Lipopolysaccharide Induces Epithelium- and Prostaglandin E₂-Dependent Relaxation of Mouse Isolated Trachea through Activation of Cyclooxygenase (COX)-1 and COX-2

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ABSTRACT

Lipopolysaccharide (LPS), a Toll-like receptor (TLR) 4 agonist, causes airway hyperreactivity through nuclear factor-κB (NF-κB). Because NF-κB induces cyclooxygenase-2 (COX-2) to increase synthesis of prostaglandins (PGs), including the potent airway anti-inflammatory and smooth muscle relaxant PGE₂, we investigated whether LPS causes short-term PGE₂-dependent relaxation of mouse isolated trachea. In rings of trachea contracted submaximally with carbachol, LPS caused slowly developing, epithelium-dependent relaxations that reached a maximum within 60 min. Fluorescence immunohistochemistry revealed TLR4-like immunoreactivity localized predominantly to the epithelium. The LPS antagonist polymixin B; the nonselective COX inhibitor indomethacin; and the selective COX-1 and COX-2 inhibitors 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC560) and 4-[5-(4-chlorophenyl)-1-(trifluoromethyl)-1H-pyrazol-1-y1]benzenesulfonamide (SC293), respectively; the transcription inhibitor actinomycin D; the translation inhibitor cycloheximide; the p38 mitogen-activated protein kinase (p38 MAPK) inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580); and a combination of the mixed PDE1/PDE2 receptor antagonist 6-isopropoxy-9-xanthone-2-carboxylic acid (AH6809) and the EP4 receptor antagonist 4'-[3-butyl-5-oxo-1-(2-trifluoromethyl-phenyl)-1-5-dihydro-[1,2,4]triazol-4-ylmethyl]-biphenyl-2-sulfonic acid (3-methyl-thiophene-2-carboxyl)-amide (L-161982) all abolished relaxation to LPS, whereas the NF-κB proteasomal inhibitor Z-Leu-Leu-Leu-aldehyde (MG-132) had no effect on the relaxation in the first 20 min, after which it reversed the response to a contraction. In conclusion, our data indicate that LPS activates airway epithelial TLR4 to cause release of PGE₂ and subsequent EP2 and EP4 receptor-dependent smooth muscle relaxation. Activation of both COX-1 and COX-2 seems to be essential for this novel response to LPS, which also involves cPLA₂, p38 MAPK, NF-κB, and an unidentified NF-κB-independent, labile regulatory protein.

Lipopolysaccharide (LPS), a product of the Gram negative bacterial cell wall, potently stimulates the innate immune system, eliciting both pro- and anti-inflammatory responses (Ulevitch and Tobias, 1995). Mediation of cellular activation in response to LPS is known to occur through Toll-like receptor (TLR) 4, a member of the Toll receptor family (Tapping et al., 2000). TLR4 shares a common proinflammatory signal transduction pathway with other proinflammatory mediators such as IL-1, which leads to the nuclear translocation of NF-κB and transcription of various genes (Vogel et al., 1999). In addition, LPS induces signaling via c-Jun NH₂-terminal kinase, p38 mitogen-activated protein kinase (p38 MAPK), and extracellular signal regulated kinase (ERK) 1/2 (Han et al., 1994; Liu et al., 1994; Hambleton et al., 1996). Activation of NF-κB causes enhanced expression of genes encoding inflammatory cytokines, such as IL-1, IL-6, and TNF-α. NF-κB also stimulates the transcription of genes required for the synthesis of PGs (Kobayashi et al., 2001). Among these are COX-1 and COX-2, which catalyze the conversion of arachidonic acid to PGs. COX-1 is constitutively expressed in many tissues, providing a signaling mechanism for maintaining basal levels of PGs. COX-2 is induced in response to LPS and is responsible for the increased production of PGs observed in the airways of animals and humans with inflammatory diseases (Liu et al., 1994). In addition, COX-2 is a potential target for the development of new anti-inflammatory drugs (Pietropaolo et al., 1999). The role of COX-1 and COX-2 in the airways is not fully understood, but it is clear that both enzymes contribute to the development of airway inflammation and hyperreactivity. The balance between COX-1 and COX-2 activity is thought to be important in determining the outcome of LPS-induced airway responses. In this study, we investigated whether LPS causes short-term PGE₂-dependent relaxation of mouse isolated trachea. We also examined the role of NF-κB, PDE1, and PDE2 in mediating these responses.
flammatory cytokines, acute phase proteins, immunoreceptors, and chemokines important in the recruitment of neutrophils, eosinophils, macrophages, and lymphocytes (Hirano, 1997; Becker et al., 2000). For example, the gene for cyclooxygenase (COX)-2 has NF-xB binding sequences in its promoter region, which are critical for transcriptional activation (Schmedtje et al., 1997). Thus, enhanced COX-2 activity induced by LPS in rat alveolar macrophages, the major cell type thought to mediate most of the responses to inhaled LPS (Koay et al., 2002), is due to the selective increase in levels of COX-2 protein, suggesting that up-regulation of COX-2 is responsible for increased synthesis of prostanoids (PG) and thromboxanes (Lee et al., 2004; Johnston et al., 2004). In addition, LPS has been shown to cause a decrease in the forced expiratory volume in 1 s in asthmatic patients (Michel et al., 1996). By contrast, short-term (30-min) pretreatment of guinea pig isolated trachea with LPS has been shown to reduce contractions to methacholine and histamine largely in an epithelium-independent manner (Fedan et al., 1995). Here, we show in the mouse isolated trachea that LPS induces acute, slowly developing, epithelium-dependent relaxation that requires the simultaneous activation of COX-1 and COX-2 and is mediated by both EP2 and EP4 PGE2 receptors. This novel response to simultaneous activation of COX-1 and COX-2 and is mediated by recovery of active force to the prestimulation level (30% Fmax) without washing out the trypsin, until no relaxation was observed, at which point LPS was then added. The epithelium was removed from the trachea according to the method of Cocks et al. (1999). In brief, warm Krebs’ solution containing 1% Triton X-100 was gently flushed into the trachea in situ before it was dissected. To test the functional effectiveness of epithelium removal, responsiveness to the PAR2 agonist SLIGRL (single letter amino acid code and amidated at the carboxyl terminal) (Cocks et al., 1999) was tested before the addition of LPS.

Immunohistochemistry. Mouse trachea were frozen in OCT (Sakura Finetek USA, Inc., Torrance, CA), and 5-μm serial cryostat sections were cut and air-dried at room temperature for several hours and then fixed in acetone for 10 min. Sections were blocked with normal serum and then labeled with goat anti-mouse TLR4 antibody (1/50). Primary antibody was bound with biotinylated donkey anti-goat IgG (1/500) and fluorescently labeled with streptavidin–Texas Red. Sections were microscopically examined using the appropriate filter.

Materials. Sources for materials are as follows: acetylcholine, capsaicin, carbachol, cycloheximide, indomethacin, lipopolysaccharides (E. coli strain O127:B8), substance P, Triton X-100, and trypsin (Sigma-Aldrich, St. Louis, MO); nifedipine (Sapphire Bioscience, Redfern, NSW, Australia); AACOCF3, AH8809, L732128, and MG-132 (Tocris Cookson Inc., Ellisville, MO); actinomycin D and SB203580 (A.G. Scientific, San Diego, CA); SC236 and SC560 (Cayman Chemical, Ann Arbor, MI); polymixin B (Invivogen, San Diego, CA); streptavidin–Texas Red (Vector Laboratories, Burlington, CA), all antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); and PAR2-activating peptide SLIGRL (Auspep, Melbourne, VIC, Australia). L-161982 was a kind gift from Dr. Robert Young (Merk Frosst, Kirkland, QC, Canada). Acetylcholine, carbachol, indomethacin, L-161982, lipopolysaccharides, polymixin B, substance P, SLIGRL, and trypsin were made up as concentrated stock solutions in distilled water. AACOCF3, capsacin, L732138, nifedipine, SB203580, SC236, and SC560 were made up in 100% ethanol. Actinomycin D, AH8809, cycloheximide, and MG-132 were made up in dimethyl sulfoxide (DMSO). Of the above-mentioned vehicles, only DMSO had an affect on LPS-induced relaxation of mouse isolated trachea, causing a small, but significant (p < 0.05) inhibition of the response at 20 min (Fig. 5).

Data Analysis. Responses (mean ± S.E.M) are expressed as percentage of relaxations or contractions from the 30% Fmax level of active force. Comparisons between groups were made using either unpaired t tests or one-way analysis of variance, with Bonferroni’s corrections for multiple comparisons. p < 0.05 was accepted as being significant.

Results

Epithelium-Dependent Relaxation to LPS. LPS purified by either phenol or trichloroacetic acid (TCA) extraction
caused similar delayed onset, slowly developing relaxations that reached maxima of 76.4 ± 2.4 and 77.0 ± 6.9%, respectively, at 60 min (Fig. 1). The spontaneous relaxation of control tissues was 23.6 ± 7.3% at 60 min (Fig. 1). Therefore, all subsequent experiments were carried out using LPS purified by either TCA or phenol. The response to LPS was abolished following in situ perfusion of the trachea with 1% Triton X-100. We have previously demonstrated histologically that this procedure removes most of the epithelium in mouse trachea and leaves the underlying smooth muscle structurally normal (Cocks et al., 1999). Success of this method to remove most of the epithelium while not damaging the smooth muscle was determined functionally with the epithelium-dependent PAR2 peptide SLIGRL (Cocks et al., 1999). Thus, in control and Triton X-100-treated tissues 10 μM SLIGRL caused maximal relaxations of 87.4 ± 3.9 and 86.3 ± 3.9%, respectively (Fig. 2). Using a mouse-specific TLR4 antibody, extensive TLR4-specific immunofluorescence was localized to the epithelium, whereas other structures such as the cartilage and smooth muscle layers were devoid of TLR4-like staining (Fig. 3). Control sections not treated with the primary TLR4 antibody exhibited only weak autofluorescence of the epithelium (Fig. 3).

**Mechanism of LPS-Induced Relaxation.** Incubation of LPS with the LPS antagonist polymixin B (10 μg ml⁻¹) 10 min before addition to the tissue, abolished the relaxation to LPS and instead caused a slowly developing, variable contraction of 16.4 ± 14.5% at 60 min (Fig. 4). Polymixin B alone did not affect the tone of the tissue (data not shown). The sensory nerve toxin capsaicin, the selective NK₁ antagonist L732138, and desensitization of PARs with trypsin had no effect on LPS-induced relaxations that reached maxima of 79.0 ± 2.4, 86.3 ± 5.2, and 87.2 ± 5.1%, respectively, at 60 min (Fig. 4). These results indicate that the relaxation to LPS was unlikely to involve the release of sensory nerve transmitters such as substance P or activators of PAR, and PAR₂ such as mast cell tryptase (Cocks and Moffatt, 2000). The relaxation to LPS was reversed to similar, slowly developing contractions by the nonselective COX inhibitor indomethacin (3 μM) as well as the COX-1 and COX-2 selective inhibitors SC560 (0.3 μM) and SC236 (1 μM), respectively (Fig. 4). The mixed DP/EP1/EP2 receptor antagonist AH6809 (3 μM) and the specific EP4 receptor antagonist L-161982 (1 μM) both trended to inhibit the response to LPS, with relaxation reaching maxima of 52.8 ± 18.4 and 46.0 ± 4.8%, respectively, at 60 min. However, these values were not significantly different from the 60-min control response (77.3 ± 6.2%; Fig. 4). In contrast, AH6809 and L-161982 added together reversed the LPS-induced relaxation to a slowly developing contraction of 10.8 ± 4.4% at 60 min (Fig. 4). Together, these results indicate that LPS causes release from the epithelium of PGEl₂, a known potent airway smooth muscle relaxant (Lan et al., 2001; Vancheri et al., 2004).

The p38 MAPK inhibitor, SB203580 (10 μM), also reversed the LPS-induced relaxation to a slowly developing contraction of 13.3 ± 8.6% at 60 min (Fig. 5), whereas the cPLA₂ inhibitor AAcOCF₃ (10 μM) partially, but significantly (p < 0.05), inhibited the relaxation to LPS (60 min maximal response 47.1 ± 6.3% versus control 75.2 ± 7.1%; Fig. 5). MG-132 (10 μM), a NF-κB proteasomal inhibitor, had no effect on the relaxation to LPS over the first 20 min of the response, but after this time the response was reversed to a

![Fig. 1. LPS-induced relaxation of mouse isolated trachea. LPS was purified by two different techniques (phenol and TCA) (both at 10 μg ml⁻¹), and the responses over time was compared with normal (control) loss of tone. Values (means ± S.E.M from n = 7–25) are expressed as percentages of pre-LPS levels of active force induced by carbachol.](Image)

![Fig. 2.](Image)
contraction (Fig. 5). Finally the transcription inhibitor, actinomycin D (2 μM) and the translation inhibitor, cycloheximide (10 μM) both abolished the relaxation to LPS when added 40 min prior to LPS, giving instead slowly developing contractions of 14.0 ± 3.7 and 10.5 ± 5.7%, respectively, at 60 min (Fig. 6). Actinomycin D (2 μM), however, had no effect on the maximal relaxation to substance P (control, 98.1 ± 1.2%; actinomycin D, 98.4 ± 1.0%; n = 5). Neither actinomycin D nor cycloheximide had any effect on the tone of tissue (data not shown).

Effect of COX-1 and COX-2 Inhibitors on Relaxations to Substance P and SLIGRL. Cumulative relaxation curves for the known epithelium-dependent relaxants, the NK1R agonist substance P (Szarek et al., 1998) and the PAR2-selective peptide agonist, SLIGRL (Cocks et al., 1999) were markedly inhibited by either SC560 (0.3 μM) or SC236 (1 μM) for 1 h; n = 5), desensitization with trypsin (n = 8), the NK1R antagonist L732138 (0.1 μM; n = 3), the nonspecific COX-inhibitor indomethacin (3 μM; n = 4), and the LPS antagonist polymyxin B (10 μg ml⁻¹; n = 4) on the response of mouse isolated trachea to LPS. B, effects of specific COX-1 and COX-2 inhibitors SC560 (0.3 μM; n = 5) and SC236 (1 μM; n = 5) and their control (n = 8) on the response of mouse isolated trachea to LPS. C, effects of the mixed DP/EP1/EP2 antagonist AH6809 (3 μM) and the specific EP4 antagonist L-161982 (1 μM) either alone (n = 4) or combined (n = 6) on the response of mouse isolated trachea to LPS. Control, n = 8.

Fig. 4. A, effects of the sensory nerve toxin capsaicin (10 μM for 1 h; n = 5), desensitization with trypsin (n = 8), the NK1R antagonist L732138 (0.1 μM; n = 3), the nonspecific COX-inhibitor indomethacin (3 μM; n = 4), and the LPS antagonist polymyxin B (10 μg ml⁻¹; n = 4) on the response of mouse isolated trachea to LPS. B, effects of specific COX-1 and COX-2 inhibitors SC560 (0.3 μM; n = 5) and SC236 (1 μM; n = 5) and their control (n = 8) on the response of mouse isolated trachea to LPS. C, effects of the mixed DP/EP1/EP2 antagonist AH6809 (3 μM) and the specific EP4 antagonist L-161982 (1 μM) either alone (n = 4) or combined (n = 6) on the response of mouse isolated trachea to LPS. Control, n = 8.

Fig. 3. Hematoxylin and eosin staining of mouse trachea clearly showing the structure of the epithelium and cartilage (A). Fluorescence immunohistochemical staining for TLR4 in the mouse trachea with primary antibody showing TLR4 immunoreactivity in the epithelium and endogenous biotin in the cartilage (B) and without primary antibody showing endogenous biotin in the cartilage and low-level autofluorescence of the epithelium (C).
This study suggests that the TLR4 agonist bacterial LPS causes acute, sustained release of PGE₂ from mouse airway epithelial cells. Thus, using a sensitive smooth muscle contractility bioassay, we found that LPS caused epithelium-dependent relaxation of the mouse trachea and that the response was blocked by inhibitors of LPS, p38 MAPK, NF-κB, cPLA₂, COX-1 and COX-2, and EP2 and EP4 receptors as well as actinomycin D and cycloheximide. Given the importance of PGE₂ in airway barrier defense (Vancheri et al., 2004), we propose that TLR4-induced release of PGE₂ plays a fine-tuning, regulatory role in the both the innate and acquired immune responses to bacterial infection in the lungs that includes acute bronchodilatation.

The development of airway hyperreactivity after LPS administration to both humans (Michel et al., 1992) and animals (Pauwels et al., 1990; Held and Uhlig, 2000) is well documented. To our knowledge, only one previous study has shown acute inhibitory effects of LPS on airway reactivity, albeit indirectly. Thus, Fedan et al. (1995) showed that acute LPS treatment of perfused guinea pig trachea in vitro depressed contractility to both extraluminally and intraluminally applied histamine and methacholine, which they concluded was due mainly to a direct depressant effect on the trachealis smooth muscle. This is in contrast to our study, where the direct relaxation of mouse trachea to LPS was...
entirely epithelium-dependent. Apart from different species of animals and serotypes of LPS, reasons for the apparent discrepancy between our study and that of Fedan et al. (1995) remain unknown.

Epithelium-dependent airway smooth muscle relaxations caused by PAR and PAR peptide agonists (Cocks et al., 1999), previously thought to be mediated by constitutive COX-2 (Lan et al., 2001), most likely involve both COX-1 and COX-2 isoforms (Kawabata et al., 2004), similar to our finding here for LPS. Kawabata et al. (2004) suggested that, like COX-1, COX-2 is constitutively expressed in the mouse airways and that both isoforms act sequentially to produce PGE₂ in response to PAR activation in a manner analogous to that suggested for PAR₁-induced Cl⁻ secretion in cell monolayers of the intestinal cell line SCBN (Buresi et al., 2002). One way such a pathway may function is that prostanooids formed by one of the COX isoforms enhance activity of the other, as suggested by Yamada et al. (2004). We, however, favor a more simple explanation that both COX isoforms need to be activated in parallel to generate sufficient PGE₂ release from the epithelium to cause relaxation of the underlying smooth muscle. This view is supported by our finding that the concentration-relaxation curve to a combination of two high-efficacy, epithelium-dependent relaxants, the PAR₁ agonist peptide SLIGRL (Cocks et al., 1999) and the NKR agonist substance P (Szarek et al., 1998), was right-shifted approximately 100-fold by either SC560 or SC236 (the same inhibitors used to abolish LPS-mediated relaxation) alone and abolished when both antagonists were added together. Similar relaxation curves to SLIGRL and SP alone were, like the response to LPS, all but abolished by each COX isoform-selective inhibitor. Assuming selective and effective inhibition of COX-1 and COX-2 by SC560 and SC236, respectively, our findings with substance P and SLIGRL indicate that COX-1 and COX-2 produce similar amounts of PGE₂ per unit stimulation, but in amounts insufficient to activate EP₂ and EP₄ receptors on smooth muscle and cause relaxation. Relaxations due to only one COX isoform become evident only when the stimulus strength is increased such as was observed here with combined NKR and PAR₂ activation.

The relatively rapid (minutes) relaxation to LPS, together with the requirement for each isoform to be activated to observe the response, further suggests that, like COX-1, COX-2 is constitutively expressed in the mouse trachea. COX-2 has been found in the epithelium of airways without clinical or histological evidence of airway inflammation (Watkins et al., 1999), and Demoly et al. (1998) reported “constitutive” expression of COX-2 in human nasal respiratory epithelium. Recent studies have also demonstrated constitutive expression of COX-2 in brain and kidney (Harris et al., 1994; Breder et al., 1995). Given that the LPS-induced relaxation was abolished by inhibitors of both gene transcription (actinomycin D) and mRNA translation (cycloheximide) and that both COX isoforms were required to be activated for the response to occur, it remains possible that COX-2 is both rapidly up-regulated and degraded, i.e., it undergoes high turnover. This seems unlikely, however, since similar COX-2 (and COX-1)-dependent relaxations to substance P were unaffected by actinomycin D. The proteasomal (and thus NF-κB) inhibitor MG-132 (Chen et al., 1997), however, did block the latter phase of the response to LPS, suggesting some role for NF-κB-dependent gene transcription. Given NKR-mediated PGE₂ release in the same tissue does not depend on transcription, we suggest that NF-κB is involved in TLR4-mediated PGE₂ release by LPS, but not via up-regulation of COX-2. In addition, both the NF-κB-independent and -dependent early and late phases, respectively, of the LPS response most likely involve events upstream from COX-1, COX-2, and PGE₂ synthesis since downstream events, including actions of PGE₂ on the smooth muscle, would be expected to be similar for TLR4 and NKR.

The rate-limiting step in the biosynthesis of PGE₂ is the liberation of arachidonic acid through the activity of cPLA₂ that is regulated by Ca²⁺-dependent translocation from the cytosol to membranes and phosphorylation, which increases the intrinsic activity of cPLA₂ by 2- to 4-fold (Lin et al., 1993). Our results with the cPLA₂ inhibitor AAOCOF₃ indicate that cPLA₂ is involved in LPS-induced PGE₂ release from mouse airway epithelium. Lin et al. (1993) demonstrated the importance of phosphorylation of cPLA₂ by MAPK in its activation, presenting a link between the MAPK pathway and COX activity. Furthermore, Buresi et al. (2002) suggested that for PAR₁ activation in intestinal epithelium the ERK 1/2 MAPK pathway and COX pathway act in series, because they were able to block cPLA₂ phosphorylation using a mitogen-activated protein kinase kinase inhibitor, showing cPLA₂ to be dependent on the ERK 1/2 MAPK pathway. Our findings with SB203580 suggest that p38 MAPK is the main kinase involved in LPS-induced release of PGE₂ in the airway epithelium, in agreement with Kawabata et al. (2004), who similarly reported p38 MAPK was involved in COX-dependent relaxation of mouse trachea to PAR₁ and PAR₂ activation.

Regardless of the exact signaling pathways involved in LPS-induced PGE₂ release from airway epithelial cells, the reasons why TLR4 is coupled to both COX-1 and COX-2 and why the ensuing process of PGE₂ release seems to be so tightly regulated are intriguing questions. TLRs are expressed on barrier defensive cells such as airway epithelium primarily to detect invading pathogens such as bacteria, viruses, and fungi (Pasare and Medzhitov, 2005). For TLR4, detection of bacterial LPS results in an early, rapid, and predominantly tumor necrosis factor-α-mediated neutrophilia to help kill the invading bacteria. Concomitant release of PGE₂ at first seems to be counterproductive to this essential innate defense mechanism since airway neutrophil trafficking to LPS inhalation in conscious mice is inhibited by PGE₂ applied exogenously (Goncalves de Moraes et al., 1996) or released endogenously by PAR agonists (Moffatt et al., 2002). However, a case may be made for a beneficial role for LPS-induced PGE₂ release during bacterial infections if the time course of release is taken into account. We have shown here using a sensitive PGE₂ bioassay—tracheal smooth muscle relaxation (Cocks et al., 1999; Lan et al., 2001; Kawabata et al., 2004)—that release of PGE₂ from the epithelium in response to LPS seems to accumulate slowly to an apparent maximal rate over 60 min. Therefore, timing of PGE₂ synthesis and release versus release of proinflammatory cytokines may be vital for coordinating neutrophilia in response to LPS. Smooth muscle relaxation and thus dilatation of the inflamed airways would similarly be beneficial by helping maintain airflow in a setting of infection. Another possible beneficial role for delayed TLR4-mediated PGE₂ release from
airway epithelium could be to help prime the acquired immune system toward an appropriate antibody response since, like certain proinflammatory cytokines, PGE2 is a key mediator of dendritic cell activation (Kalinski et al., 1998). Again, timing between LPS-mediated PGE2 and release and release of other comodulators such as IL-6 and IL-8 (Medzhitov et al., 1997) may be vital for an appropriate delayed antibody response. It should, however, be noted that long-term exposure to LPS has been shown to cause airflow epithelial cell damage (Folks et al., 1988) and reduction in PGE2 release (Folks et al., 1989). Therefore, we suggest that any immunomodulatory effects of LPS occur over a relatively short term following initial exposure.

In conclusion, epithelium-dependent relaxation to LPS through TLR4 in mouse isolated trachea seems to involve a novel p38 MAPK- and cPLA2-dependent pathway that leads to activation of constitutive COX-1 and COX-2 and release of PGE2. In addition, although NF-κB may have a regulatory role in the later stages of this acute response to LPS, it seems to be predominantly controlled by another labile, as yet unidentified protein. We speculate that during bacterial infections, such tight control allows TLR4 to fine-tune the guinea-pig isolated trachea coincides with decreased prostaglandin E2 production. In addition, although NF-κB/H9260 is associated with the macula densa of rat kidney and increases with salt restriction.


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