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Mechanism of Hepatic Cytochrome P450 Modulation during *Listeria monocytogenes* Infection in Mice

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SUMMARY

There have been numerous reports of altered drug clearance during episodes of viral infection and during the clinical use of recombinant interferons, but there have been very few reports regarding the effect of active bacterial infections on cytochrome P450-mediated metabolism. The objective of this study was to determine the mechanism by which the Gram-positive bacteria *Listeria monocytogenes* causes a depression of cytochrome P450-mediated biotransformation in mice. After induction with β-napthoflavone, hepatic microsomal cytochrome P450 levels were reduced by 40% and ethoxyresorufin-*O*-dealkylase (EROD) activity was decreased by 65% in mice infected for 48 hr. The loss of EROD activity was accompanied by losses of cytochrome P450IA apoenzyme and cytochrome P450IA mRNA. Listeria infection did not affect total mRNA levels, as determined by oligo(dT)$_{18}$ hybridization. The time course of these effects demonstrated that an up-regulation of cytochrome P450IA preceded the loss of this isozyme and that changes in cytochrome P450IA mRNA preceded the changes in apoenzyme levels and EROD activity. In hepatic microsomes from uninduced mice, cytochrome P450 levels and the rates of dealkylation of ethoxyresorufin, benzyloxyresorufin, pentoxyresorufin, and aminopyrine were significantly reduced, by 40–60%, after 48 hr of infection. The decrease in aminopyrine-*N*-demethylase activity was accompanied by a loss of cytochrome P450IID9 mRNA after 48 hr of infection. Cytochrome P450IID9 mRNA levels returned to normal after 96 hr of infection, whereas aminopyrine-*N*-demethylase activity was still decreased at this time. No up-regulation of cytochrome P450IID9 occured before the loss of this isozyme. The results of this study indicate that the changes in the levels of cytochrome P450IA and cytochrome P450IID9 that are observed during *L. monocytogenes* infection occur at a pretranslational step. If other bacteria have a similar capacity to depress cytochrome P450 by such a mechanism, then drugs with narrow therapeutic indices should be administered with caution during infectious diseases caused by bacteria or viruses.

The ability of interferon-inducing agents, recombinant interferons, and viral infections to depress cytochrome P450-mediated drug metabolism is now well established (see Refs. 1 and 2 for a review). In 1976, Renton and Mannering (3, 4) showed that many different immunoactive interferon-inducing agents, such as tilorone, quinacrine, and poly(rI·rC), caused significant depression of hepatic microsomal cytochrome P450 content after their administration to rats. A depression of drug clearance during infectious disease in humans was first documented by Chang *et al.* (5) and was suspected to be the result of virally induced interferon production (6). Since that time, there have been several other documented examples of compromised drug metabolism in infected humans (7–12) and several reports of altered drug clearance during the clinical use of recombinant interferon-α (13–15). Recently, Okuno *et al.* (16) directly demonstrated that interferon treatment can depress hepatic microsomal cytochrome P450-dependent metabolism in humans.

There have been very few reports regarding the effect of active bacterial infections on cytochrome P450-mediated metabolism (17–19). Our laboratory has previously shown that infection with *Listeria monocytogenes* depresses cytochrome P450-mediated metabolism in mice (18). The objective of this study was to determine the mechanism by which *L. monocytogenes* causes a depression of cytochrome P450-mediated biotransformation in mice.

Experimental Procedures

Materials. Ethoxyresorufin was purchased from Sigma Chemical Co. (St. Louis, MO). Benzyloxyresorufin and pentoxyresorufin were purchased from Molecular Probes Inc. (Eugene, OR). [γ-^{32}P]ATP and GeneScreenPlus nylon filters were purchased from New England Nuclear Research Products (Boston, MA). The cytochrome P450IA1 antibody was obtained from Oxygene Inc. (Dallas, TX). The cytochrome P450IA cDNA oligomer was synthesized by the Regional DNA Synthesis Laboratory at The University of Calgary (Calgary, Alberta, Canada) and the cytochrome P450IID9 cDNA and oligo(dT)$_{18}$ probes were

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ABBREVIATIONS: poly(rI·rC), polyriboinosinic·polyribocytidylic acid; PBS, phosphate-buffered saline; APND, aminopyrine-*N*-demethylase; βNF, β-naphthoflavone; BROD, benzyloxyresorufin-*O*-dealkylase; CFU, colony-forming units; EROD, ethoxyresorufin-*O*-dealkylase; PROD, pentoxyresorufin-*O*-dealkylase; SSC, standard saline citrate; SSPE, sodium chloride-sodium dihydrogen orthophosphate-ethylenediamenetetraacetic acid.

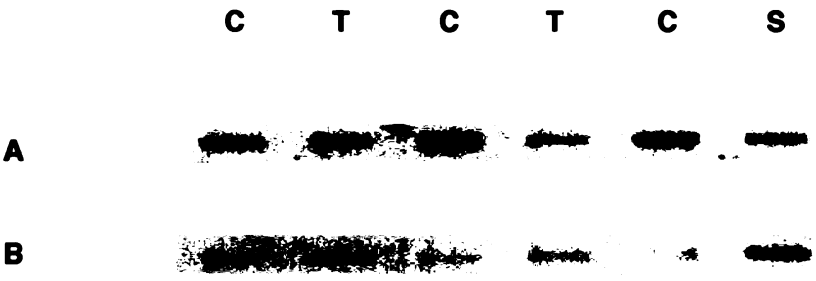

Fig. 1. Effect of 48 hr of infection with listeria on cytochrome P450IA mRNA and total mRNA levels. Mice were infected with 1×10^6 CFU of 15U listeria for 48 hr after induction with βNF (as described in Experimental Procedures); control mice were also induced with βNF and received an equal volume of sterile PBS. Hepatic RNA from control (*C*) and listeria-infected (*T*) mice, as well as an RNA standard (*S*), was bound to nylon filters and hybridized with the cytochrome P450IA cDNA (A) or oligo(dT)$_{18}$ (B) probes, and the filters were exposed in an X-ray cassette with intensifying screens (Cronex Lightning Plus).

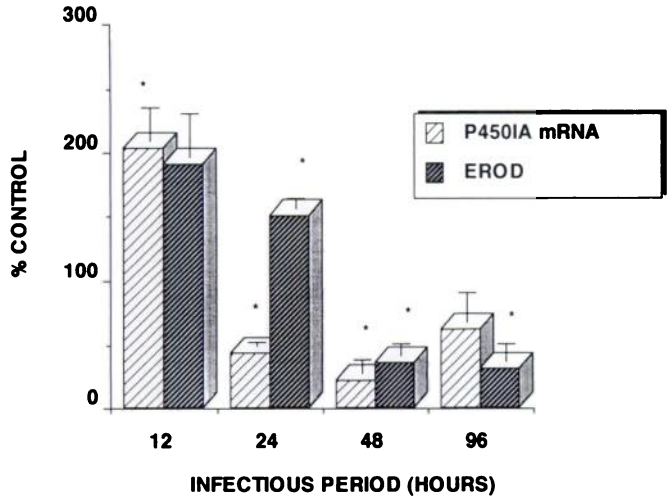

Fig. 2. Time course of the effect of infection with listeria on cytochrome P450IA mRNA levels and EROD activity. Mice were infected with 1×10^6 CFU of 15U listeria after induction with βNF. EROD activity was determined in hepatic microsomes and cytochrome P450IA mRNA levels were determined from slot blots (as described in Experimental Procedures); control mice were also induced with βNF and received an equal volume of sterile PBS. All results are expressed as percentage of control. The mean control EROD values for 12, 24, 48, and 96 hr were 300 ± 94, 430 ± 80, 532 ± 113, and 810 ± 158 pmol of resorufin/mg/min, respectively. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test. * Significantly different from control ($P < 0.05$).

synthesized by The Marine Gene Probe Laboratory of Dalhousie University (Halifax, Nova Scotia, Canada).

Animals and treatments. Male Swiss BALB/c mice (25–35 g) were obtained from Charles River Laboratories (Montreal, Quebec, Canada). The mice were maintained (five/cage) on clay chip bedding and allowed to acclimatize in our facility for at least 1 week before being used for experiments. Animals were fed standard Purina Rat Chow and water *ad libitum*. During the period of infection, mice were maintained in cages covered with protective filter bonnets, in a laminar flow hood. *L. monocytogenes* strain 15U (serotype 4b), isolated from an infected patient, was supplied by Dr. Robert Bortolussi (20), Izaak Walton Killam Hospital (Halifax, Nova Scotia, Canada). Aliquots of listeria were stored at −70° in brain-heart infusion broth containing 20% glycerol. For each experiment, an aliquot of listeria was thawed and 50 μl were added to 10 ml of brain-heart infusion broth and grown for 18 hr at 37°. The bacterial suspension was then centrifuged at 1000 × *g* for 15 min and the bacteria were resuspended in sterile PBS, pH 7.4. The bacteria were then washed three additional times using 5-min centrifugation periods. Bacterial concentrations were determined by measuring the absorbance of suspensions, using a wavelength of 620 nm; 0.6 absorbance unit is equivalent to 5×10^8 CFU/ml. Mice were infected by the intraperitoneal administration of 1×10^6 CFU of listeria suspended in PBS.

Mice were induced with βNF (40 mg/kg) using the following treatment protocol. Mice infected for 12, 24, or 48 hr were treated with five daily injections of βNF and were killed 24 hr after the final βNF injection, with the *Listeria* bacteria being administered for the final period. Mice infected with listeria for 96 hr were treated with daily injections of βNF for 7 days and were killed 24 hr after the final βNF injection; *Listeria* bacteria were administered on day 4, 96 hr before the mice were killed. In those studies that investigated the effect of listeria infection on cytochrome P450IID9, uninduced mice were infected with listeria for 12-, 48-, or 96-hr periods. Viable *Listeria* bacteria were present in the livers of all infected mice at the time of sacrifice, indicating the presence of active infection.

Preparation of hepatic microsomes and total hepatic RNA. Mice were killed by cervical dislocation and livers were removed immediately and rinsed in sterile filtered KCl (1.15%). The liver tissue was homogenized in a glass homogenizer, and all homogenates were kept on ice until centrifugation at 10,000 × *g* for 10 min at 4° in a Beckman J2-21 centrifuge. The supernatants were then centrifuged at 100,000 × *g* for 40 min at 4° in an IEC/B-60 ultracentrifuge. The microsomal pellets were resuspended (50% suspension by liver weight) and homogenized in glycerol buffer (25% 200 mM KH_2PO_4, pH 7.4, 20% glycerol, 0.23% KCl). Microsomes were stored at −70°. Total hepatic RNA was prepared from the same livers by the method of Chomczynski and Sacchi (21) and was stored in diethylpyrocarbonate-treated water at −20°.

Hepatic microsomal cytochrome P450 content and microsomal drug biotransformation. Microsomal cytochrome P450 content was determined by the method of Omura and Sato (22), and microsomal protein was determined by the method of Lowry *et al.* (23). EROD, BROD, and PROD activities were determined by the method of Burke *et al.* (24). Microsomal protein concentrations ranged from 25 to 125 μg/ml and were varied to ensure linear reaction rates for at least 3 min. A resorufin standard was used to calibrate the instrument and activities are expressed as nmol of resorufin formed/min/mg of microsomal protein. Hepatic microsomal APND activities were determined by the method of Sladek and Mannering (25); the concentration of HCHO produced during the reaction was calculated from a formaldehyde standard curve obtained at 412 nm, and microsomal activities were expressed as nmol of HCHO formed/mg of microsomal protein/hr.

Northern blot and slot blot analysis of RNA. Total hepatic RNA (20 μg) was separated electrophoretically on 2.2 M formaldehyde-1.3% agarose gels with subsequent blotting or was bound to nylon filters (GeneScreenPlus) by using a Bio-Rad Bio-Dot slot blot apparatus after denaturation and heating at 60° for 15 min. A range of concentrations of each RNA sample were prepared by 2-fold serial dilution with diethylpyrocarbonate-treated water. The final slot blot loads (0.2 ml/slot) ranged from 3.0 to 0.012 μg for slot blots that were hybridized with the cytochrome P450IA probe and from 10.0 to 0.16 μg for blots that were hybridized with the cytochrome P450IID9 cDNA probe.

The cytochrome P450IA, cytochrome P450IID9, and oligo(dT)$_{18}$ cDNA oligomers were labeled with [γ-^{32}P]ATP by the method of Maxam and Gilbert (26). The cytochrome P450IA cDNA probe is a complement to nucleotides 1259–1281 of the murine cytochrome P450IA1 mRNA sequence (27). This probe sequence is also complementary to the sequence of the mouse cytochrome $P_3$450 mRNA and has a single mismatch with the complementary sequence of the mouse cytochrome $P_2$450 mRNA (cytochrome P450IA2). The cytochrome

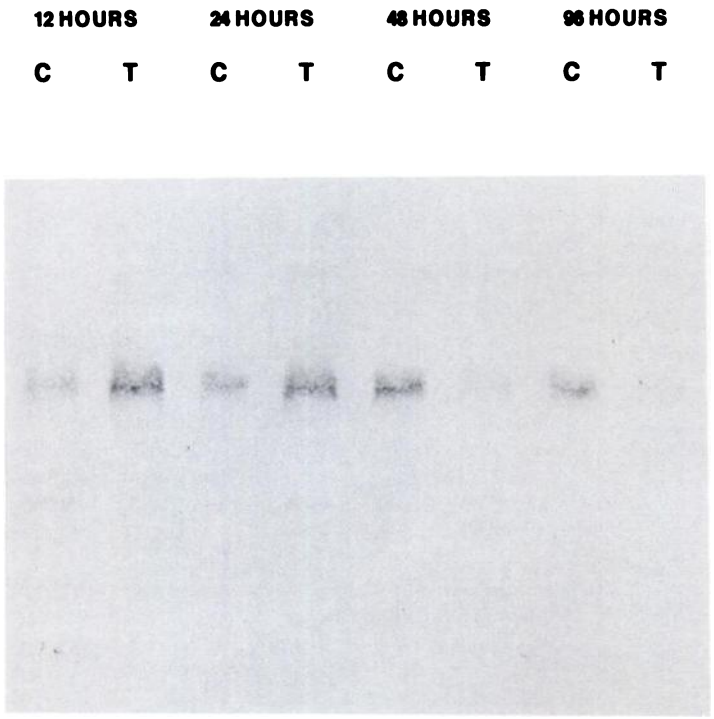

Fig. 3. Time course of the effect of infection with listeria on cytochrome P450IA apoenzyme levels. Mice were infected with listeria as described for Fig. 2. After electrophoretic separation of hepatic microsomal proteins on a polyacrylamide gel, the proteins were transferred to nitrocellulose membranes and the resulting Western blot was hybridized with an antibody directed against cytochrome P450IA apoenzyme (as described in Experimental Procedures). A pair of samples are shown for each time point. *C*, Control microsomes isolated from βNF-induced mice treated with sterile PBS; *T*, microsomes from βNF-induced mice infected with listeria.

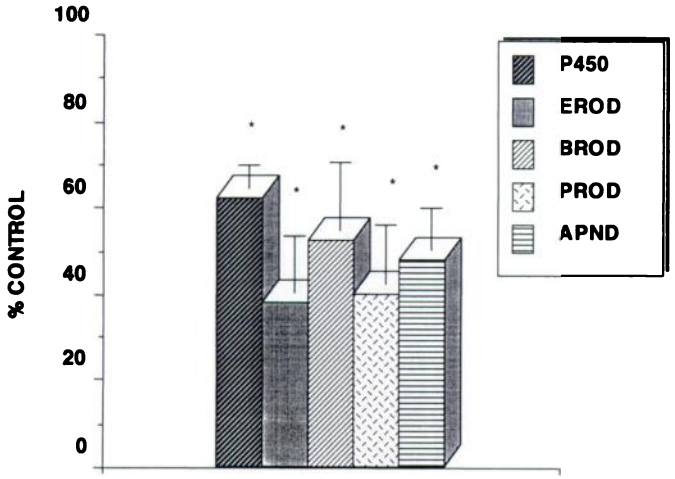

Fig. 4. Effect of 48 hr of infection with listeria on cytochrome P450 and related substrate metabolism in uninduced mice. Uninduced mice were infected with 1×10^6 CFU of 15U listeria for 48 hr. Hepatic microsomal cytochrome P450 content and EROD, BROD, PROD, and APND activities were determined and expressed as percentages of the mean control levels determined in mice treated for the same period with an equal volume of sterile PBS. The mean control levels were as follows: cytochrome P450, 0.70 ± 0.02 nmol/mg of protein; EROD, 27.8 ± 0.3 pmol of resorufin/mg/min; BROD, 4.6 ± 0.4 pmol of resorufin/mg/min; PROD, 1.7 ± 0.2 pmol of resorufin/mg/min; APND, 253 ± 18.7 μmol of HCHO/mg/hr. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (five mice/group).* Statistically different from control ($P < 0.05$).

P450IID9 cDNA probe is a complement to nucleotides 1429–1461 of the murine cytochrome P450IID9 mRNA sequence (28).

Slot blots and Northern blots were prehybridized at 42° for 4 hr in a volume of 4.5 ml or 12.5 ml, respectively, containing 6× SSPE (20× SSPE contains 3 M NaCl, 0.2 M NaH_2PO_4, and 0.02 M EDTA, pH 7.4), 1% sodium dodecyl sulfate, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, and 0.1 mg/ml sheared salmon sperm DNA, in a hybridization water bath. Hybridization of blots with the cytochrome P450IA or P450IID9 cDNA oligomers was carried out in a solution containing the same components as the prehybridization solution except that the salmon sperm DNA was replaced with 5 pmol of ^{32}P-labeled cytochrome P450IA or P450IID9 cDNA probe. Hybridizations were carried out for 18 hr at 48° for the cytochrome P450IA probe and for 3 hr at 60° for the cytochrome P450IID9 probe. Blots were washed in 2× SSC (10× SSC contains 1.5 M NaCl and 0.15 M sodium citrate) at room temperature for 10 min, followed by four 10-min washes at 64° or 60° for the cytochrome P450IA and P450IID9 probes, respectively.

The effect of listeria infection on total mRNA levels was determined by a modification of the method of Harley (29), using an oligo(dT)$_{18}$ probe. Slot blots were prehybridized at 42° for 1 hr in 4.5 ml of a solution containing 6× SSPE, 1% sodium dodecyl sulfate, 0.1% Ficoll, 0.1% bovine serum albumin, and 0.1 mg/ml sheared salmon sperm DNA. After the prehybridization period, this solution was replaced with 6 ml of a solution containing 5× SSC, 0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone, 8.6 mg of Na_2HPO_4, and 36 pmol of ^{32}P-labeled oligo(dT)$_{18}$ probe. The slot blots were hybridized at ambient temperature for 1 hr and were then washed for 4 × 5 min with 2× SSC at ambient temperature.

Blots were visualized on X-ray film (Kodak XAR-5) and the intensities of the bands on the autoradiograms were determined using a video densitometer (Bio-Rad model 620) and the 1-D Analyst software program. Duplicate slot blots were prepared for each experiment; one blot of each pair was hybridized with the cytochrome P450IA or P450IID9 cDNA probe and the second blot of each pair was used to ensure equal total mRNA loading, by hybridization with an oligo(dT)$_{18}$ probe. Autoradiographic exposure was adjusted to ensure that at least three of the dilutions were in the linear portion of the densitometric scan, and the optical density of a single band within the linear region was expressed as a ratio of the optical density of the RNA standard of that blot. The same procedure was repeated for the second blot of the pair, which was hybridized with the oligo(dT)$_{18}$ probe. The cytochrome P450 mRNA content was then corrected for total mRNA loading and this final ratio was used for statistical analysis.

Western blot analysis of microsomal protein. Hepatic microsomal proteins were separated by electrophoresis and the resulting Western blot was probed with an antibody directed against rat cytochrome P450IA1 apoenzyme. Preliminary studies demonstrated that this rabbit anti-rat cytochrome P450IA1 antibody also binds to murine

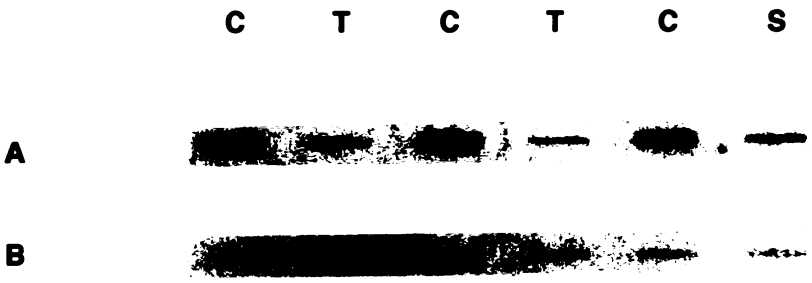

Fig. 5. Effect of 48 hr of infection with listeria on cytochrome P450IID9 mRNA and total mRNA levels. Mice were infected with listeria as described in Experimental Procedures. Hepatic RNA from control (*C*) and listeria-infected (*T*) mice, as well as an RNA standard (*S*), was bound to nylon filters and hybridized with the cytochrome P450IID9 cDNA (A) or oligo(dT)$_{18}$ (B) probes, and filters were exposed in an X-ray cassette with intensifying screens (Cronex Lightning Plus).

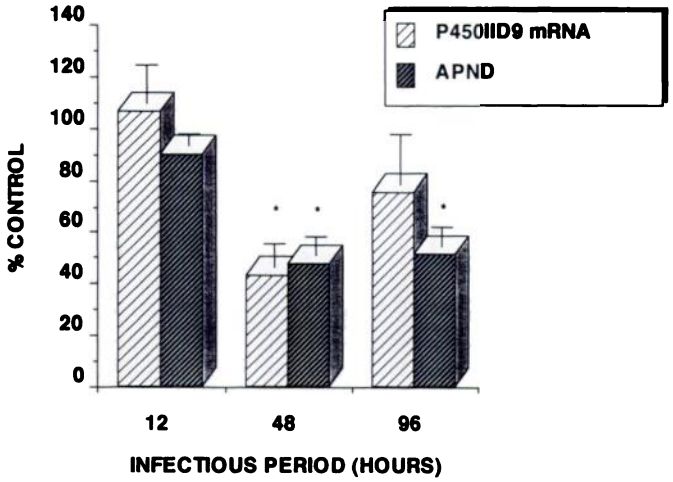

Fig. 6. Time course of the effect of infection with listeria on cytochrome P450IID9 mRNA levels and APND activity. Mice were infected with 1 × 10^6 CFU of 15U listeria and were killed at the specified times. APND activity was determined in hepatic microsomes and cytochrome P450IID9 mRNA levels were determined from slot blots as described in Experimental Procedures. All results are expressed as percentage of control. The mean control APND values for 12, 48, and 96 hr were 255.1 ± 8.0, 260.4 ± 9.6, and 267.2 ± 14.2 μmol of formaldehyde produced/mg of protein/hr, respectively. Statistical analysis was carried out on the raw data using an unpaired Student's *t* test (five mice/group).* Statistically different from control ($P < 0.05$).

cytochrome P450IA1 apoenzyme. Virtually no binding was evident in lanes containing microsomal protein from uninduced mice (data not shown) but intense bands were present in lanes containing microsomal protein from βNF-induced mice. The apparent molecular weight of the target protein was consistent with the molecular weight of murine cytochrome P450IA1 apoenzyme (30). Because the molecular weight of murine cytochrome P450IA2 is similar to that of cytochrome P450IA1, it must be recognized that we could not determine whether the antibody cross-reacted with cytochrome P450IA2.

Hepatic microsomal protein (20–25 μg) was added to each well in a volume of 10 μl and proteins were separated by polyacrylamide gel electrophoresis (7.5% polyacrylamide running gel and 3% stacking gel); protein stacking was carried out at 75 V for approximately 20 min and proteins were then separated in the running gel at 125 V. Proteins were transferred from the gel to nitrocellulose membranes using a semi-dry electrophoretic transfer system (Tyler Research Instruments) at 25 V for 15 min. The resulting Western blot analysis was then carried out using the cytochrome P450IA1 primary antibody (Oxygene) and an anti-rabbit IgG secondary antibody (Sigma) linked to alkaline phosphatase for detection. Membranes were incubated overnight at 4° with the primary antibody and were washed for 3 × 10 min with 0.05% Tween 20 in PBS. The secondary antibody was then added and incubated with the membrane for 1 hr at room temperature, followed by three 10-min washes with 0.05% Tween 20 in PBS.

Statistical methods. The unpaired Student's *t* test was used for statistical comparison between the treated group and corresponding control group for all experiments; a p value of <0.05 was considered statistically significant. All error bars represent the standard error.

Results

Total hepatic microsomal cytochrome P450 levels were decreased by 40% (0.46 ± 0.02 versus 0.74 ± 0.03 nmol/mg of protein; five mice/group, $p < 0.01$) and EROD activity was reduced by 60% (186 ± 48 versus 532 ± 113 pmol of resorufin/mg/min; five mice/group, $p = 0.02$) in βNF-induced mice infected for 48 hr with 1 × 10^6 CFU of listeria. During this infectious period, the mice did not demonstrate any obvious behavioral changes and histological examination of liver sections did not demonstrate pathological changes. No significant changes in total liver protein or microsomal protein concentrations were observed in these mice.

The levels of cytochrome P450IA mRNA in listeria-infected livers were decreased by 78% (1.3 ± 0.7 versus 5.8 ± 1.3 arbitrary absorbance units/μg of RNA; five mice/group, $p = 0.02$), as determined by slot blotting (Fig. 1). Northern blot analysis of hepatic RNA from the same mice demonstrated that the apparent molecular size of the mRNA that hybridized to the cytochrome P450IA cDNA probe was consistent with the size of murine cytochrome P450IA mRNA (27). Approximately equal hybridization levels were observed in the slot blots hybridized with the oligo(dT)$_{18}$ probe (Fig. 1), indicating that listeria infection did not affect total mRNA levels.

The time course for the effect of listeria infection on the loss of cytochrome P450IA mRNA and EROD activity in βNF-induced mice infected for 12, 24, 48, or 96 hr is shown in Fig. 2. The levels of cytochrome P450IA mRNA were increased 12 hr after infection, which was followed by a decrease at 24 and 48 hr after infection; the levels were not significantly different from control after 96 hr of infection (Fig. 2). EROD activity was significantly elevated 24 hr after infection but was significantly decreased at 48 and 96 hr (Fig. 2). The levels of cytochrome P450IA apoenzyme were elevated at 12 and 24 hr of infection and were depressed at 48 and 96 hr (Fig. 3).

In uninduced mice, total hepatic microsomal cytochrome P450 content and the rates of dealkylation of ethoxyresorufin, benzyloxyresorufin, pentoxyresorufin, and aminopyrine were significantly reduced, by approximately 40–60%, after 48 hr of infection with listeria (Fig. 4). The direct incubation of listeria (1 × 10^4 CFU) with hepatic microsomes for 24 hr at 37° had no effect on total cytochrome P450 or EROD, BROD, or PROD activities; however, there was a 70% decrease in the cytochrome P450 content of control microsomes during this incubation period. The levels of cytochrome P450IID9 mRNA were decreased by 50% (0.91 ± 0.20 versus 2.10 ± 0.37 arbitrary absorbance units/μg of RNA; five mice/group, $p = 0.02$), as determined by slot blotting (Fig. 5). Northern blot analysis of hepatic RNA from the same mice demonstrated that the ap-

parent molecular size of the mRNA that hybridized to the cytochrome P450IID9 cDNA probe was consistent with the size of murine cytochrome P450IID9 mRNA (28); no binding occurred in lanes containing hepatic RNA from female mice, consistent with the lack of this isozyme in female murine liver (31). Approximately equal hybridization levels were observed in the slot blots hybridized with the oligo(dT)$_{18}$ probe (Fig. 5), indicating that listeria infection also did not affect total mRNA levels in uninduced mice.

The time course for the loss of cytochrome P450IID9 mRNA and APND activity in mice infected for 12, 48, or 96 hr is summarized in Fig. 6. Listeria infection for 12 hr did not affect cytochrome P450IID9 mRNA levels nor did it have any effect on APND activity. Infection for 48 hr resulted in a significant decrease in both cytochrome P450IID9 mRNA levels and APND activity; cytochrome P450IID9 mRNA levels recovered to normal levels by 96 hr but APND activity was still significantly reduced at this time.

Discussion

The results of this study provide evidence that listeria infection depresses the drug-metabolizing capacity of several hepatic cytochrome P450 isozymes in mice. Total hepatic microsomal cytochrome P450 content and the rates of oxidation of four substrates (EROD, BROD, PROD, and APND activities) were all significantly reduced after 48 hr of infection. The isozymes that support these activities have not been fully characterized in uninduced mice; however, in uninduced rats EROD activity has been shown to be significantly inhibited in the presence of antibodies directed against cytochrome P450IA2 (32), as well as cytochromes P450IIC6 and P450IIC11 (33). PROD activity in uninduced rats was significantly inhibited by an antibody directed against cytochrome P450IIC11 (33); aminopyrine is a substrate that is known to be demethylated by many noninducible and inducible cytochrome P450 isozymes (34). If a similar pattern of isozyme activity occurs in mice, it appears that listeria infection results in significant depression of at least several and possibly all cytochrome P450 isozymes. Depression of several but not all isozymes has been observed in studies after the administration of recombinant interferons, interferon-inducing agents, and purified endotoxins (2, 35–40). In the present study, listeria infection did not cause overt liver damage, it had no effect on total liver protein or microsomal protein, and reactive species produced by the bacteria were not capable of degrading cytochrome P450 apoenzymes *in vitro*. These findings indicate that a generalized hepatotoxic effect of listeria is not responsible for the depression of cytochrome P450 levels.

L. monocytogenes infection resulted in significant depression of the βNF-inducible cytochrome P450IA isozyme. A significant decrease in hepatic microsomal EROD activity, as well as cytochrome P450IA apoenzyme levels, was observed after 48 hr of infection in βNF-induced mice. Slot blot analysis indicated that this loss of apoenzyme was accompanied by a loss of cytochrome P450IA mRNA that could entirely account for the loss of apoenzyme and EROD activity that was observed at the same time. This study demonstrates that listeria infection decreases cytochrome P450IA levels predominantly by pretranslational inhibition of the synthesis of the apoenzyme. A previous report from our laboratory indicated that the administration of poly(rI·rC) to clofibrate-treated rats resulted in a significant decrease in hepatic cytochrome P450IVA1 mRNA levels, total cytochrome P450 content, and microsomal lauric acid hydroxylase activities (2). Several other groups have confirmed that recombinant interferons and interferon-inducing agents can affect hepatic mRNA levels coding for other cytochrome P450 isozymes (36, 37, 39). The administration of *Escherichia coli* endotoxin to male rats significantly suppressed the levels of hepatic cytochrome P450IIC11 mRNA to approximately 15% of control levels (35) and resulted in 95% inhibition of cytochrome P450IIC11 gene transcription rates determined in hepatic nuclei (38). The present study is the first to report that cytochrome P450 mRNA is lost during an active infection.

Total mRNA levels, as determined by oligo(dT)$_{18}$ hybridization, were not affected during listeria infection. This demonstrates that listeria has a relatively selective effect on hepatic protein synthesis; the rate of synthesis of many proteins appeared to be unaffected, whereas the synthesis of some proteins such as cytochrome P450 were significantly depressed. This type of selectivity has also been documented after the administration of poly(rI·rC) to mice and of recombinant interferon-α to hamsters (41, 42). If the effect of listeria results predominantly from a decrease in cytochrome P450 mRNA levels, then the decrease in mRNA levels must be observed before the loss of apoenzyme and/or enzyme activity. Surprisingly, a significant increase in cytochrome P450IA mRNA was observed after 12 hr of infection, accompanied by a 2-fold increase in EROD activity at 12 and 24 hr. This apparent up-regulation of induction has been observed by others during immune stimulation but the mechanism involved is unknown (43–45). After the inductive phase, both cytochrome P450IA mRNA and EROD activity were significantly depressed as the infection proceeded. The levels of cytochrome P450IA apoenzyme paralleled the changes in EROD activity. The entire time course demonstrated that any changes in cytochrome P450IA mRNA preceded the changes in EROD activity and that mRNA levels returned to normal levels before the reversal of the effect on EROD activity. The results are consistent with a mechanism involving the modulation of cytochrome P450IA at a pretranslational step in βNF-treated mice. The decrease in cytochrome P450 mRNA during listeria infection could result from a decrease in the rate of mRNA transcription or from an alteration in mRNA stability.

Cytochrome P450$_{16α}$ shows high APND activity in rats (46), and APND was used to assess the functional status of cytochrome P450$_{16α}$ (cytochrome P450IID9) in mice during listeriosis. Cytochrome P450IID9 differed from cytochrome P450IA in its response to listeria, in that there was no up-regulation before the loss of this particular isozyme. Listeria had no effect on cytochrome P450IID9 mRNA or APND activity after 12 hr of infection, but significant decreases in both were observed after 48 hr of infection. Cytochrome P450IID9 mRNA levels in infected mice were not significantly different from control after 96 hr, whereas APND activity remained significantly suppressed at this time. The magnitude of the suppression of cytochrome P450IID9 mRNA throughout the time course could entirely account for the loss of isozyme activity, indicating that a pretranslational effect on the synthesis of cytochrome P450IID9 apoenzyme is responsible for the loss of this isozyme. These observations paralleled our findings that the modulation of an induced form (cytochrome P450IA) results from changes in mRNA levels.

In summary, murine listeriosis results in significant changes

in several cytochrome P450 isozymes. The cytochrome P450IA and P450IID9 isozymes are depressed at a pretranslational step involving a loss of mRNA coding for the apoenzyme portion of these isozymes. Augmentation of cytochrome P450 occured early in the infection but was specific for cytochrome P450IA. This is the first report that demonstrates that an active infection of any kind results in a significant change in cytochrome P450 mRNA. If the effect of *L. monocytogenes* on cytochrome P450 is shared by other bacteria, then potential drug reactions are likely to occur in infected patients.

Acknowledgments

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