vortexed briefly, and kept on ice for 5 min. The lysate was centrifuged at 500 × g for 5 min at 4°C, washed in 10 ml of ice-cold Nuclei EZ lysis buffer, resuspended in storage buffer (50 mMTris-HCl [pH 8.3], 40% glycerol, 5 mM MgCl₂, and 0.1 mM EDTA) at 1 × 10⁷/ml and immediately frozen in liquid nitrogen.

The nuclear run-on assay was performed according to the method of Schubeler et al. (23). Briefly, nuclei were thawed on ice, 2 × 10⁸ nuclei in 200 µl were mixed with 60 µl of 5× run-on buffer (25 mMTris-HCl [pH 8.0], 12.5 mM MgCl₂, 750 mM KCl, 1.25 mM each of ATP, CTP, and GTP, and 100 µCi of [α-³²P]UTP (3000 Ci/mmol). Twenty microliters of a 15× sarkosyl solution was also added to each sample. The mixture was incubated for 30 min at 30°C, then 30 µl of DNase I (1 U/µl) was added and incubation was continued for another 15 min. RNA was isolated in a single step using TRIzol reagent according to the manufacturer’s instructions. The precipitated RNA was dissolved in 100 µl of deionized H₂O and stored at −70°C. Plasmid fragments containing specific cDNA (5 µg/slot) were blotted directly to the membrane using a slot blot apparatus (Bio-Rad). The blots were prehybridized for 12 h at 60°C in 1% SDS, 10% dextran sulfate, 1.4 M NaCl, and 325 µg of yeast tRNA and preincubated for 10 min with 500 U RNasin plus 40 mM DTT. The radiolabeled RNA (5 × 10⁶ cpm) was hybridized for 48 h at 45°C. The filters were washed sequentially in 1× SSC, 1% SDS, 30 min at 65°C and 2 times in 0.1 × SSC/0.1% SDS for 30 min at room temperature and exposed to film. The GAPDH and KC cDNAs were used as internal standard and positive control, respectively.

Results

LPS induces FPR1 mRNA via transcriptional and posttranscriptional mechanisms

LPS is known to modulate the level of FPRs present on the surface of phagocytes in a rapid (10–30 min) fashion by promoting relocalization from intracellular storage sites to the plasma membrane (24, 25). In addition, several recent articles demonstrate that LPS can stimulate the de novo expression of FPR1 or FPR2 mRNA in mouse mononuclear phagocytes (9–11). To characterize the response of FPR1, primary elicited peritoneal neutrophils and both resident and elicited peritoneal macrophages were treated with LPS for 4 h. Total RNA was isolated and used to assess levels of FPR1 mRNA by Northern blot hybridization (Fig. 1A). FPR1 mRNA was weakly expressed in unstimulated resident and elicited macrophages and neutrophils and was significantly increased following LPS treatment (Fig. 1A). In a detailed time course experiment, incubation of mouse TG-elicited peritoneal macrophages with LPS resulted in detection of elevated FPR1 mRNA within 2 h and reached a maximum by 8 h (Fig. 1B). Over the next 16 h, levels of FPR1 mRNA began to decline though remained significantly higher than in unstimulated cells. Levels of FPR1 protein were also increased after 8 h of stimulation with LPS and returned to near baseline by 24 h afterward (Fig. 1C). This is consistent with previous studies showing that LPS treatment can increase the chemotactic response of macrophages to formylated peptides (10, 11). Changes in mRNA levels may occur either through modulation of the rate of production (generally the frequency of transcriptional initiation) or the rate of mRNA decay. To assess the impact of LPS treatment on FPR1 gene transcription, nuclear run-on analysis was conducted. Nuclei were isolated from primary macrophages either without treatment or after stimulation with LPS for 2 or 4 h, and transcription initiated in vivo was allowed to continue in the presence of [α-³²P]UTP. RNA was prepared and hybridized with slot-blotted cDNAs encoding FPR1, CXCL1 (KC), or GAPDH. In these cells, the magnitude of FPR1 gene transcription is nearly undetectable in untreated cells and increases significantly at both 2 and 4 h after stimulation with LPS (Fig. 2). This film was quantified by densitometry using the NIH Image software program and values for both FPR1 and KC were normalized to the level of GAPDH. CXCL1 gene transcription is known to be dramatically increased in response to LPS (26, 27) and this was confirmed in this experiment. The increase in transcription from CXCL1 was more transient and of markedly greater magnitude than that seen with the FPR1 gene.

FPR1 mRNA has been reported to exhibit rapid decay (t₁/₂ of 90 min) in unstimulated TG-elicited mouse peritoneal macrophages and in differentiated HL-60 cells (18, 19). This finding suggests
that LPS might regulate FPR1 mRNA levels at a posttranscriptional level. To test this hypothesis, elicited peritoneal macrophages (Fig. 3A) or neutrophils (Fig. 3B) were either untreated or stimulated with LPS for 4 h. ActD was then added to prevent further transcription, and the levels of residual FPR1 mRNA were determined by Northern blot hybridization following the indicated additional incubation times. The band intensity in the autoradiographs was quantified by image analysis, the levels of FPR1 mRNA were normalized with those of GAPDH mRNA, and the rates of decay are illustrated graphically. In both cell populations, the decay of the small amount of FPR1 mRNA present in unstimulated cells is appreciably faster than that seen in cells treated with LPS for 4 h. The half-life in unstimulated cells is between 60 and 90 min whereas in LPS-treated cells it is at least 7 h.

Because the effects of LPS on FPR1 mRNA accumulation decline between 8 and 20 h after stimulation, we assessed the duration of enhanced mRNA stability. Macrophages were stimulated for either 8 or 20 h with LPS, followed by addition of ActD to block further transcription. Levels of residual mRNA were then determined by Northern blot hybridization (Fig. 4). Similar to the result shown in Fig. 3, the half-life of FPR1 mRNA in cells treated with LPS for 8 h was >7 h. In contrast, by 20 h of LPS treatment, the half-life had returned to just under 2 h. Therefore, the response to LPS at the level of FPR mRNA stability is transient in time and a return to prestimulation decay rate coincides with the decline in FPR1 mRNA.

**LPS can induce FPR1 expression indirectly**

Although many of the changes in gene expression observed in LPS-stimulated macrophages are known to be direct and immediate consequences of LPS-initiated signaling pathways, there are several well-documented examples where the action of LPS is mediated through the intermediate expression of another stimulus (28–30). For example, LPS-induced expression of a number of IFN-responsive genes such as inducible NO synthase or the T cell chemokine IFN-γ-inducible protein10 depend on the intermediate production of type I IFN via the MyD88-independent signaling pathway utilized by TLR4 (28–30). To determine whether the effects of LPS on FPR1 gene transcription and mRNA stability are direct or indirect, several experimental strategies were used. First, we assessed whether the LPS-stimulated increase in FPR1 mRNA would occur in cells in which protein synthesis was blocked with the inhibitor CHX. Peritoneal macrophages were treated with LPS or CHX either alone or in combination for 4 h before measurement...
of FPR1 mRNA levels by Northern blot hybridization. Interestingly, CHX alone resulted in a significant increase in FPR1 mRNA although the magnitude of this effect was significantly less than that seen with LPS (Fig. 5A). This is likely to result from the stabilization of FPR1 mRNA since CHX is known to promote enhanced stability of a number of short-lived mRNAs (31, 32). Moreover, we have previously reported that CHX does not alter the rate of FPR1 gene transcription (18). When macrophages were treated with both LPS and CHX, the ratio of FPR1:GAPDH was approximately the same as achieved with CHX alone. This suggests that a substantial portion of the response to LPS is blocked by preventing continuing protein synthesis and would be consistent with an indirect stimulatory mechanism.

In a second strategy, we assessed the possibility that secretory products from LPS-treated macrophages would have the capability to stimulate enhanced expression of FPR1 mRNA. Peritoneal macrophages were treated with LPS for 4 h and the supernatant medium was harvested and cleared of intact cells and particulate debris by centrifugation. The supernatant had the capacity to stimulate enhanced expression of FPR1 mRNA (Fig. 5B). This is likely to result from the stabilization of FPR1 mRNA since CHX is known to promote enhanced stability of a number of short-lived mRNAs (31, 32). Moreover, we have previously reported that CHX does not alter the rate of FPR1 gene transcription (18). When macrophages were treated with both LPS and CHX, the ratio of FPR1:GAPDH was approximately the same as achieved with CHX alone. This suggests that a substantial portion of the response to LPS is blocked by preventing continuing protein synthesis and would be consistent with an indirect stimulatory mechanism.

FIGURE 6. Role of TLRs in promoting FPR1 mRNA expression. A, TG-elicited macrophages (Mφ) from either C3H/HeN or C3H/HeJ mice were used to prepare supernatants following stimulation with LPS as described in the legend to Fig. 5. Cultures of naive macrophages from either C3H/HeN or C3H/HeJ were stimulated with nothing (NT), LPS, or supernatants (Sup) for 4 h as indicated in the figure before analysis of FPR1 and GAPDH mRNAs by Northern blot hybridization. B, TG-elicited macrophages from C57BL/6 mice were stimulated with lipopolysaccharide (LPS, 100 ng/ml), Pam3Cys (P3C, 10 ng/ml), or poly(I:C) (pIC, 100 μg/ml), as indicated, for 4 h before analysis of FPR1 or GAPDH mRNAs by Northern blot hybridization. Similar results were obtained in at least two separate experiments. NT, No treatment.

The role of TLR4 in LPS-stimulated FPR1 mRNA expression was evaluated by comparing the ability of supernatants from LPS-treated C3H/HeN-derived macrophages (LPS sensitive) to stimulate FPR1 mRNA expression in macrophages derived from C3H/HeN mice (TLR4 deficient, LPS insensitive) (Fig. 6A). Although C3H/HeN macrophages responded well to LPS and their supernatants could stimulate FPR1 expression in both HeN- and HeJ-derived naive macrophages, HeJ macrophages showed no direct response to LPS treatment and their supernatants were also inactive when used to stimulate macrophages from HeN mice. Thus, the effects of LPS are operating through the TLR4 receptor complex. Moreover, since macrophages from TLR4-deficient mice were unable to respond to LPS, it is clear that the signal initiating the process is LPS and not a contaminant material that might act through a separate TLR or pattern recognition receptor. We also wished to determine whether other TLRs might be capable of stimulating FPR1 gene expression. To test this possibility, macrophages from C57BL/6 mice were treated with ligands exhibiting specificity for TLR4 (lipid A), TLR2 (Pam3Cys), or TLR3 (poly[I:C]) for 4 h and analyzed for expression of FPR1. Although the TLR4 agonist was clearly the most potent stimulus, the other stimuli were both competent to produce a significant increase in FPR1 mRNA levels.

As a first attempt to define the nature of macrophage secretory products that could act to stimulate FPR1 gene expression, we surveyed a selection of cytokines known to be produced in peritoneal macrophages in response to LPS stimulation. Peritoneal macrophages were treated with LPS, IL-1α, TNF-α, IL-6, and IFN-β for 4 h before harvest of RNA and analysis of FPR1 mRNA levels (Fig. 7A). Of the four different cytokines, only TNF-α was able to promote enhanced expression of FPR1 mRNA, although this was substantially less potent than LPS itself. To assess the relative contribution of TNF-α in LPS-induced FPR1 expression, we evaluated the ability of neutralizing Ab against TNF-α to block the response to LPS (Fig. 7B). Although TNF-α was able to promote enhanced expression of FPR1 mRNA, neutralization of TNF-α did not appreciably diminish the response to either LPS or supernatant from LPS-treated macrophages. The Ab did effectively neutralize the response to TNF-α itself. The limited effect of TNF-α suggests that additional factors are involved in regulating FPR1 expression, although their identity remains unknown.

To determine whether the secretory products inducing elevated mRNA in response to LPS are also acting by modulating FPR1 mRNA stability, the half-life of FPR1 mRNA was compared in cultured macrophages stimulated with polymixin B sulfate-treated supernatants for either 8 or 20 h (Fig. 8A). As seen previously, the half-life of FPR1 mRNA in untreated cells was between 1 and 2 h. At 8 h following stimulation with polymixin B-treated supernatant for 8 h resulted in an increase in half-life to >6 h and this declined again to ~2 h after 20 h of stimulation. Indeed, LPS, TNF-α, and the supernatant from LPS-treated macrophages all exhibited the capacity to stabilize FPR1 mRNA after a 4-h stimulation period (Fig. 8B). These findings demonstrate that the secretory product(s) utilize the same molecular mechanisms to promote the
lated macrophages (prepared as described in the legend to Fig. 5) either in
lates elevated expression of the
we wished to examine the mechanism through which LPS stimu-
exhibits constitutive instability (9, 10, 18, 19). In the present study,
elevated in response to LPS and furthermore that the FPR1 mRNA
reports have demonstrated that expression of different FPRs can be
expression of the receptor-encoding genes (5, 6, 9, 10). Several
of receptor function, subcellular localization, and, more recently,
known to be regulated at a number of levels, including modulation
mRNA as described in the legend to Fig. 1. B, TG-elicited macrophages
were plated at 1 × 10⁷ cells per 100-mm dish and treated for 4 h with LPS
(100 ng/ml), TNF-α (10 ng/ml), or supernatants (Sup) from LPS-stimu-
lated macrophages (prepared as described in the legend to Fig. 5) either in
the presence or absence of neutralizing Ab to TNF-α as indicated. Levels
of FPR1 and GAPDH mRNA were determined as described in the legend
to Fig. 1. Similar results were obtained in two separate experiments. NT, No treatment.

increased levels of FPR1 mRNA as seen in cells treated directly
with LPS.

Discussion
The trafficking of inflammatory leukocytes to sites of injury or
infection is an important determinant of both the nature and mag-
nitude of the ensuing inflammatory response. Although the process
is complex and involves many steps, the expression and function
of both chemoattractants and their receptors are likely to be pri-
mary contributing components. Chemoattractant receptors such as
those recognizing formyl-methionine terminated peptides are
known to be regulated at a number of levels, including modulation
of receptor function, subcellular localization, and, more recently,
expression of the receptor-encoding genes (5, 6, 9, 10). Several
reports have demonstrated that expression of different FPRs can be
elevated in response to LPS and furthermore that the FPR1 mRNA
exhibits constitutive instability (9, 10, 18, 19). In the present study,
we wished to examine the mechanism through which LPS stimu-
lates elevated expression of the FPR1 gene. The data clearly dem-
onstrate that FPR1 mRNA levels are dramatically elevated in both
macrophages and neutrophils following exposure to LPS and that
this results both from elevated transcription and from stabilization
of the FPR1 mRNA. In addition, the ability of LPS to enhance
FPR1 expression levels appears to be at least partly a result of the
action of one or more intermediate secreted products.

We have recently reported that FPR1 mRNA is constitutively
unstable and exhibits a half-life of between 60 and 90 min (18),
consistent with an earlier study of FPR1 expression in human
HL-60 cells (19). Although both macrophages and neutrophils ex-
press the FPR1 protein under “resting” conditions, the marked in-
stability of the mRNA provides an opportunity to achieve rapid
modulation of gene expression. Thus, reduction in the rate of tran-
scription would lead to rapid disappearance of short-lived mRNAs,
whereas increasing transcriptional activation would have only
modest impact if the half-life of the message was not also pro-
longed. Although LPS appears to increase the transcription of the
FPR1 gene, it is likely that the documented change in mRNA
decay is also an important mechanism for achieving the increased
FPR1 mRNA levels seen in LPS-stimulated cells.

LPS, along with other proinflammatory stimuli, is known to
induce enhanced expression of multiple short-lived mRNAs by pro-
moting increased stability (33, 34). This process appears to depend
upon AU-rich sequence elements generally located within the 3’
untranslated region. Interestingly, the FPR1 mRNA has a very
short 3’ untranslated region (~100 nt) which appears to be devoid
of AU-rich sequences. This suggests that there will be distinct
nucleotide motifs within the FPR1 mRNA sequence that confer
both instability and sensitivity to stimulation with LPS. Further-
more, the mechanisms through which LPS promotes enhanced sta-
bility are likely to be different from those operating on AU-rich
sequence element-containing mRNAs. It is noteworthy that ex-
pression of several chemokine receptors including CCR1, CCR2,
and CXCR1 have been reported to exhibit significant regulatory
control at the level of mRNA decay, in particular following stim-
ulation with LPS (15–17). The regulatory sequences responsible
for both instability and sensitivity to LPS for these mRNAs are
also unknown. However, considering that this behavior is the op-posite of that seen with FPR1, it is also likely that it will be mech-anistically distinct from that operating to control the degradation of FPR1 mRNA.

Many changes in gene expression seen in LPS-treated macro-phages are known to be direct and not dependent on intermediate events. The induction of type I IFN-stimulated genes such as inducible NO synthase and IFN-γ-inducible protein 10 in response to stimulation with LPS (the MyD88-independent pathway) is, however, a well-documented example of an indirect response to LPS (28–30). The effects of LPS on FPR1 expression do not appear to utilize this specific MyD88-independent pathway since IFN-β has no effect on FPR1 mRNA levels. Moreover, ligands that stimulate macrophages through TLR2 or TLR3 are also capable of induced FPR1 expression. Since in these two TLRs are each depend-ent on distinct and non-overlapping downstream signaling adap-tor proteins (MyD88 and Toll-IL-1R adapter protein for TLR2 and Toll receptor IFN-inducing factor for TLR3) (35–37), the pathway leading to FPR1 modulation does not correlate with either the MyD88-dependent or MyD88-independent pathways.

Multiple lines of evidence support the hypothesis that LPS stimula-tion leads to the production of secretory products that are able to independently stimulate the elevated expression of FPR1 mRNA. The ability of CHX to partially compromise the effects of LPS is consistent with the possibility that the LPS-mediated change in FPR1 depends on or requires this intermediate event. The finding that the time course of response is not different be-tween secreted activity and LPS, and that LPS is noticeably the inducing factor and lipopolysaccharide regulate the expression of interleukin 8 receptors on polymorphonuclear leukocytes. J. Biol. Chem. 270: 28188–28192.


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