MKP-1 switches arginine metabolism from nitric oxide synthase to arginase following endotoxin challenge

Leif D. Nelin,1 Xianxi Wang,1 Qun Zhao,1 Louis G. Chicoine,2 Tamara L. Young,1 Dionna M. Hatch,1 B. Keith English,3 and Yusen Liu1

Centers for 1Perinatal Research and 2Gene Therapy, Columbus Children’s Research Institute, Department of Pediatrics, The Ohio State University, Columbus, Ohio; and 3Department of Pediatrics, Children’s Foundation Research Center at Le Bonheur Children’s Medical Center, University of Tennessee Health Sciences Center at Memphis, Memphis, Tennessee

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L-ARGININE (L-arg) is the substrate for both nitric oxide (NO) synthase (NOS) and arginase. Metabolism of L-arg by NOS produces L-citrulline and NO, whereas metabolism of L-arg by arginase produces L-ornithine and urea. There are three described isoforms of NOS, neuronal NOS, inducible NOS (iNOS), and endothelial NOS, of which iNOS is abundantly expressed in macrophages (1, 7). The NO produced by iNOS has a wide variety of physiological functions in the inflammatory response, including vasodilation and viral and bacterial killing (2). There are two described isoforms of arginase (26). Arginase I has been referred to as the hepatic isoform, although recent studies demonstrate that arginase I expression can be induced by lipopolysaccharide (LPS), IL-13, and alterations in oxygen tension in a wide variety of cells and tissues (5, 10, 27, 28, 30). Arginase II has been referred to as an extra-hepatic isoform, and arginase II expression is also inducible by a variety of factors, including LPS, TNF-α, IFN-γ, 8-bromo-cGMP, and hyperoxia (10, 26, 27, 28). The L-ornithine produced by arginase is a precursor for polyamine and L-proline synthesis, which are vital to tissue repair processes following injury (35). Thus it has been postulated that, in inflammatory diseases, NO production from L-arg is involved in the initial host response, whereas L-ornithine production from L-arg is involved in healing (17, 23).

It has been found in macrophages that T-cell helper (Th) 1 cytokines, such as TNF-α and IFN-γ, result in iNOS expression and Th2 cytokines, such as IL-13, result in arginase expression (16). The idea that NOS and arginase may have important yet divergent roles in the immune response led us to postulate that switching mechanisms may exist that allow macrophages to redirect L-arg metabolism from NOS to arginase. Previously, it has been reported that the mitogen-activated protein kinases (MAPK) contribute to iNOS induction in LPS-stimulated RAW264.7 cells (4, 7). We have recently shown that macrophages overexpressing MKP-1 have decreased total expression and a shorter duration of p38 and JNK activation following LPS stimulation (32, 36). Thus we hypothesize that MKP-1 attenuates LPS-induced iNOS expression, thus acting as a switch to change L-arg metabolism from the production of NO and L-citrulline to L-ornithine and urea production. To test this hypothesis, we utilized RAW264.7 cell lines stably transfected with an MKP-1 expression vector in thioglycollate-elicited peritoneal macrophages harvested from wild-type and Mkp-1−/− mice, as well as in vivo in wild-type and Mkp-1−/− mice. We found that overexpression of MKP-1 resulted in lower iNOS expression and NO production but greater urea production in response to LPS. Although deficiency of MKP-1 resulted in greater iNOS expression and NO production and lower urea production in response to LPS, neither the overexpression nor the deficiency of MKP-1 had any substantial effect on the expression of the arginases.
METHODS

RAW264.7 culture. RAW264.7 macrophages were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FCS (HyClone Laboratories, Logan, UT) at 37°C in a humidified atmosphere containing 5% CO2. Cells were transfected with either a MKP-1 expression construct (pSRα-FLAG-MKP-1) together with pcDNA3 (Invitrogen) or pcDNA3 alone using FuGENE6 transfection reagent (Roche, Indianapolis, IN) according to the manufacturer’s specifications and as previously described (32). Cells were selected in medium containing G418, and resistant clones were isolated. One clone expressing exogenous MKP-1 was designated D3, whereas a clone harboring pcDNA3 was designated D3. LPS (E. coli, O55:B5) was purchased from Sigma Chemicals (St. Louis, MO) dissolved in serum-free medium and added to the medium.

Mice. The generation of MKP-1 knockout mice was described previously (14). Cryopreserved embryos of Mkp-1 knockout mouse (−/−) on a C57BL6/129 mixed background were kindly provided by Bristol-Myers Squibb Pharmaceutical Research Institute (Lawrenceville, NJ) and were regenened into mice in The Jackson Laboratory (Bar Habor, ME). These mice were bred in house to yield both wild-type and Mkp-1−/− mice. All mice were maintained at 24°C with a relative humidity between 30 and 70% on a 12-h day-night cycle. Mice were fed Harlan Teklad irradiated diet (Harlan Sprague-Dawley) ad libitum. All animals received humane care in accordance with the guidelines of the National Institutes of Health under a protocol approved by the Institutional Animal Care and Use Committee of the Columbus Children’s Research Institute.

Peritoneal macrophage isolation and culture. Peritoneal macrophages were obtained from Mkp-1−/− mice and their wild-type littermates as previously described (32). Briefly, mice were injected with 2 ml of 3% brewer thioglycollate medium (BD Diagnostics, Sparks, MD) intraperitoneally. Four days later, cells were harvested by lavage with cold RPMI 1640 medium (Invitrogen) containing 5% FBS and plated into tissue culture plates. Cells were allowed to adhere by lavage with cold RPMI 1640 medium (Invitrogen) containing 5% FBS and plated into tissue culture plates. Cells were allowed to adhere for 2 h, washed free of nonadherent cells, and maintained in RPMI 1640 medium containing 5% FBS.

Protein isolation. Protein was isolated from the RAW264.7 cells by 10.220.33.3 on April 19, 2017 http://ajpcell.physiology.org/ Downloaded from
phenylmethylsulfonyl fluoride]. The lysis buffer was sterile filtered in a syringe and added to each plate of cells. The cells were scraped and centrifuged at 12,000 g for 10 min. Total protein concentration was determined by the Bradford method using a commercially available assay kit (Bio-Rad, Hercules, CA).

**RNA isolation.** RNA was isolated as previously described (32, 36). Briefly, 1 ml of Trizol (Invitrogen) was added to each plate containing the cells and incubated for 5 min at room temperature. Chloroform (0.2 ml) was added and the tubes shaken for 15 s and then incubated at room temperature for 3 min. The mixture was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was transferred to a fresh 1.5-ml tube. Isopropanol alcohol (0.5 ml) was added and the mixture incubated at room temperature for 10 min, then centrifuged at 12,000 g for 15 min at 4°C. The supernatant was discarded, and the pellet was washed with 75% ethanol and centrifuged at 7,500 g for 5 min at 4°C. The supernatant was discarded, and the pellet was partially dried, dissolved in RNAse free water, and stored at −70°C.

**Nitrite assay.** The medium samples were assayed in duplicate for nitrite using a chemiluminescence NO analyzer (model 280, Sievers Instruments, Boulder, CO) as previously described (10, 27, 28). Briefly, 100 µl of sample was placed in a reaction chamber containing a mixture of NaI in glacial acetic acid to reduce nitrite to NO. The NO gas was carried into the NO analyzer using a constant flow of He gas. The analyzer was calibrated using a NaNO2 standard curve.

**Urea assay.** The medium samples were assayed in duplicate for urea colorimetrically as previously described (10, 27, 28). Briefly, 100 µl of sample was added to 3 ml of chromogenic reagent [5 mg of thiosemicarbazide, 250 mg of diacetyl monoxime, 37.5 mg of FeCl3 in 150 ml 25% (vol/vol) H2SO4, 20% (vol/vol) H3PO4] or the same reagents with 0.5 U urease added. After 1 h at 37°C, the mixtures were vortexed and then boiled at 100°C for 5 min. The mixtures were cooled to room temperature, and the difference in absorbance (530 nm) with and without urease was determined and compared with a urea standard curve.

**Western blotting.** The protein lysate from the cells was assayed for MKP-1, FLAG, iNOS, arginase I, and/or arginase II proteins by Western blot analysis, as previously described (27, 32, 36). Aliquots of cell lysate containing equal amounts of protein were diluted 1:1 with SDS sample buffer, heated to 80°C for 15 min, and then centrifuged at 10,000 g at room temperature for 2 min. Aliquots of the supernatant were used for SDS-polyacrylamide gel electrophoresis. The proteins were transferred to polyvinylidene difluoride membranes and blocked overnight in PBS with 0.1% Tween containing 5% nonfat dried milk and 3% albumin. The membranes were then incubated with the primary antibody MKP-1 (1:500; Santa Cruz Biotehnologies, Santa Cruz, CA), FLAG (1:4,000; Berkeley Antibody, Richmond, CA), iNOS (1:5,000; BD Transduction Laboratories, San Diego, CA), arginase I (1:1,000, Abcam, Cambridge, MA), or arginase II (1:200, Santa Cruz Biotechnology) for 4 h and then washed three times with PBS-Tween with 1% nonfat dried milk. The membranes were then incubated with the biotinylated IgG secondary antibody (1:5,000; Vector Laboratories; Burlingame, CA) for 1 h, washed, and then incubated with streptavidien-horseradish peroxidase conjugate (1:1,500; Bio-Rad) for 30 min. The protein bands were visualized using chemiluminescence (ECL reagent; Amersham Pharmacia Biotech, Piscataway, NJ) and quantified using densitometry (Sigma Gel, Jandel

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**Fig. 3.** MKP-1 overexpression does not alter LPS-induced arginase mRNA expression. A: arginase I mRNA expression is not affected by MKP-1 overexpression. A representative RT-PCR gel for arginase I mRNA is shown. The bottom gel represents 18S rRNA that serves as an input control. B: the graph represents the relative densities of the arginase I bands (arginase I OD/18S OD). Values are means ± SE; n = 6. *Significantly different from control (P < 0.05). C: arginase II mRNA expression is not affected by MKP-1 overexpression. Representative RT-PCR gel for arginase II mRNA is shown. The bottom gel is 18S rRNA, which serves as a loading control. D: the graph shows the means ± SE of the relative densities of the arginase II bands (arginase II OD/18S OD); n = 3–4. *Significantly different from control (P < 0.05).
To control for protein loading, the blots were then stripped using a stripping buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, and 100 mM 2-mercaptoethanol), and the blots were reprobed for β-actin or IgG (1:10,000; Abcam), as described above.

Reverse-transcription PCR. Reverse transcription PCR was performed as previously described (10, 27, 28). Briefly, 2 μg of total RNA were reverse transcribed in a 40-μl reaction containing 2.5 μM dT16 (Applied Biosystems), 20 U AMV-RT, 1 mM dNTP, 1X buffer (Promega), and RNase-free water. The samples were incubated in a PCR-iCycler (Bio-Rad) at 42°C for 60 min, followed by 95°C for 5 min. PCR reactions were carried out in 50-μl reactions containing 5 μl of reverse-transcription product, 1 mM MgCl2, 1.25 U AmpliTaqGold (Applied Biosystems), 0.2 mM dNTP (Promega), and 15 μM forward and 15 μM reverse primers. iNOS was amplified using forward primer (5′-TCCAGAACGAAGATGTGACC-3′) and reverse primer (5′-GGACCAGCCAAAATCCAGT-3′). Arginase I was amplified using forward primer (5′-AGGTTGAGTGTTGCCGAACG-3′) and reverse primer (5′-GGTTGAGTTCCGAACACGCAAGGG-3′). Arginase II was amplified using forward primer (5′-ACACGTTTCTGTCAGCTCTCCT-3′) and reverse primer (5′-TCAAGGTTCATCCAGTTATGTC-3′). The mixed samples were heated to 94°C for 4 min, and followed by 94°C for 1 min, 53°C for 1 min, and 72°C for 2 min for 35 cycles for iNOS, 40 cycles for arginase I, and 37 cycles for arginase II. The PCR products were sized by electrophoresis in 2.0% agarose gel and poststaining with Syber Gold (Molecular Probes, Eugene, OR) for 30 min. The gels were scanned and densitized using a MultiGenius Bio Imaging System (Syngene, Frederick, MD), and band density analysis was performed on a personal computer with SigmaGel (Jandel Scientific) software.

Statistical analysis. Values are means ± SE. One-way ANOVA was used to compare the data between the groups. Significant differences were identified using a Neuman-Keuls post hoc test. Differences were considered significant when P < 0.05.

RESULTS

MAP kinases are involved in LPS-induced NO production in RAW264.7 cells. Treatment with LPS increased NO production from 9.2 ± 3.1 nmol/mg protein in control RAW264.7 cells to 156.1 ± 18.6 nmol/mg protein in LPS-treated RAW264.7 cells (P < 0.0005). Interestingly, the LPS-induced increase in NO production was attenuated by ~35% by the p38 inhibitor SB20358 (Fig. 1). Moreover, the LPS-induced increase in NO production was attenuated by ~60% by the JNK inhibitor SP600125 (Fig. 1). In contrast, the MEK1/2 inhibitor U0126 had no discernible effect on the LPS-induced increase in NO production (Fig. 1). These results demonstrate that p38 and JNK activation are involved in the LPS-induced NO production in macrophages.

MKP-1 overexpression attenuates LPS-induced iNOS expression. In both D3 and PC53 cells, LPS-treatment resulted in a time-dependent increase in MKP-1 protein levels (Fig. 2A). The FLAG-MKP-1 was detected only in PC53 cells (Fig. 2A), and there was greater total MKP-1 expression at 90 min in PC53 cells than in D3 cells, such that the PC53 cells had approximately twice as much MKP-1 as the D3 cells. Confirming our previous report that MKP-1 protein half-life increases with LPS treatment (8), the protein level of exogenous FLAG-MKP-1 was also increased on LPS stimulation in PC53 cells (Fig. 2A).

Unretreated D3 and PC53 cells expressed low levels of iNOS mRNA. On LPS treatment, iNOS mRNA expression was significantly increased in both D3 and PC53 cells. However, compared with LPS-treated D3 cells, iNOS mRNA levels in LPS-treated PC53 cells were ~38% lower (Fig. 2C), indicating that enhanced MKP-1 expression inhibits the induction of iNOS mRNA by LPS. In the absence of LPS stimulation, both D3 and PC53 cells had trace amounts of iNOS protein (Fig. 2D). LPS treatment resulted in substantially greater iNOS protein levels in both D3 and PC53 cells. However, compared with iNOS protein levels in LPS-stimulated D3 cells, iNOS protein levels in LPS-stimulated PC53 cells were 40% lower (Fig. 2E), further confirming the observation that MKP-1 attenuates the expression of iNOS.

MKP-1 overexpression had little effect on arginase expression. Arginase I mRNA bands were only readily detectable in cells treated with LPS, regardless of whether the cells expressed exogenous MKP-1 (Fig. 3). On LPS treatment, arginase I mRNA levels were increased by ~16-fold. There was no significant differences in arginase I mRNA levels between the LPS-treated D3 and PC53 cells (Fig. 3B). Arginase II mRNA, was present in both control and LPS-stimulated D3 and PC53 cells. The relative induction of arginase II by LPS treatment was much less than for arginase I in these cells (only about twofold in both D3 and PC53 cells; Fig. 3D). These data indicate that LPS-treatment induced arginase I and II expression and that MKP-1 overexpression had little effect on the LPS-induced arginase I or II expression.

![Graph A](http://ajpcell.physiology.org/)

**A**. Production of nitrite (NO_2^-; nmol/mg protein) in response to LPS was attenuated in cells expressing exogenous MKP-1 (PC53 cells). Values are means ± SE from 6–8 independent experiments. *LPS treated were significantly different from no LPS treatment by 10.2 ± 0.3. 3.3 on April 19, 2017 http://ajpcell.physiology.org/ Downloaded from **B**. Production of urea (nmol/mg protein) after LPS stimulation was enhanced in cells expressing exogenous MKP-1 (PC53 cells). Values are means ± SE from 6–8 independent experiments. *LPS treated were significantly different from no LPS treatment by 10.2 ± 0.3. 3.3 on April 19, 2017 http://ajpcell.physiology.org/ Downloaded from
MKP-1 overexpression decreases NO production and increases urea production. LPS-treatment resulted in a marked increase in NO production in both D3 and PC53 cells. Interestingly, the LPS-induced increase in NO production in PC53 cells was only ~50% that of D3 cells (Fig. 4A), indicating that MKP-1 overexpression attenuated LPS-induced NO production. LPS treatment resulted in a substantial increase in urea production in both D3 and PC53 cells (Fig. 4B). Interestingly, despite no significant differences in either arginase I or II mRNA expression between LPS-treated PC53 and D3 cells, LPS-treated PC53 cells had ~50% more urea production than did LPS-treated D3 cells (Fig. 4B).

MKP-1 deficiency increases NO production and decreases urea production in primary macrophages. Treatment of primary thioglycollate elicited macrophages from Mkp-1<sup>-/-</sup> mice with LPS resulted in substantially greater iNOS protein levels at 24 h than in LPS-treated macrophages from wild-type mice (Fig. 5A). We determined the half-life of iNOS protein in LPS-treated macrophages using cycloheximide (10 μg/ml) and found that there was no difference in iNOS protein half-life in wild-type (1.84 h) vs. Mkp-1<sup>-/-</sup> (1.94 h) macrophages, suggesting that the increase in iNOS protein levels in Mkp-1<sup>-/-</sup> macrophages were due to increased transcription. Protein levels of arginase I (Fig. 5B) and II (Fig. 5C) were induced by LPS.
treatment in macrophages from both wild-type and Mkp-1−/− mice, although there appeared to be greater arginase I protein levels at 24 h in the macrophages from Mkp-1−/− mice than in the macrophages from wild-type mice (Fig. 5B).

Addition of LPS to the medium resulted in a time-dependent increase in NO production beginning at ~6 h after challenge (Fig. 5D). The LPS-induced NO production was greater in peritoneal macrophages from Mkp-1−/− mice than in peritoneal macrophages from wild-type mice at 6, 8, 14, and 24 h post-LPS treatment (Fig. 5D). Treatment with LPS resulted in greater urea production in macrophages harvested from both wild-type and Mkp-1−/− mice beginning ~4–6 h after LPS treatment (Fig. 5E). Contrary to what was observed for NO production, the LPS-induced urea production was greater in the wild-type macrophages than in Mkp-1−/− macrophages (Fig. 5E). Thus, in the absence of Mkp-1, NO production was enhanced, whereas urea production was attenuated following LPS stimulation. Given that iNOS protein levels were substantially greater and that levels of arginase I and II were not less following LPS-challenge in the Mkp-1−/− macrophages, these results suggest that the greater NO production resulted in decreased L-arg bioavailability to arginase in the LPS-stimulated Mkp-1−/− macrophages.

Mkp-1 knockout mice exhibit increased iNOS expression and NO production following LPS challenge. The plasma concentrations of nitrates were greater in Mkp-1−/− mice than in their wild-type littermates, whereas the plasma concentrations of urea were lower in Mkp-1−/− mice than in their wild-type littermates 24 h after LPS challenge (Fig. 6A). Western blot analysis of tissue homogenates revealed a remarkable increase in iNOS protein levels in the lungs and livers from Mkp-1−/− mice but not in tissues from their wild-type littermates (Fig. 6B). However, the levels of arginase I protein were not different in the lungs and livers between LPS-treated Mkp-1−/− mice and their wild-type littermates (Fig. 6C). In the lungs, there was no difference in arginase II protein levels between Mkp-1−/− and wild-type mice (Fig. 6D). We were unable to reliably detect arginase II protein in the livers from either LPS-treated Mkp-1−/− mice or LPS-treated wild-type mice.

**DISCUSSION**

The major findings of this study were that Mkp-1 overexpression resulted in 1) decreased LPS-induced iNOS mRNA and protein expression, 2) no significant effect on LPS-induced arginase I or arginase II mRNA expression, 3) decreased LPS-induced NO production, and 4) increased LPS-induced urea production in a macrophage cell line. Mkp-1 deficiency in thioglycollate-elicted peritoneal macrophages resulted in 1) increased LPS-induced NO production and iNOS protein expression and 2) decreased LPS-induced urea production, with no decrease in the protein levels of arginase I or II. Finally, LPS-treated Mkp-1−/− mice had 1) greater plasma NO concentrations and lower plasma urea concentrations, 2) greater liver and lung iNOS protein levels, and 3) similar levels of lung and liver arginase I and arginase II proteins compared with LPS-treated wild-type mice. These findings support our hypothesis that Mkp-1 attenuates LPS-induced iNOS expression and NO production. The finding that Mkp-1 overexpression had little effect on LPS-induced arginase expression but led to increased LPS-induced urea production suggests that, in the PC53 cells, more L-arg was available to arginase. Conversely, the finding that Mkp-1 deficiency did not decrease LPS-induced arginase expression and yet decreased urea production suggests that less L-arg was available to arginase in the peritoneal macrophages from Mkp-1−/− mice and in vivo. Taken together, these findings support our hypothesis that Mkp-1 may act as a switching mechanism for redirecting L-arg metabolism from NO production to urea and l-ornithine production.

The greater production of urea in the PC53 cells after LPS challenge may be due to increased bioavailability of L-arg to arginase, given that there was little change in arginase protein expression. It has been suggested that the co-induction of iNOS and arginase is a mechanism to limit NO production in macrophages to avoid NO overproduction (6). Although the concept that NOS and arginase compete for a common pool of L-arg is somewhat curious given that the L-arg Km for NOS is ~10 μM and for arginase is ~1 mM, these Km values would suggest that there should be adequate L-arg concentrations in...
the cell medium and plasma to maintain adequate l-arg bioavailability to both enzymes. However, in cultured cell studies where either NOS (33) or arginase (23) were overexpressed, the activities of the other enzyme, whose expression was unaltered by the treatment, were decreased. In studies in cytokine-stimulated pulmonary arterial endothelial cells and macrophages, NO production was significantly enhanced by inhibition of arginase (5, 6, 10). Therefore, taken together, these studies are consistent with the concept that arginase and NOS compete for a common pool of intracellular l-arg.

On the other hand, the decreased urea production following LPS in the Mkp-1−/− macrophages may be secondary to inhibition of arginase activity. It has been found that arginase can be inhibited by an intermediate in the l-arg-NO pathway, N⁶-hydroxy-l-arginine (NOHA), during high-output NO synthesis. For example, Buga et al. (3) demonstrated that when cytokine-induced NO production was increased by 20-fold in rat aortic endothelial cells, intracellular levels of NOHA increased and arginase activity was inhibited. Furthermore, inhibition of NOS decreased levels of NOHA and increased urea production. The IC₅₀ for NOHA inhibition of arginase has been found to be 10–40 μM (3, 12). Waddington et al. (34), found that NOHA inhibited arginase activity in macrophages at NO concentrations of 20 and 200 μM but not at an NO concentration of 2 μM. In our in vitro studies in macrophages, the media concentration of nitrite reached 50 μM in some studies at 24 h, and therefore it is likely that the decrease in urea production in LPS-treated Mkp-1−/− macrophages may have been due at least in part to inhibition of arginase activity by NOHA.

MKP-1 has been shown to prefer JNK and p38 as substrates (9, 32, 36). We found in this study that LPS-induced NO production is mediated, at least in part, by JNK and p38 and that overexpression of MKP-1 attenuated LPS-induced iNOS mRNA expression. Given the substrate preference of MKP-1, it is likely that overexpressing MKP-1 resulted in lower levels of phosphorylated p38 and/or JNK due to accelerated dephosphorylation of these kinases, as we have recently demonstrated (9, 36). This accelerated dephosphorylation of p38 and JNK would ultimately result in lower levels of iNOS mRNA expression. Conversely, knockout of MKP-1 would result in delayed dephosphorylation of p38 and JNK, as we have recently demonstrated (37), which would have resulted in prolonged activation and greater levels of iNOS mRNA and protein. Thus alterations in p38 and JNK phosphorylation were likely involved in the alterations in iNOS mRNA levels we found. It has recently been reported that LPS stimulates NO production via activation of NF-κB in RAW264.7 macrophages (12) and that p38 activation is involved in this signaling pathway (11). Thus alterations in NF-κB signaling caused by prolonged p38 activation due to Mkp-1 knockout may have also contributed to changes in the production of iNOS mRNA we found in this study. Regardless of which mechanism(s) were responsible, alterations in iNOS mRNA expression were associated with similar alterations in iNOS protein expression and NO production. For example, in the Mkp-1-overexpressing cells, both iNOS mRNA and iNOS protein levels were decreased (Fig. 2). Given the recently described relatively short half-life of iNOS protein (22), the observed decrease in iNOS protein levels are consistent with the decrease in iNOS transcription found in these cells.

In terms of the LPS effect on arginase, treatment of RAW264.7 macrophage cell lines with LPS led predominantly to the upregulation of arginase I mRNA expression, a finding that is consistent with recent studies (15, 21, 29). The induction of arginase I has been reported to be controlled by activation of an enhancer 3 kb downstream of the basal promoter (29). The net effect of arginase I and/or arginase II induction by LPS was a dramatic increase in urea production. The biological significance of enhanced arginase activities in LPS-treated macrophages remains unclear. We speculate that arginase could represent a molecular mechanism used by macrophages to attenuate NO production and thereby dampen inflammatory responses (6, 19).

We have recently reported that exogenous iNOS and native arginase compete for a common pool of l-arg in endothelial...
cells (33). Since iNOS and arginase compete for their common substrate, l-arg, the bioavailability of l-arg to iNOS would be limited by upregulating arginase, thereby resulting in a reduction in NO production. Further support for this concept comes from the reported finding that pharmacological inhibition of arginase leads to enhanced NO production (5, 10). Augmenting arginase activities also favors the formation of polyamines and/or L-proline from l-ornithine, which are important in cellular proliferation and repair after injury (19, 23). Thus we propose that arginase induction following endotoxin represents a change in macrophage phenotype following LPS stimulation, from a state favoring an acute inflammatory response to a state favoring repair and healing (31). This concept is supported by studies reporting that Th2 cytokines are potent inducers of arginase, whereas Th1 cytokines are potent inducers of iNOS (6, 25, 26). Given the key role of arginase in polyamine and proline synthesis, the induction of arginase by Th2 cytokines may be vital to attenuate aggressive inflammatory responses and allow for cellular proliferation (23). Consistent with this concept is a recent study by Ignarro et al. (18) in which vascular smooth muscle cells transfected with arginase I exhibited enhanced cellular proliferation. Therefore, we propose that the upregulation of arginase following LPS stimulation serves two complimentary roles that allow for host defense without causing damage to the host: first, arginase serves as an important negative regulator of NO production to limit tissue damage; and second, arginase facilitates tissue repair through participating in polyamine and proline synthesis.

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