Regulation of the genes for arginase isoforms and related enzymes in mouse macrophages by lipopolysaccharide

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Salimuddin, Akitoshi Nagasaki, Tomomi Gotoh, Hirotaka Isobe, and Masataka Mori. Regulation of the genes for arginase isoforms and related enzymes in mouse macrophages by lipopolysaccharide. *Am. J. Physiol.* 277 (*Endocrinol. Metab.* 40): E110–E117, 1999.—Arginase exists in two isoforms, the hepatic (arginase I) and extrahepatic types (arginase II). Arginase I is markedly induced in rat peritoneal macrophages and rat tissues in vivo by bacterial lipopolysaccharide (LPS). In contrast, both arginase I and arginase II are induced in LPS-activated mouse peritoneal macrophages. In the present study, expression of arginase isoforms and related enzymes was studied in mouse tissues in vivo and in peritoneal macrophages with RNA blot and immunoblot analyses and enzyme assay. When mice were injected intraperitoneally with LPS, inducible nitric oxide synthase (iNOS) and arginase II were induced early in the lung and spleen. mRNAs for argininosuccinate synthase (AS) and ornithine decarboxylase (ODC) were also induced early. In comparison, arginase I was induced later in the lung. Early induction of iNOS, arginase II, AS, ODC, and cationic amino acid transporter 2 and late induction of arginase I were observed in LPS-activated peritoneal macrophages. These results indicate that the genes for the two arginase isoforms are regulated differentially. Possible roles of the arginase isoforms in the regulation of nitric oxide production and in polyamine synthesis are discussed.

arginase I; arginase II; nitric oxide; nitric oxide synthase

ARGININE plays a central role in the biosynthesis of various products, including nitric oxide (NO), urea, creatine phosphate, proline, and polyamines. The cellular production of NO absolutely depends on the availability of arginine because no other physiological amino acid or guanidino-containing compound can substitute as a substrate for NO synthase (NOS). Arginine is metabolized by NOS to NO and citrulline and by arginase to urea and ornithine. Ornithine is a substrate for the synthesis of polyamines and proline, whereas citrulline, a coproduct of the NOS reaction, is utilized for the synthesis of arginine via the "citrulline-NO cycle" (14, 15, 22, 26, 27, 37). The intracellular level of arginine is maintained and replenished by endogenous sources like intracellular protein degradation or by exogenous sources from the kidney, where arginine is synthesized from citrulline by the enzymes argininosuccinate synthase (AS) and argininosuccinate lyase (AL; Ref. 24). Because arginase and NOS share a common substrate, NO production is likely to be linked to the regulation of arginase activity, especially in nonhepatic tissues where the complete urea cycle is not present (3). In fact, it was reported that the balance of arginine metabolism between these two enzymes has important pathophysiological effects (1, 13, 19, 30).

Arginase exists in two isoforms, the hepatic type (arginase I) and the extrahepatic type (arginase II). The cDNA and the gene for arginase I were isolated, and the regulation of the gene was studied (10, 34). cDNAs for human and rat arginase II were also isolated (12, 25, 35), and the human gene was mapped to chromosome 14q24.1–24.3 (9). Wang et al. (36) and Gotoh et al. (12) found that arginase II and the inducible form of NOS (iNOS) are coinduced in murine macrophage-like RAW 264.7 cells by bacterial lipopolysaccharide (LPS), which suggests that arginase II is involved in downregulation of NO production. On the other hand, we found that arginase I and iNOS are coinduced by LPS in the cultured rat peritoneal macrophages and in the lung and spleen in vivo (33). More recently, Louis et al. (21) showed that both arginase I and arginase II are induced in activated mouse peritoneal macrophages. To understand the roles of these two arginase isoforms in mouse macrophages in vivo, we analyzed the expression of the genes for these isoforms in mouse tissues after LPS treatment.

Here, we report that iNOS and arginase II are induced early in the lung and spleen of LPS-treated mice. On the other hand, arginase I is induced late in the lung and is not induced in the spleen. In LPS-treated mouse peritoneal macrophages like lung, iNOS and arginase II were induced early, whereas arginase I was induced later. Changes in the mRNAs for metabolically related enzymes are also reported.

MATERIALS AND METHODS

Materials. A monoclonal antibody against mouse iNOS was purchased from Transduction Laboratories (Lexington, KY), and an antibody against human arginase I was raised in a rabbit, as described previously (16). An antibody against rat arginase II was raised in a rabbit by injection of the synthetic peptide corresponding to residues 336–354 of the enzyme (DDBJ/EMBL/GenBank databases, accession no. D86928) that had been conjugated with keyhole limpet hemocyanin.

Animals and LPS treatment. Specific pathogen-free male BALB/c mice (5–7 wk of age) were given *Escherichia coli* LPS (serotype 0127:B8, Sigma, St. Louis, MO) intraperitoneally at 10 mg/kg of body weight and were killed at the indicated times after anesthetization with ether.

Preparation and culture of mouse peritoneal macrophages. Male BALB/c mice (7 wk of age) were given 2 ml of 10% polypeptone (Difco Laboratory, Detroit, MI) intraperitoneally,

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and peritoneal cells were harvested 3 days after the injection (8). The cells were seeded in 10-cm culture dishes at 1×10^7 cells/dish in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. After incubation for 3 h, nonadherent cells were removed by being washed three times with phosphate-buffered saline, and the adherent cells were cultured in the absence or presence of LPS (10 μg/ml; Ref. 33) at 37°C under 5% CO_2 in air for the indicated time periods. Practically all adherent cells prepared by the same procedure were shown to be macrophages (26).

RNA blot analysis. Total RNAs from mouse tissues were prepared by the guanidinium thiocynate-phenol-chloroform extraction procedure (4). Total RNA from peritoneal cells (1×10^7 cells) was isolated with the Trizol reagent (GIBCO/BRL, Gaithersburg, MD) according to the recommendations of the manufacturer. After electrophoresis in formaldehyde-containing agarose gels, RNAs were transferred to nylon membranes. Hybridization was performed with the following probes: digoxigenin-labeled antisense RNAs for mouse iNOS (26), rat arginase I (33), rat arginase II (12), rat AS (38), mouse ornithine decarboxylase (ODC), and rat cationic amino acid transporter 2 (CAT-2). The template plasmids for the last two proteins were prepared as follows. Partial cDNAs for mouse ODC (nt 477–1046; GenBank, accession no. M10624; Ref. 17) and mouse CAT-2 (nt 1289–1834; GenBank, accession no. L11600; Ref. 5) were isolated by PCR with mouse kidney RNA and rat liver RNA, respectively, and inserted into the *Hin*c II site of pGEM-3Zf(+) (Promega, Madison, WI), yielding pGEM-mODC-1 and pGEM-rCAT-2–1, respectively. Chemiluminescence signals derived from hybridized probes were detected on X-ray films with the digoxigenin luminescence detection kit (Boehringer Mannheim, Mannheim, Germany), and the band intensities were quantified densitometrically with the MacBas bioimage analyzer (Fuji Photo Film, Tokyo, Japan).

Immunoblot analysis. Mouse tissues and peritoneal cells were homogenized in 9 vol of 20 mM potassium HEPES buffer, pH 7.4, containing 0.5% Triton X-100, 1 mM dithiothreitol, 10% (wt/vol) glycerol, 50 μM antipain, 50 μM leupeptin, 50 μM chymostatin, and 50 μM pepstatin. The homogenates were centrifuged at 25,000 *g* for 30 min at 4°C, and the supernatants were used as tissue or cell extracts. The extracts were subjected to SDS-10% polyacrylamide gel electrophoresis, and proteins were electrotransferred to nitrocellulose membranes. Immunodetection was performed with the enhanced chemiluminescence kit (Amersham Life Science, Buckinghamshire, UK) according to the protocol of the manufacturer. Chemiluminescence signals were detected on X-ray films and were quantified with the MacBas bioimage analyzer.

iNOS activity. The tissue extracts were prepared as described in *Immunoblot analysis*. iNOS activity was assayed by measuring conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline essentially as described by Lui et al. (20). In brief, 30 μl of tissue extracts were incubated in a mixture (150 μl) containing 6 mM potassium HEPES, pH 7.6, 1 mM L-[^{3}H]arginine (3 mCi/mmol; Amersham Life Science), 1 mM NADPH, 30 nM calmodulin, 3 μM (6R)-5,6,7,8-tetrahydrobiopterin, 20 μM FAD, 20 μM flavin mononucleotide or FMN, and 60 mM L-valine (to inhibit arginase activity) at 37°C for 20 min. The reaction was stopped by adding 300 μl of 100 mM potassium HEPES, pH 5.5, containing 2 mM EDTA, and 2 mM EGTA. The reaction mixture was applied to a Dowex AG50W-X8 (Na^+ form) column (2 ml; Bio-Rad Laboratories, Hercules, CA), and eluted L-[^{3}H]citrulline was measured in a scintillation counter.

Arginase activity. The tissue extracts were prepared as described in *Immunoblot analysis*. Arginase was preactivated by incubation of the tissue extracts with 50 mM $MnCl_2$ at 37°C for 30 min. Arginase I and arginase II activities were measured by immunodepletion with the anti-arginase I antibody. Preactivated tissue extracts (22.5 μl) were incubated with 2.5 μl of anti-arginase I serum or nonimmune rabbit serum (control) in 50 μl of 6 mM potassium HEPES, pH 7.4, for 10 min at 25°C. The mixture was then incubated with 100 μl of protein A Sepharose (Pharmacia Biotech, Uppsala, Sweden; ×2 suspension) for 10 min at 25°C, and antigen-antibody complex was removed by centrifugation. Supernatant was used to measure the enzyme activity according to the method of Schimke (29). Immunodepleted and nondepleted activities were regarded as arginase I and arginase II activities, respectively. Under these conditions, arginase activity in the liver extract was completely depleted, whereas the activity in the kidney extract was not. The anti-arginase II antibody used in the immunoblot analysis could not be used for immunodepletion.

Another method. Protein was determined with the protein assay reagent (Bio-Rad Laboratories) with bovine serum albumin as a standard.

RESULTS

Distribution of mRNAs for arginase isoforms and related enzymes in mouse tissues. Expression of mRNAs for arginase I, arginase II, AS, and ODC in adult mouse tissues was studied by RNA blot analysis (Fig. 1). Arginase I mRNA of ~1.7 kb was expressed most strongly in the liver where urea synthesis takes place, was expressed weakly in the heart and spleen, and was not detected in other tissues. In contrast, arginase II mRNA of ~1.8 kb was expressed most strongly in the small intestine, followed by large intestine and kidney. mRNA of ~1.5 kb for AS, which is involved in the synthesis of arginine and urea, was expressed strongly in the liver, moderately in the testis, kidney, and heart, and at very low levels in other tissues. mRNA (~2.6 kb) for ODC, an enzyme that catalyzes a key step of polyamine synthesis from ornithine, was expressed strongly in the kidney, less strongly in the testis, spleen, small intestine and large intestine, and weakly in other tissues.

Induction of iNOS, arginase isoforms, and related enzymes in the mouse lung by LPS. We analyzed the induction of arginase isoforms and related enzymes by LPS in the mouse tissues in vivo by RNA blot, immunoblot, and activity analyses. The amount of LPS was first titrated at 1, 10, 25, and 50 mg/kg body wt by assessment of induction of iNOS mRNA. Nearly maximal induction was obtained by LPS at 10 and 25 mg/kg body wt. Induction was less marked at 1 mg/kg body wt, and mice died partly at 50 mg/kg body wt. Thus LPS at 10 mg/kg was chosen as the minimal dose to give nearly maximal induction of iNOS mRNA. The results for the lung are shown in Fig. 2. In RNA blot analysis, iNOS mRNA of ~4.5 kb started to increase at 3 h after the LPS treatment, reached a maximum at 6 h, decreased sharply at 12 h, and then slowly declined thereafter (Fig. 2, *A* and *B*). We found that both arginase I and arginase II mRNAs were induced by LPS. Arginase I mRNA, which was present at a very low level before the

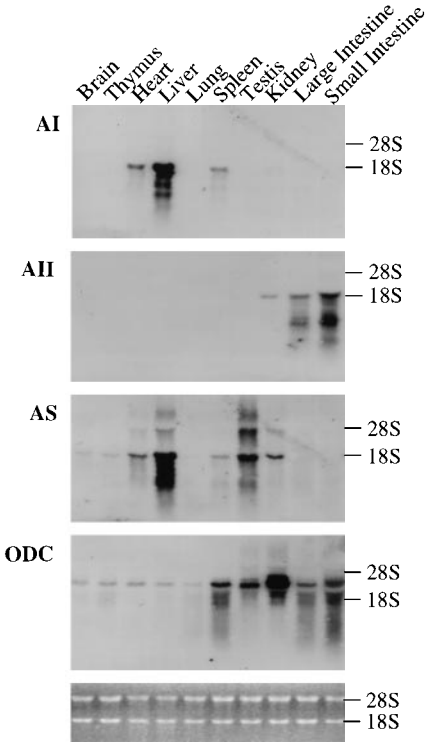

Fig. 1. RNA blot analysis for arginase I (AI), arginase II (AII), argininosuccinate synthase (AS), and ornithine decarboxylase (ODC) in mouse tissues. Total RNAs (3.0 μg) from indicated tissues were electrophoresed in formaldehyde-containing 1% agarose gels and transferred to nylon membranes. Filters were hybridized with digoxigenin-labeled antisense RNA probes for arginase I, arginase II, AS, and ODC as described in MATERIALS AND METHODS. Detection was accomplished with a digoxigenin chemiluminescence detection kit (Boehringer Mannheim). Positions of 28S (4.6 kb) and 18S (1.9 kb) rRNAs are shown on *right*. Integrity of rRNAs was verified by the apparently identical intensities of 28S and 18S rRNA bands stained with ethidium bromide after elecrophoresis (*bottom*).

LPS treatment, started to increase at 12 h and continued to rise up to 36 h. In contrast, arginase II mRNA, which was not detectable before LPS treatment, increased at 3 h, reached a maximum at 6 h, and decreased slowly thereafter. ODC mRNA was induced with similar kinetics as iNOS and arginase II mRNAs. AS mRNA was induced strongly but a little later than mRNAs for iNOS, arginase II, and ODC, reaching a maximum at 12 h and decreasing slowly thereafter.

In immunoblot analysis, arginase I protein of ~38 kDa became detectable at 24 h and increased somewhat at 36 h (Fig. 2*C*). On the other hand, arginase II protein of ~38 kDa was present at a very low level before the LPS treatment, started to increase at 3 h, and increased further up to 36 h. The induced level of arginase II protein at 36 h was close to that in the kidney.

NOS activity in the lung, which was barely detectable before the LPS treatment, increased up to 12 h and then decreased (Fig. 2*D*). Between the two arginase isoforms, arginase II activity increased early and reached a maximum at 24 h. The activity at 24 h was close to that in the kidney. In contrast, arginase I activity became detectable at 24 h and increased at 36 h. Activity of arginase I at 36 h was lower than that of arginase II. Thus the changes of activities of iNOS and arginase isoforms in the lung agreed with those of mRNAs and proteins for these enzymes.

Induction of iNOS, arginase isoforms, and related enzymes in the mouse spleen by LPS. Expression of the enzymes involved in arginine metabolism in the spleen after the LPS treatment is shown in Fig. 3. iNOS mRNA was induced early, increased up to 12 h, and then decreased gradually until 36 h where it still remained higher than basal levels (Fig. 3, *A* and *B*). In sharp contrast to the lung, induction of arginase I mRNA was not observed (data not shown). Arginase II mRNA started to increase at 3 h, reached a plateau at 6 h, and remained high even up to 36 h after LPS injection. ODC mRNA increased almost linearly with time up to 12 h and then decreased slowly. AS mRNA was detectable before the treatment, increased at 6 h by about twofold, and remained at similar levels thereafter.

In immunoblot analysis, arginase II protein, which was barely detectable before the LPS treatment, was evident at 3 h, increased up to 12 h, and then decreased slowly. The level of arginase II protein at 12 h was similar to that in the kidney. Arginase I protein was not detected during the LPS treatment.

NOS activity increased sharply up to 6 h and then slowly up to 24 h and decreased at 36 h (Fig. 3*D*). Arginase II activity increased in a similar kinetic manner as NOS activity. The activity of arginase II at 24 h was similar to that in the kidney. Arginase I activity was not detected during the LPS treatment. Thus the changes of activities of iNOS and arginase isoforms in the spleen agreed again with those of mRNAs and proteins for these enzymes.

Induction of iNOS, arginase isoforms, and related enzymes in mouse peritoneal macrophages by LPS. Mouse peritoneal macrophages were cultured in the presence of LPS, and the expression of mRNAs and proteins for iNOS, arginase I, arginase II, AS, and CAT-2 was studied (Fig. 4). iNOS mRNA species of ~4.5 and 7 kb, which were undetectable before the treatment, were induced early, reached a maximum at 3 h, and remained high up to 36 h (Fig. 4*A*). Arginase I mRNA, which was present before LPS treatment, remained much unchanged up to 9 h, increased markedly at 24 h, and increased further at 36 h. Arginase II mRNA was also present before the treatment, increased up to 9 h, and remained high up to 36 h. AS mRNA was induced very similarly as iNOS mRNA. ODC mRNA increased weakly at 3 h and markedly at 9 h and then decreased to basal levels by 24 h. mRNA species for CAT-2 of ~4.5 and 8 kb was induced at 3 h, increased further at 9 h, and then decreased to a barely detectable level at 24 h.

Induction of iNOS and arginase proteins in LPS-treated mouse peritoneal macrophages was studied by immunoblot analysis (Fig. 4*B*). iNOS protein of ~130 kDa was detected 3 h after the LPS treatment and increased gradually up to 24 h. Thus the increase of the protein was later than that of its mRNA, as expected.

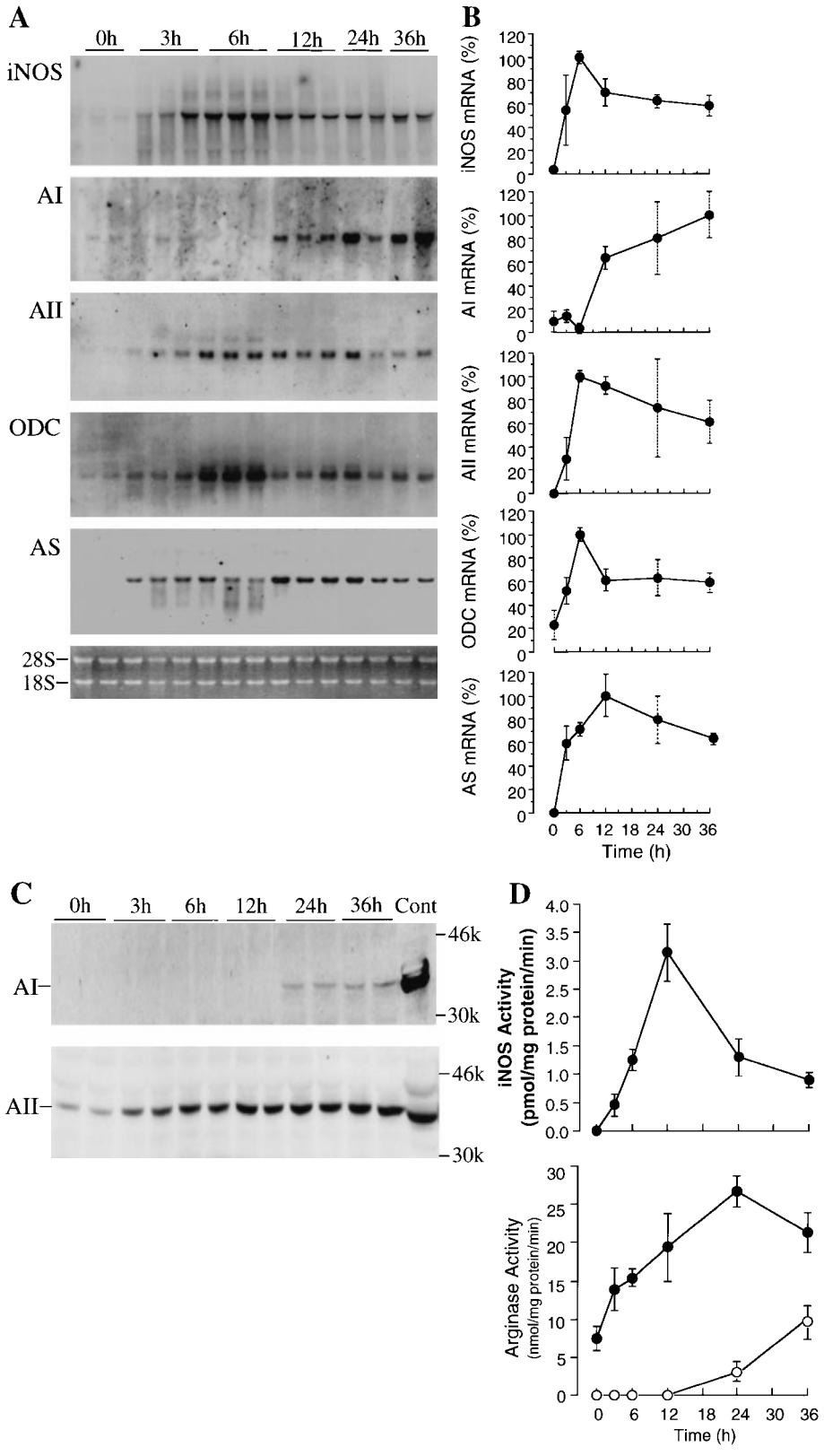

Fig. 2. Time course of induction of inducible nitric oxide synthase (iNOS), arginase I (AI), arginase II (AII), ODC, and AS in mouse lung after lipopolysaccharide (LPS) treatment. *A*: total RNAs were isolated from mouse lung at indicated times after LPS injection. Two mice at 0 (before LPS injection), 24, and 36 h and 3 mice at 3, 6, and 12 h were used. RNAs (2 μg) were subjected to blot analysis. *B*: results in *A* were quantified and are means ± SD (solid line; $n = 3$) or means ± ranges (dotted line; $n = 2$). Maximal values are set at 100%. *C*: lung extracts (20 μg of protein) from 2 mice at each time were subjected to immunoblot analysis for arginase isoforms. Cont, control. Mouse liver extract (10 μg of protein) was included for arginase I, and mouse kidney extract (10 μg of protein) was included for arginase II. Molecular mass markers (Rainbow protein molecular size markers, Amersham) are ovalbumin [46 kDa (k)] and carbonic anhydrase (30 kDa). *D*: activities of iNOS, arginase I (○), and arginase II (●) were measured as described in MATERIALS AND METHODS. Values are means ± SD; $n = 3$.

Two polypeptides of ~35 and 38 kDa, which immunoreacted with the arginase I antibody and comigrated with those in the liver, were detected before the LPS treatment, remained much unchanged up to 24 h, and increased much at 36 h. These two polypeptides of arginase I have often been observed and apparently arose from the two initiation codons located 30 bp apart, as reported for the rat enzyme (18, 28, 32). The concentration of arginase I protein in the LPS-treated peritoneal macrophages at 36 h was close to that in the

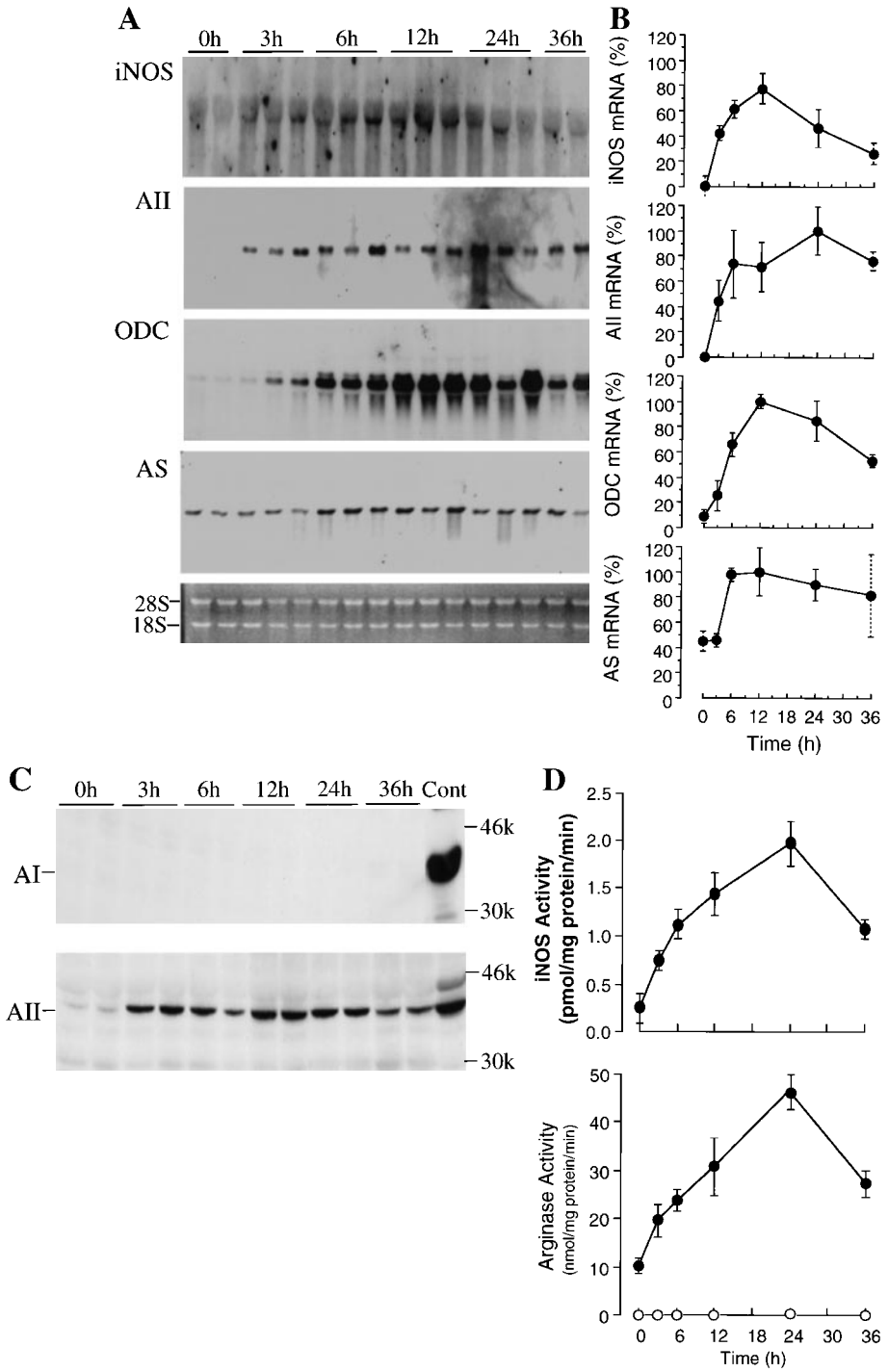

Fig. 3. Time course of induction of iNOS, arginase I (AI), arginase II (AII), ODC, and AS in mouse spleen after LPS treatment. *A*: total RNAs were isolated from mouse spleen, and RNA blot analysis was performed as described for Fig. 2. Three mice were used at 24 h instead of 2 mice. *B*: results in *A* were quantified and are means ± SD (solid line; $n = 3$) or means ± ranges (dotted line; $n = 2$). Maximal values are set at 100%. *C*: spleen extracts (20 μg of protein) from 2 mice at each time were subjected to immunoblot analysis for arginase isoforms. Cont, control. Mouse liver extract (10 μg of protein) was included for arginase I, and mouse kidney extract (10 μg of protein) was included for arginase II. Molecular mass markers (Rainbow protein molecular size markers, Amersham) are ovalbumin [46 kDa (k)] and carbonic anhydrase (30 kDa). *D*: activities of iNOS, arginase I (○), and arginase II (●) were measured as described in MATERIALS AND METHODS. Values are means ± SD; $n = 3$.

liver, where this enzyme is expressed most strongly under normal conditions. Arginase II protein of ~38 kDa was detected before the treatment and increased gradually up to 36 h. The concentration of arginase II in the activated peritoneal macrophages was somewhat higher than that in the kidney where this enzyme is expressed moderately under basal conditions.

DISCUSSION

It is known that arginase activity increases when macrophages are stimulated by LPS and cytokines (2, 7, 19, 23, 30, 32). Recent molecular cloning of extrahepatic type arginase II (12, 25, 35) in addition to hepatic arginase I (18, 28) has enabled us to differentiate between the two distinct isoforms and to determine the kinetics of their expression. Considerable confusion exists concerning the relative expression of these isoforms in macrophages and tissues during the inflammatory response. In LPS-stimulated mouse macrophage-like RAW 264.7 cells, only arginase II was induced (12, 36), whereas only arginase I was induced in LPS-stimulated rat peritoneal macrophages and in rat

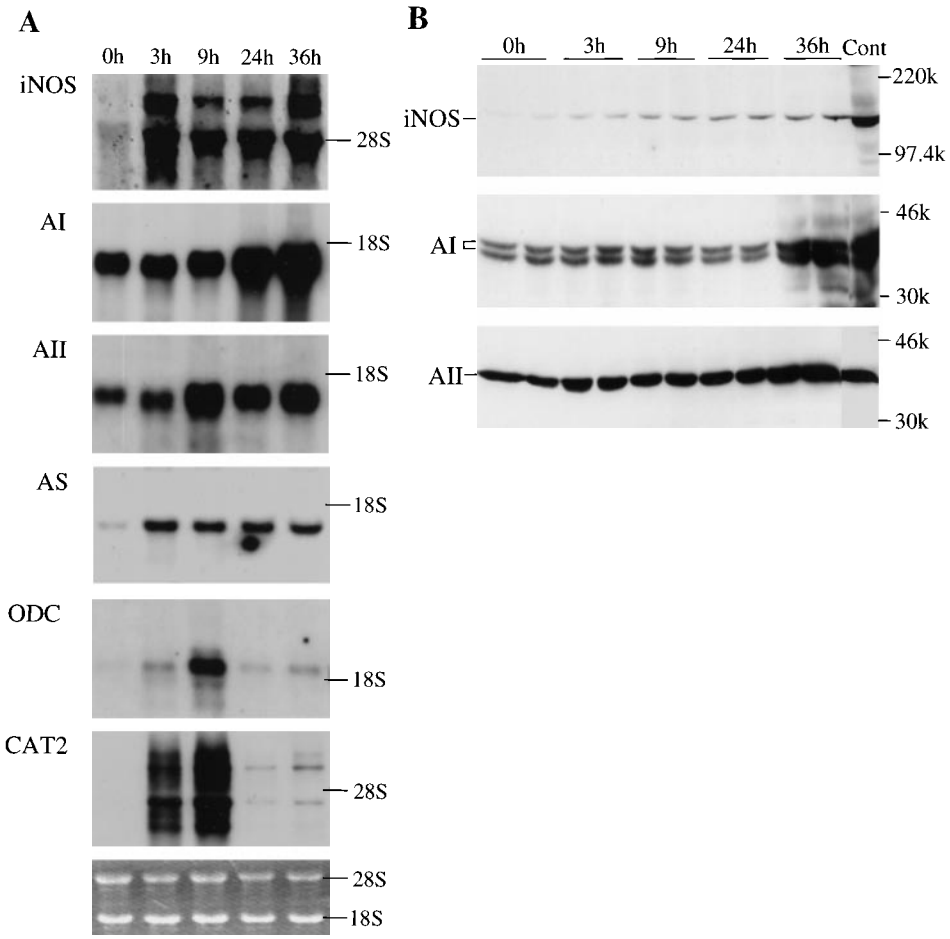

Fig. 4. Time course of induction of iNOS, arginase I (AI), arginase II (AII), ODC, AS, and cationic amino acid transporter 2 (CAT-2) in mouse peritoneal cells after LPS treatment. *A*: cells were collected from 8 mice and were pooled and treated with 10 μg/ml of LPS, and total RNAs were isolated at indicated times after LPS treatment. 0 h, before LPS treatment. RNAs (2 μg) were subjected to blot analysis. *B*: cells were collected from 16 mice and were pooled and treated with 10 μg/ml of LPS for indicated periods, and cell extracts (10 μg of protein) were subjected to SDS-10% polyacrylamide gel electrophoresis. Proteins were electrotransferred to nitrocellulose membranes, and membranes were immunoblotted with mouse monoclonal antibody against mouse iNOS (0.25 μg/ml) or rabbit antisera against arginase I or arginase II (5,000- and 500-fold dilution, respectively). Cont, control. Mouse macrophage extract in iNOS detection kit (Transduction Laboratories; 10 μg of protein) was included for iNOS, mouse liver extract (10 μg of protein) was included for arginase I, and mouse kidney extract (10 μg of protein) was included for arginase II. Molecular mass markers (Rainbow protein molecular size markers, Amersham) are myosin [200 kDa (k)], phosphorylase *b* (97.4 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa).

tissues in vivo (33). Recently, Louis et al. (21) found that only arginase I is induced in rat peritoneal macrophages, whereas both arginases I and II are induced in mouse peritoneal macrophages. The present study demonstrates that both isoforms are induced by LPS in the mouse lung in vivo. Thus expression of arginase isoforms differs between rat and mouse. Kinetic analysis of their expression patterns in mice showed that arginase II is induced early after the LPS treatment both in the lung and spleen in vivo and in peritoneal macrophages in culture, whereas arginase I is induced later in the lung and peritoneal macrophages. Arginase I was not induced by the LPS treatment in the spleen, whereas arginase II was seen to be markedly induced. These findings point to the clear conclusion that the genes for the two arginase isoforms are regulated differently in response to LPS treatment in various tissue types.

Experiments with peritoneal macrophages show that the enzymes of arginine and NO metabolism are induced in the same cells by LPS and suggest strongly that these enzymes are also induced in macrophages in the mouse lung and spleen in vivo. However, induction kinetics in response to LPS treatment differed between in vivo and cultured macrophages. In the cultured cells, LPS stimulates the cells immediately after addition and continues to do so throughout the course of the experiment. In vivo, on the other hand, LPS-induced cytokines in addition to LPS transported from the abdominal cavity to the tissues may contribute to the induction. Furthermore, different populations of macrophages (resident and nonresident) may exist in different tissues of LPS-administered mice. In addition, other inflammatory cells such as neutrophils and lymphocytes, which are present in the tissues, may modulate expression of these genes.

Based on the present kinetic studies in vivo, we speculate the following events in the endotoxemia. CAT-2, iNOS, and arginase II are induced early in activated macrophages. Under these circumstances, extracellular arginine will be actively taken up by the macrophages and utilized by both iNOS and arginase II. These metabolic changes are cytotoxic to infected bacteria in two ways. First, NO produced by the iNOS reaction is cytotoxic to the bacteria. Second, the depletion of extracellular arginine by its active uptake is thought to exert static action on bacterial growth. In fact, plasma arginine concentration was reported to decrease in septic patients (6). At the same time, AS is induced and recycles citrulline formed by the iNOS reaction to arginine. On the other hand, arginase I is induced later than arginase II. Overproduction of NO is toxic to macrophages and neighboring cells. Therefore, there must be a mechanism to prevent sustained overproduction of NO. CAT-2 and iNOS mRNAs and iNOS activity decreased after early induction in vivo,

which suggests that arginine uptake and NO production decrease in the late stage of endotoxemia. Furthermore, arginase I is induced later and may function in downregulating NO production. In fact, arginase has been shown to modulate NO production in activated macrophages (3, 23, 30) and endothelial cells (2). More recently, we showed that arginase downregulates NO production and prevents NO-mediated apoptosis in macrophage-like RAW 264.7 cells (11). Ornithine, which is formed by arginases I and II in endotoxemia, may be utilized for synthesis of polyamines and proline (and thus collagen), which are required for cell growth and tissue repair.

We have recently shown that the induction of the arginase I gene in LPS-stimulated rat macrophages is mediated, at least partly, by the preceding induction of CAAT/enhancer binding protein-β (10, 33) . Molecular cloning of the arginase II promoter region was reported (31), and studies on the transcriptional regulation of this gene remain to be performed. Insights into the molecular mechanisms responsible for the divergent expression patterns of the arginase isoforms are in need of elucidation.

The first two authors contributed equally to this work.

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