Heme oxygenase-1 (HO-1) is an inducible enzyme that catalyzes the first and rate-limiting step in the oxidative degradation of free heme into ferrous iron, carbon monoxide, and biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase (1, 2). In mammalian cells, three genetically distinct isozymes have been identified. HO-1 is an inducible form, whereas HO-2 and HO-3 are constitutively expressed forms. HO-1 expression is induced in various cell lines by a range of stress stimuli, including lipopolysaccharide (LPS), lipoteichoic acid, peptidoglycan, and proinflammatory cytokines (3–7). The increased HO-1 expression induced by these stress stimuli is thought to be an adaptive mechanism that protects the cells from immunopathogenesis or stress damage (8). For instance, Rushworth et al. reported that LPS-induced HO-1 and NAD(P)H:quinone oxidoreductase (NQO1) protected against excessive inflammatory responses in human monocytes (4, 9). Very recently, HO-1 has been reported to regulate the immune response to influenza virus infection and vaccination in aged mice (10). In addition, the HO-1 metabolites carbon monoxide, bilirubin, and ferritin play cytoprotective roles in many kinds of organ injury (11, 12). It has been reported that carbon monoxide, a product of HO-1, augments caveolin-1 (cav-1)/Toll-like receptor 4 (TLR4) interactions to downregulate proinflammatory signaling upon LPS stimulation (13). Furthermore, biliverdin from HO-1 protects against endotoxin-induced acute lung injury in rats (14). These studies suggest that HO-1 and its metabolites play important roles in suppressing deleterious increases in inflammation and oxidative injury.

Monocytes and macrophages play essential roles in inflammation and the mobilization of host defenses against mycoplasma infection. Mycoplasma lipoproteins are regarded as major virulence factors that contribute to the pathogenesis of mycoplasmas by the production of various inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor α (TNF-α) in monocytes and macrophages (15, 16). Macrophage-activating lipopeptide-2 (MALP-2), a molecular component of the surface membrane of Mycoplasma fermentans, is a potent inducer of chemokines and cytokines (17). MALP-2 activates gene transcription by binding to its membrane receptor, TLR2/6, on circulating monocytes and other cell lines, inducing the signal transduction pathways leading to the phosphorylation of kinases, including the IκB kinases and mitogen-activated protein kinases (MAPKs), and the activation of NF-κB and AP-1 families (18, 19). MALP-2 activation of monocytes results in a wide range of responses, including the secretion of proinflammatory factors, expression of chemoattractant proteins, and release of prostaglandins via cyclooxygenase (COX) pathways (17, 20). Nevertheless, MALP-2 is also reported to facilitate dendritic cell maturation and modulate proteasome composition and activity (21), and its synthetic derivative S-[2,3-bispalmitoyloxy-(2R)-propyl]-R-cysteinyl-amido-monomethoxy polyethylene glycol (BPP) exhibits some adjuvant effects for cross-priming against cellular antigens (22). Moreover, MALP-2 can induce endothelial cell proliferation and migration and a strong secretion of granulocyte-macrophage colony-stimulating factor to enhance or restore blood flow and recruit immune response cells. The production of various inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, and TNF-α, by monocytes and macrophages in response to MALP-2 stimulation (13) further suggests that MALP-2 may play a role in the activation of the innate immune system and the initiation of the inflammatory response to mycoplasma infection.

The role of heme oxygenase in mycoplasma infection is not well understood. Heme oxygenase-1 (HO-1) is a stress-inducible enzyme that confers cytoprotection against oxidative injury and performs a vital function in the maintenance of cell hemostasis. Increasing numbers of reports have indicated that mycoplasma-derived membrane lipoproteins/lipopeptides, such as macrophage-activating lipopeptide-2 (MALP-2), function as agents that stimulate the immune system by producing various inflammatory mediators, such as cytokines and cyclooxygenase 2 (COX-2), which play roles in the pathogenesis of inflammatory responses during mycoplasma infection. Here, we report that MALP-2 induced HO-1 mRNA and protein expression and upregulated HO-1 enzyme activity in THP-1 cells. Specific inhibitors of mitogen-activated protein kinases (MAPKs), SB203580, PD98059, and SP600125, significantly abolished HO-1 expression. In addition, MALP-2 also induced NF-E2-related factor 2 (Nrf2) translocation, and the silencing of Nrf2 expression in THP-1 cells decreased the levels of MALP-2-mediated HO-1 expression. Furthermore, COX-2 protein expression levels were upregulated in THP-1 cells in response to MALP-2, and transfection with small interfering RNAs of HO-1 significantly increased COX-2 accumulation. These results demonstrate that MALP-2 induces HO-1 expression via MAPKs and Nrf2 pathways and, furthermore, that MALP-2-induced COX-2 expression was modulated by HO-1 in THP-1 cells.

Mycoplasma fermentans MALP-2 Induces Heme Oxygenase-1 Expression via Mitogen-Activated Protein Kinases and Nrf2 Pathways To Modulate Cyclooxygenase 2 Expression in Human Monocytes

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Mycoplasma fermentans MALP-2 can induce endothelial cell proliferation and migration, and the mobilization of host defenses against mycoplasma infection. Mycoplasma lipoproteins are regarded as major virulence factors that contribute to the pathogenesis of mycoplasmas by the production of various inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor α (TNF-α) in monocytes and macrophages (15, 16). Macrophage-activating lipopeptide-2 (MALP-2), a molecular component of the surface membrane of Mycoplasma fermentans, is a potent inducer of chemokines and cytokines (17). MALP-2 activates gene transcription by binding to its membrane receptor, TLR2/6, on circulating monocytes and other cell lines, inducing the signal transduction pathways leading to the phosphorylation of kinases, including the IκB kinases and mitogen-activated protein kinases (MAPKs), and the activation of NF-κB and AP-1 families (18, 19). MALP-2 activation of monocytes results in a wide range of responses, including the secretion of proinflammatory factors, expression of chemoattractant proteins, and release of prostaglandins via cyclooxygenase (COX) pathways (17, 20). Nevertheless, MALP-2 is also reported to facilitate dendritic cell maturation and modulate proteasome composition and activity (21), and its synthetic derivative S-[2,3-bispalmitoyloxy-(2R)-propyl]-R-cysteinyl-amido-monomethoxy polyethylene glycol (BPP) exhibits some adjuvant effects for cross-priming against cellular antigens (22). Moreover, MALP-2 can induce endothelial cell proliferation and migration and a strong secretion of granulocyte-macrophage colony-stimulating factor to enhance or restore blood flow and recruit immune response cells. The production of various inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, and TNF-α, by monocytes and macrophages in response to MALP-2 stimulation (13) further suggests that MALP-2 may play a role in the activation of the innate immune system and the initiation of the inflammatory response to mycoplasma infection.

The role of heme oxygenase in mycoplasma infection is not well understood. Heme oxygenase-1 (HO-1) is a stress-inducible enzyme that confers cytoprotection against oxidative injury and performs a vital function in the maintenance of cell hemostasis. Increasing numbers of reports have indicated that mycoplasma-derived membrane lipoproteins/lipopeptides, such as macrophage-activating lipopeptide-2 (MALP-2), function as agents that stimulate the immune system by producing various inflammatory mediators, such as cytokines and cyclooxygenase 2 (COX-2), which play roles in the pathogenesis of inflammatory responses during mycoplasma infection. Here, we report that MALP-2 induced HO-1 mRNA and protein expression and upregulated HO-1 enzyme activity in THP-1 cells. Specific inhibitors of mitogen-activated protein kinases (MAPKs), SB203580, PD98059, and SP600125, significantly abolished HO-1 expression. In addition, MALP-2 also induced NF-E2-related factor 2 (Nrf2) translocation, and the silencing of Nrf2 expression in THP-1 cells decreased the levels of MALP-2-mediated HO-1 expression. Furthermore, COX-2 protein expression levels were upregulated in THP-1 cells in response to MALP-2, and transfection with small interfering RNAs of HO-1 significantly increased COX-2 accumulation. These results demonstrate that MALP-2 induces HO-1 expression via MAPKs and Nrf2 pathways and, furthermore, that MALP-2-induced COX-2 expression was modulated by HO-1 in THP-1 cells.

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cells for pathogen defense and tissue regeneration (23). These studies have demonstrated that MALP-2 exhibits multiple effects on the immune system. However, whether mycoplasma-derived MALP-2 induces the expression of HO-1 to serve as a cytoprotective agent is still unknown.

HO-1 has been reported to have cytoprotective effects in mediating cellular homeostasis as a general inducible protein (24). For this reason, considerable research efforts have focused on characterizing the molecular mechanisms that regulate the expression of HO-1. When activated, NF-E2-related factor 2 (Nrf2) and Nrf2 transcription factors, induces the expression of a host of cytoprotective and detoxification genes such as HO-1 and NQO1 (4, 9). In this study, we investigated the mechanisms underlying MALP-2-induced HO-1 expression in THP-1 cells and the potential effects of HO-1 in modulating MALP-2-induced COX-2 expression. Our results demonstrate that MALP-2 can activate MAPKs and Nrf2 pathways to induce the expression of HO-1. Silencing the expression of HO-1 prolongs MALP-2-induced COX-2 expression.

MATERIALS AND METHODS

Materials. MALP-2 was purchased from Alexis Biochemicals (product number ALX-162-027-C050). M. fermentans (PG18, ATCC 19889) was obtained from the ATCC. Real-time PCR primers for HO-1 and β-actin were synthesized by Invitrogen. The MAPK-specific inhibitors SB203580, SP600125, PD98059, and anti-β-actin antibody were purchased from Sigma-Aldrich. Anti-HO-1, anti-Nrf2, and anti-COX2 monoclonal antibodies were products of Cell Signaling Technology, Inc. Horseradish peroxidase (HRP)-labeled secondary antibodies (goat anti-rabbit IgG and goat anti-mouse IgG) and polyvinylidene difluoride (PVDF) membranes were purchased from Millipore. The TATA binding protein (TBP) polyclonal antibody was a product of Proteintech. The heme oxygenase-1 enzyme activity assay kit was obtained from GenMed Sciences (Shanghai, China). Radioimmunoprecipitation assay (RIPA) buffer and NE-PER nuclear and cytoplasmic extraction reagents were purchased from Pierce Biotechnology. Protease and phosphatase inhibitors were purchased from Roche. Nrf2, HO-1 small interfering RNA (siRNA), and control (Con) siRNA were purchased from Ribobio Co. Ltd. (Guangzhou, China). All of the cell culture flasks and plates were purchased from Corning, and the endotoxin-free consumables were obtained from Xygen and Gilson.

Cell culture and MALP-2 stimulation. Human monocytic THP-1 cells were purchased from ATCC and cultured in RPMI 1640 medium (HyClone) supplemented with 10% fetal bovine serum (FBS) ( Gibco), 2 mM 1-glutamine, 100 µg ml⁻¹ penicillin, and 100 µg ml⁻¹ streptomycin. Cells were maintained in a humidified atmosphere at 37°C and 5% CO₂. For stimulation experiments, THP-1 cells were seeded in serum-free medium in 6-well plates (1 × 10⁶ well⁻¹) and allowed to cultivate overnight. Then cells were stimulated with MALP-2 for appropriate time intervals according to the protocols.

Mycoplasma culture and inactivation preparation. M. fermentans cells were cultivated in medium containing 20% horse serum, 10% freshly prepared yeast extract, 1% glucose, and 1,000 µM penicillin G under the conditions of 37°C and 5% CO₂ and quantified as described previously (25). For heat inactivation, the M. fermentans (10⁸ color-changing units [CCU] ml⁻¹) cells were isolated and resuspended in Hayflick medium, followed by incubation at 60°C for 30 min; no growth was observed over a 2-week period. The endotoxin levels of the heat-inactivated mycoplasma (HIM) preparations were <60 pg ml⁻¹, as determined by a Limulus amebocyte lysate assay (Pyrochrome).

Inhibitor treatment. THP-1 cells were cultivated in serum-free medium in 6-well plates (10⁶ well⁻¹) with SB203580 (30 µM), PD98059 (30 µM), SP600125 (30 µM), and a vehicle control (dimethyl sulfoxide [DMSO] <0.1%) for 30 min. Then the THP-1 cells were washed twice with warm phosphate-buffered saline (PBS) by centrifugation at 1,000 × g for 5 min, followed by further culturing for 16 h in complete medium in the presence or absence of MALP-2. For all inhibitors, the concentrations used were those recommended by Davies et al. (26). Cell viability was assessed by a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The inhibitors did not induce obvious cell toxicity.

RNA extraction and real-time PCR. To extract total RNA from 1 × 10⁶ cells, we used the TRizol reagent (Invitrogen) according to the manufacturer’s instructions. Reverse transcription of 3 µg total RNA was performed according to the instructions provided by Promega. Sequences of primers were as follows: HO-1, 5’-ATGGCTTCCCT GTACACCATC-3’ (forward) and 5’-TGTGTCGCTAATCTCTC CT-3’ (reverse); β-actin, 5’-CATCCTGGTCTGACCTTG-3’ (forward) and 5’-TAATGTCACCCAGATTTCC-3’ (reverse). Relative quantitative real-time PCR used SYBR green technology (ABI) on cDNA generated from the reverse transcription of purified RNA. After preamplification (50°C for 2 min and 95°C for 10 min), the PCR mixtures were amplified for 40 cycles (95°C for 15 s and 58°C for 1 min) on a real-time PCR detection system (ABI7500). We used the comparative cycle threshold method to normalize the expression levels for each mRNA against the expression levels for β-actin mRNA.

HO-1 enzyme activity assay. Our determined HO-1 activity in micromolar fractions from THP-1 cells by monitoring the conversion of heme into ferrous iron, carbon monoxide, and biliverdin according to the manufacturer’s instructions. Briefly, cell pellets were centrifuged at 8,000 × g for 3 min in 1.5-ml tubes and homogenized in proper GenMed lysates. Homogenates were vortexed at 10-s intervals of 5 min, total 30 min. Supernatants were centrifuged at 10,000 × g for 15 min. When tested, the tubes were marked as sample and background. Then 340 µl of GenMed buffer, 20 µl of reaction liquid, and 20 µl of substrate were added to the sample tubes and a background tube. A GenMed negative control was then added to the background tube and the samples were added to the sample tubes, and then the tubes were vortexed and incubated at 37°C for 1 h. The reaction was stopped with the addition of 0.4 ml of chloroform, and the extraction of the chloroform layer was measured using a spectrophotometer. Bilirubin formation was calculated from the difference in absorption between 464 nm and 530 nm. The HO-1 enzyme activity level was indicated as nanomoles of bilirubin formed per milligram of protein per hour.

Western blotting. Cells were pelleted by centrifugation at 2,500 × g for 5 min and then washed twice with ice-cold PBS, lysed on ice for 15 min in 100 µl of RIPA buffer, and centrifuged at 14,000 × g for 15 min. Cytoplasmic and nuclear extracts were prepared using the NE-PER nuclear and cytoplasmic extraction reagents according to the manufacturer’s instructions. Supernatants were collected and protein concentrations were determined by a Bradford protein assay. Aliquots of cell lysates (80 µg of proteins) were boiled for 5 min in SDS-containing sample buffer and were separated by SDS-PAGE on a 10% to 12% polyacrylamide gel. Blots were transferred to a PVDF membrane and Western blot analyses were performed with the indicated antibody according to their manufacturer’s guidelines. Proteins were detected using an enhanced chemiluminescence (ECL) Western blotting system (Thermo), and the band intensity was measured by densitometric analysis using ImageJ software.

Transient transfection with small interfering RNAs. THP-1 cells (1 × 10⁶ well⁻¹) were transfected with a final concentration of 100 nM Nrf2 siRNA, HO-1 siRNA, or control siRNA for 24 h using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Transfected cells were left unstimulated or treated with MALP-2 (2.5 ng ml⁻¹) for the indicated times. The samples were then prepared for Western blot analysis as described above.

Statistical analyses. Data were assessed as means ± standard deviation (SD) of the results for at least three experiments. One-way analysis of variance (ANOVA) and Student’s t test were used for statistical evaluation. Differences with P values of <0.05 were considered statistically significant.
RESULTS

MALP-2 induces HO-1 mRNA expression in human THP-1 cells. MALP-2, with a structure of Pam2CGNND8SNISFKEK, has been shown to be a potent activator of macrophages and monocytes. To determine the effect of MALP-2 treatment on HO-1 mRNA expression, THP-1 cells were treated with various concentrations of MALP-2 for 12 h or treated with 5.0 ng ml\(^{-1}\) of MALP-2 at various time points, and the mRNA expression levels of HO-1 were determined by real-time PCR analysis. As shown in Fig. 1A, MALP-2 induced HO-1 mRNA accumulation in a concentration-dependent manner. The level of HO-1 mRNA was highest when the concentration of MALP-2 reached 5 ng ml\(^{-1}\). HO-1 mRNA expression induced by MALP-2 was significantly elevated at 4 h, peaked at 8 h, and became attenuated at 12 to 24 h (Fig. 1B). As controls, THP-1 cells were treated with various concentrations of HIM for 12 h or infected with 10^6 CCU ml\(^{-1}\) of HIM for 0, 4, 8, 12, or 24 h, and then induced HO-1 mRNA expression was measured by real-time PCR. HO-1 mRNA expression was normalized to \(\beta\)-actin mRNA levels. Data represent means ± SD from at least three independent experiments. *, \(P < 0.05\), and #, \(P < 0.01\), compared with the basal level.

MALP-2 induces HO-1 protein expression and upregulates its enzymatic activity in THP-1 cells. HO-1 protein plays a pivotal role in monocytes activating inflammation, which counteracts immunopathogenic damage caused by stress stimuli (27, 28). To confirm the mRNA results, we examined HO-1 protein expression by Western blotting. As shown in Fig. 2A, the HO-1 protein level was very low in unstimulated cells; however, HO-1 protein expression was sharply increased in response to 0.01 to 5 ng ml\(^{-1}\) of MALP-2. In addition, the level of HO-1 protein peaked at 12 h, was sustained up to 16 h, and became attenuated at 24 h (Fig. 2B). Given that the HO-1 protein expression level does not always represent its enzymatic activity, we further determined the HO-1 enzymatic activity by measurement of the formation of bilirubin per milligram of protein. As shown in Fig. 2C, a significant increase in HO-1 activity caused by MALP-2 occurred in a dose-dependent manner. These data suggest that the increase in HO-1 protein expression is accompanied by enhanced enzymatic activity.

MALP-2-induced HO-1 expression requires ongoing transcription and translation. How HO-1 is induced in response to MALP-2 is still unknown. To determine whether MALP-2-induced HO-1 expression required transcription or translation, we first examined the accumulation of HO-1 in response to MALP-2 in the absence or presence of a translational level inhibitor, cycloheximide (CHX), or the RNA synthesis inhibitor actinomycin D (ActD). As shown in Fig. 3, MALP-2-mediated induction of HO-1 expression was abolished by either CHX or ActD in a dose-dependent manner. Taken together, these results suggest that in response to MALP-2, HO-1 protein is synthesized de novo in THP-1 cells.

MAPKs pathways involved in MALP-2-induced HO-1 expression in THP-1 cells. MALP-2 has been reported to activate many kinase pathways in THP-1 cells, such as MAPKs (19, 20). The potential role of the MAPK pathways in MALP-2-induced HO-1 expression was examined in THP-1 cells using kinase-specific inhibitors. These included a p38 inhibitor (SB203580), a
MEK1/2 inhibitor (PD98059), and a c-Jun N-terminal kinase (JNK) inhibitor (SP600125). THP-1 cells were pretreated with these inhibitors for 30 min prior to MALP-2 incubation. Total protein was extracted and determined by Western blotting. Figure 4 shows that the levels of HO-1 upregulation by MALP-2 were partially inhibited by SB203580 (38%), PD98059 (44%), and SP600125 (56%). These results indicate that the p38, ERK1/2, and JNK pathways participate in the MALP-2-mediated induction of HO-1.

MALP-2-induced HO-1 upregulation is mediated via Nrf2 signaling. Nrf2 is a redox-sensitive basic leucine zipper transcrip-
tion factor of the NADPH oxidase complex that is activated by oxidative stresses and translocates into the nucleus (29). The activation of Nrf2 has been reported to play an important role in the antioxidant response element (ARE)-driven expression of several detoxifying and antioxidant enzymes, including HO-1 (9). To confirm whether MALP-2 induced Nrf2 translocation, THP-1 cells were treated with MALP-2 for various times, and then Nrf2 cytosolic and nuclear protein expression was prepared and analyzed by Western blotting. As illustrated in Fig. 5A, Nrf2 was present in the cytosol in unstimulated THP-1 cells but had almost disappeared by 120 min in response to MALP-2. Conversely, Nrf2 was undetectable in the nuclei of unstimulated THP-1 cells but, in response to MALP-2, significantly increased at 60 min and continued to increase at 120 min. To further determine the role of Nrf2 in MALP-2-dependent HO-1 induction, cells were transiently transfected with Nrf2 siRNA. As illustrated in Fig. 5B and C, transfection with Nrf2 siRNA downregulated the protein expression of Nrf2 and subsequently decreased the HO-1 expression induced by MALP-2.

Silencing HO-1 enhances MALP-2-induced COX-2 expression. MALP-2 has been shown to induce COX-2 accumulation in human placental trophoblast cells and macrophages (20, 30). Little is known, however, about whether HO-1 is involved in MALP-2-induced COX-2 expression. In this study, we found that MALP-2 significantly induced COX-2 expression in THP-1 cells (Fig. 6A). To confirm the effect of HO-1 on MALP-2-mediated COX-2 expression, THP-1 cells were transfected with HO-1 siRNA. As illustrated in Fig. 6B, transfection of THP-1 cells with HO-1 siRNA resulted in the significant knockdown of HO-1 protein upregulation and caused significant induction of COX-2 attributable to MALP-2.

**DISCUSSION**

LPS has been shown to be an important regulator of bacterial infections in monocytes and macrophages. In response to LPS, mononuclear phagocytes also synthesize intracellular cytoprotective proteins, including HO-1 and NQO1, to protect against excessive inflammatory responses in human monocytes (4, 9). In this study, our present data clearly demonstrate that MALP-2, like LPS, induces HO-1 mRNA and protein expression and increases its enzymatic activity levels in THP-1 cells. Moreover, MALP-2-dependent HO-1 expression is mediated by MAPKs and Nrf2
antioxidant response elements (AREs) (4). Nrf2 has been shown to be a key factor in the ARE-mediated induction of antioxidant proteins and the regulation of inflammatory responses in response to various stimuli (37, 38). Rushworth et al. reported that Nrf2 regulates LPS-induced HO-1 and NQO1 protein production and thus modulates the LPS-induced proinflammatory response (9). Other studies have shown that Nrf2 activation inhibits proinflammatory cytokine-induced adhesion molecule expression in endothelial cells. Conversely, exposure to endotoxin leads to higher levels of TNF and interleukin-6 expression in Nrf2-deficient mice than in wild-type animals (39). In this study, we have demonstrated that MALP-2 also activates Nrf2 translocation to induce HO-1 accumulation, and furthermore, that suppressing Nrf2 production with siRNA attenuates HO-1 protein expression.

COX-2 is an inducible form of cyclooxygenase, and its expression level regulates endogenous prostaglandin synthesis. More and more studies have shown that COX-2-induced pathways play important roles in modulating inflammatory reactions (40). For example, the induction of COX-2 expression by cigarette smoke contributes to the proinflammatory effects of prostaglandin E2 (PGE₂) in the airways of subjects with chronic obstructive pulmonary disease (COPD) (41). In human placental trophoblasts, the expression of COX-2 and production of PGE₂ caused by MALP-2 may be involved in the mechanism of preterm labor (30). COX-2-mediated pathways were involved in the production of CXC motif chemokine ligand 8 (CXCL8), CXCL1, CXCL5, and vascular endothelial growth factor (VEGF), which may have important roles in the pathogenesis of pulmonary fibrotic disorders (42). Therefore, elucidating the mechanism by which COX-2 expression is regulated will be crucial to our understanding of these COX2-regulated pathophysiological processes. Recently, the induction of HO-1 has been reported to attenuate LPS-induced COX-2 expression in mouse brain endothelial cells (43). Furthermore, some COX-2-selective inhibitors suppressed the expression of COX-2 by the induction of HO-1 expression (44, 45). In the current study, we found that HO-1 modulates MALP-2-induced COX-2 expression in THP-1 cells, suggesting a multifunctional role of HO-1 in balancing the relation of anti-inflammation and proinflammation.

The mechanism by which many inducers induce HO-1 production is a matter of much discussion (27, 36, 46, 47). This report adds new insight to the discussion because we have observed that upon activation with MALP-2, HO-1 expression is dependent on the activation of MAPKs and Nrf2. HO-1 protein subsequently contributes to the regulation of monocyte responses to MALP-2, so that monocytes are less likely to generate an excessive inflammatory response. Recently, MALP-2 has been found to improve lung host defenses against infections with mycobacteria (48) and improve reendothelialization in a murine model of experimental vascular injury (49). In addition, MALP-2 can reduce bacteremia and improve bacterial clearance in lung parenchyma (50) and can accelerate wound healing in diabetic mice (51). The induction of Nrf2 translocation to the nucleus to control excessive inflammation is a process that might be important to our understanding of the pharmaceutical applications of MALP-2. In this regard, MALP-2 analogues developed without any inflammatory reactions to host cells are expected to eliminate the possibility of the emergence of some infections. Thus, further understanding of the functions of MALP-2 and its analogues that regulate Nrf2 and its gene products may lead to the identification of novel therapeu-
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