Platelet-activating factor (PAF) is a potent proinflammatory phospholipid mediator that causes hypotension, increases vascular permeability, and has been implicated in anaphylaxis, septic shock and several other inflammatory responses. PAF is hydrolyzed and inactivated by the enzyme PAF-acetylhydrolase. In the intact rat, a mesenteric vein infusion of lipopolysaccharide (LPS) served as an acute, liver-focused model of endotoxemia. Plasma PAF-acetylhydrolase activity increased 2-fold by 24 h following LPS administration. Ribonuclease protection experiments demonstrated very low levels of plasma-type PAF-acetylhydrolase mRNA transcripts in the livers of saline-infused rats; however, 24 h following LPS exposure, a 20-fold induction of PAF-acetylhydrolase mRNA was detected. In cells isolated from endotoxin-exposed rat livers, Northern blot analyses demonstrated that Kupffer cells but not hepatocytes or endothelial cells were responsible for the increased PAF-acetylhydrolase mRNA levels. In Kupffer cells, plasma-type PAF-acetylhydrolase mRNA was induced by 12 h, peaked at 24 h, and remained substantially elevated at 48 h. Induction of neutropenia prior to LPS administration had no effect on the increase in PAF-acetylhydrolase mRNA seen at 24 h. Although freshly isolated Kupffer cells contain barely detectable levels of plasma-type PAF-acetylhydrolase mRNA, when Kupffer cells were established in culture, PAF-acetylhydrolase expression became constitutively activated concomitant with cell adherence to the culture plates. Alterations in plasma-type PAF-acetylhydrolase expression may constitute an important mechanism for elevating plasma PAF-acetylhydrolase levels and an important component in minimizing PAF-mediated pathophysiology in livers exposed to endotoxemia.

Platelet-activating factor (PAF) is a potent proinflammatory phospholipid (1-O-alkyl-2-acyl-sn-glycero-3-phosphocholine) involved prominently in diverse pathophysiological episodes. In fact, PAF has been implicated as a mediator of inflammation, allergic reactions, and shock (for review, see Chao and Olson (1)). Tissue PAF levels are modulated by regulation of key steps in both the biosynthetic and degradative pathways. The degradation of PAF occurs through the hydrolysis of the acetyl group at the sn-2 position of PAF and produces biologically inactive lyso-PAF. PAF-acetylhydrolase catalyzes the hydrolytic reaction, and this enzyme is present in mammalian blood (2–4), blood cells (5–7), and various tissues (5, 8, 9). Also, PAF-acetylhydrolase has been isolated from the peritoneal cavity of guinea pigs after endotoxin shock (10). Both intracellular and extracellular PAF-acetylhydrolase isoforms have been described. The molecular cloning and characterization of the human plasma PAF-acetylhydrolase was recently reported (11). This 44-kDa protein was isolated from human plasma, and amino acid sequence analysis led to the screening of a macrophage cDNA library where a positive cDNA clone was isolated. The cDNA encoded a 441-amino acid protein, which contained a secretion signal sequence. Tew et al. (12) have purified and cloned a cDNA for the human plasma PAF-acetylhydrolase and demonstrated it to be a glycosylated protein ranging in size from 43 to 67 kDa. In addition to the extracellular enzyme, the molecular characterization of two intracellular PAF-acetylhydrolases has been reported. Bovine brain PAF-acetylhydrolase, isoform 1b, is a heterotrimeric enzyme composed of 29-, 30-, and 45-kDa subunits (13, 14). Moreover, the cDNA sequences for a bovine and a human intracellular PAF-acetylhydrolase, isoform II, were published recently (15). The intracellular isoform II exhibited 43% amino acid identity to the human plasma PAF-acetylhydrolase.

The source of serum PAF-acetylhydrolase is not known, but a likely source is the liver since the liver secretes several plasma proteins in abundance, including lipoproteins. Cultured hepatocytes and the human hepatoma cell line, Hep G2, secrete PAF-acetylhydrolase into the culture media (16–18). Also, macrophages secrete large amounts of PAF-acetylhydrolase (19, 20), but whether macrophages contribute to the level of the circulating plasma enzyme has not been proven. Northern blot analyses of different human tissues demonstrated the presence of plasma-type acetylhydrolase mRNA in thymus and tonsil, tissues which contain macrophages in abundance (11). Even though hepatocytes secrete the plasma-type PAF-acetylhydrolase in culture, Tjoelker et al. (11) found no detectable level of PAF-acetylhydrolase RNA in normal human liver. It is quite possible that the abundance of PAF-acetylhydrolase mRNA is so low as to be undetectable in whole liver total RNA. Alternatively, as a mechanism to avoid PAF-induced pathophysiology, PAF-acetylhydrolase expression may achieve detectable levels only in response to a need for greater degradation of increased PAF levels following tissue injury.

Previous work in our laboratory has demonstrated that the isolated perfused rat liver responds to PAF with significant effects on both vascular resistance and glucose output (21–23).
We have demonstrated that the liver can produce PAF in response to particulate (24, 25) or endotoxin stimulation and that the Kupffer cells were the site of PAF synthesis (26, 27). Also, we specifically demonstrated the presence of PAF receptors (28) and PAF receptor RNA (17) in Kupffer cells. We have extended our research to intact animal models and have demonstrated that PAF accumulates in the intact liver exposed to various types of injury, including ischemia-reperfusion (29), obstructive jaundice (30), and endotoxin exposure (31). It may be appropriate to assume that increased PAF levels signal an increase in the level of PAF-acetylhydrolase for PAF degradation. For this reason, we have investigated whether plasma-type PAF-acetylhydrolase expression in the liver is regulated in response to endotoxin challenge in the intact rat.

A diverse set of pathophysiological responses accompanies exposure to lipopolysaccharide (LPS) including the induction of endotoxic shock and activation of the immune system and the complement cascade (32). Several biological responses to LPS are thought to be mediated by the release of proinflammatory substances such as cytokines and lipid mediators. Using a liver-focused model of endotoxemia, we have demonstrated for the first time the presence of plasma-type PAF-acetylhydrolase mRNA in rat liver. The expression in the liver was cell type-specific and was limited to Kupffer cells. After endotoxin exposure, the expression of PAF-acetylhydrolase mRNA increased 20-fold. Concurrent with the increase in liver plasma-type PAF-acetylhydrolase mRNA, there was a 2-fold increase in the circulating plasma PAF-acetylhydrolase activity.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Collagenase (type IV from Clostridium histolyticum), trypsin E (type XIV from Streptomyces griseus) and bovine serum albumin (fraction V, essentially fatty acid-free) were purchased from Sigma. Metrizamide (2-(3-acetamido-5-methyl (fraction V, essentially fatty acid-free) were purchased from Sigma. Mrini (38 °C), used for isolation and expansion of hepatocytes, was obtained from Nyegaard and Co. (Oslo, Norway). The rat cDNA homologue of the human plasma-type PAF-acetylhydrolase was kindly provided by ICOS (Bothell, WA). Unless specifically stated otherwise, any reference to PAF-acetylhydrolase refers to the plasma-type PAF-acetylhydrolase.

**LPS-stimulated Expression of PAF-Acetylhydrolase**—Male Sprague-Dawley rats weighing between 225 and 300 g, fed a standard laboratory chow ad libitum, were anesthetized by an intramuscular injection of 0.35 ml of a xylazine/ketamine mixture. A 1-cm lower midline abdominal incision was made, and a single loop of intestine was removed from the abdomen. LPS (Escherichia coli serotype O111:B4; 3 mg/kg) was dissolved in a solution of 0.9% NaCl and administered i.v. into a tail vein 4 days prior to exposure to LPS. LPS was administered as described above and Kupffer cells were isolated on day 5 following LPS administration, a time at which the circulating plasma PAF-acetylhydrolase activity was maximal.

**Liver—**Male Sprague-Dawley rats weighing between 225 and 300 g, fed a standard laboratory chow ad libitum, were anesthetized by an intramuscular injection of 0.35 ml of a xylazine/ketamine mixture. A 1-cm lower midline abdominal incision was made, and a single loop of intestine was removed from the abdomen. LPS (Escherichia coli serotype O111:B4; 3 mg/kg) was dissolved in a solution of 0.9% NaCl and administered i.v. into a tail vein 4 days prior to exposure to LPS. LPS was administered as described above and Kupffer cells were isolated on day 5 following LPS administration, a time at which the circulating plasma PAF-acetylhydrolase activity was maximal.

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**Ribonuclease Protection Assays—**Ribonuclease protection experiments, a full-length rat plasma-type PAF-acetylhydrolase cDNA was modified to generate an appropriate antisense RNA probe. A 700-bp EcoRI fragment was removed from the 3′ end of the PAF-acetylhydrolase cDNA. The remaining cDNA plus vector was agarose gel-purified and religated with T4 DNA ligase. This deletion construct placed the T3 RNA polymerase promoter adjacent to the EcoRI site at nucleotide 1125. The 3′-truncated PAF-acetylhydrolase cDNA was linearized with ClaI (nucleotide 950) and T4 RNA polymerase was used to create a 245-bp [α-32P]UTP labeled antisense RNA probe (MaxiScript, Ambion, Austin, TX). As an internal control, a 355-bp rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antisense RNA probe was generated using T3 RNA polymerase from the pTRI-GAPDH template (Ambion). Because of the extreme difference in mRNA abundance between GAPDH and PAF-acetylhydrolase, the specific activity of the GAPDH antisense RNA probe was reduced by greater than 1000-fold. Eighty micrograms of liver total RNA were hybridized in solution with both antisense RNA probes (RPAII Kit, Ambion). After ribonuclease digestion, the samples were separated on a denaturing 5% polyacrylamide, 8 M urea gel. Differences in the amount of PAF-acetylhydrolase and GAPDH mRNA were visualized using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Yeast tRNA was included as a negative control.

**Isolation of Liver Cells—**Hepatocytes were isolated from collagenase-digested livers by low speed centrifugation. Endothelial cells and Kupffer cells were isolated from rat livers using a modification of the centrifugal elutriation procedure of Knoop and Sleyster (33) as described previously (34). The viability of the liver cell preparations was greater than 95% as determined by trypan blue exclusion.

**RNA Isolation and Northern Blot Analyses—**All RNA isolation procedures were based on the method of Chomczynski and Sacchi (35). After the addition of 1 ml of chloroform and phase separation, the RNA was precipitated with 2.5 ml of isopropyl alcohol. For RNA obtained from hepatic cell types, the freshly isolated hepatocytes, endothelial cells, and Kupffer cells were immediately homogenized in TRIzol after centrifugal elutriation. For cultured Kupffer cells, 1 ml of TRIzol was added per 60-mm plate at the appropriate times. For Northern blots, 10 μg of total RNA was loaded on a 5% agarose, 2.2 M formaldehyde MOPS gel and electrophoresed at 70 V for 4 h. RNA was transferred to a Magna nylon membrane (Micron Separations Inc., Westborough, MA) and hybridized with a 32P-labeled PAF-acetylhydrolase cDNA prepared by random priming (36). Hybridizations were performed at high stringency (50% formamide, 1 M NaCl, 10% dextran sulfate, 50 mM Tris, pH 7.5, 0.1% sodium pyrophosphate, and 0.2% Denhardt’s solution) at 42 °C for 16 h and the membranes were washed twice in 2× SSC, 1% SDS at 65 °C for 20 min and once in 0.1× SSC, 0.1% SDS at room temperature for 15 min. After hybridization with the PAF-acetylhydrolase cDNA, the membranes were stripped and rehybridized with a 32P-end-labeled oligonucleotide complimentary to 18 S RNA to verify sample loading and the integrity of the RNA. Northern blots were visualized and quantitated using the PhosphorImager (Molecular Dynamics).

**Neutropenic Rats—**Rat neutrophils were depleted by an injection of vinblastine sulfate as described previously (30). Briefly, vinblastine sulfate (0.75 mg/kg) was dissolved in physiological saline and injected i.v. into a tail vein 4 days prior to exposure to LPS. LPS was administered as described above and Kupffer cells were isolated on day 5 following vinblastine treatment, a time at which the vinblastine sulfate has induced maximal neutrophil depletion (37).

**PAF-Acetylhydrolase Assay—**Rat serum was diluted 1/10 with phosphate-buffered saline containing 0.1% BSA. The diluted serum (50 μl) was incubated with 40 μl of 1.25 × 10−3 M unlabeled PAF (50 nmol) and 10 μl of 1 × 10−3 M [3H]Acetyl-PAF in a glass tube at 37 °C for 10 min. The reaction was stopped by cooling on ice. Forty microliters of a 7% BSA solution were added to the reaction mixture and incubated for 5 min at 0 °C. Sixty microliters of a 30% trichloroacetic acid solution were added, and the mixture was incubated for an additional 10 min at 0 °C. To separate the denatured protein, the reaction mixture was centrifuged for 5 min at 3800 × g. 100 μl of the supernatant were mixed with 10 μl of scintillation mixture, and the amount of liberated radioactive acetate was counted in a liquid scintillation counter. The control values of released acetate acid were obtained for both free serum and serum heated for 10 min in boiling water (38).

**Primary Culture of Rat Kupffer Cells—**After enzymatic digestion of the rat liver and centrifugal elutriation of Kupffer cells, isolated Kupffer cells were maintained at 37 °C in RPMI 1640 culture medium (Life Technologies, Inc.), supplemented with 25 mMol/liter HEPES, t-glutamine, 10% fetal bovine serum (Hyclone Laboratories, Inc., Logan, UT), 112 units/ml penicillin, and 112 units/ml streptomycin in 60-mm tissue culture dishes. Cells were plated at a density of 10 million cells per 60-mm culture dish. All cells were incubated in 90% air and 10% CO2. On the second day of culture, the RPMI medium was changed.

**RESULTS**

**Plasma-type PAF-Acetylhydrolase mRNA Levels in Whole Liver—**Under pathophysiological conditions, endotoxin exposure can occur through increased absorption of endotoxin from the gastrointestinal tract leading to systemic endotoxemia. The liver is critical in protecting the systemic circulation from gut-derived LPS. Increased hepatic absorption of LPS from the gastrointestinal tract has been associated with instances of...
Northern blot analyses of these RNA samples (Fig. 2) indicated chemical induced liver injury (39), partial hepatectomy (40), and intestinal ischemia/reperfusion (41–43). The infusion of LPS directly into a rat mesenteric vein was employed as a liver-focused model of endotoxemia. The effects of this type of endotoxin exposure on plasma-type PAF-acetylhydrolase expression in whole liver and isolated hepatic cells and on PAF-acetylhydrolase activity in circulating blood were investigated at times ranging from immediately after LPS exposure to 48 h later.

We assayed whole liver for the presence of plasma-type PAF-acetylhydrolase mRNA in both saline- and LPS-infused rats. A ribonuclease protection assay was employed to investigate liver PAF-acetylhydrolase expression at various times after endotoxin exposure (Fig. 1). Both a 245-bp antisense PAF-acetylhydrolase RNA and a 355-bp antisense GAPDH RNA were hybridized in solution with 80 μg of total liver RNA. After RNase digestion, the protected fragments were distinguished by their sizes in kilobases are shown on the right panel. The Northern blot was stripped and reprobed with an 18 S RNA probe (lower panel). The Northern blot shown is a representative blot of three independent experiments.

PAF-Acetylhydrolase mRNA Levels in Isolated Hepatic Cells—To determine the cell type responsible for the increase in PAF-acetylhydrolase expression, hepatocytes, Kupffer cells, and endothelial cells were isolated from endotoxin-exposed livers 24 h after treatment. Total RNA was obtained from these cells immediately after completion of the isolation process. Northern blot analyses of these RNA samples (Fig. 2) indicated that Kupffer cells and endothelial cells but not hepatocytes contained a 1.8-kb transcript which hybridized at high stringency to the full-length PAF-acetylhydrolase cDNA. Freshly isolated sinusoidal endothelial cells (not cultured) contain approximately 10% contamination by Kupffer cells; therefore, we concluded that Kupffer cells were the primary cell type responsible for the elevated PAF-acetylhydrolase expression detected in whole liver 24 h after LPS exposure. A larger 4.4-kb band was determined to be nonspecific binding to the 28 S RNA. After hybridization of the Northern blot with the PAF-acetylhydrolase cDNA, the membrane was stripped and reprobed with a 32P-labeled oligonucleotide complimentary to 18 S RNA. The 18 S oligonucleotide hybridized with relatively equal intensity to all three hepatic cell types.

In Vivo Time Course of PAF-Acetylhydrolase mRNA Induction in Isolated Kupffer Cells—Kupffer cells isolated from livers of rats exposed to LPS for 24 h showed a pronounced increase in expression of PAF-acetylhydrolase. To determine the time course of PAF-acetylhydrolase RNA induction, rats were exposed to LPS as before. At 3, 6, 12, 24, and 48 h after exposure, Kupffer cells were isolated and examined for the presence of PAF-acetylhydrolase transcripts. Northern blot analyses (Fig. 3) demonstrated a small increase in RNA levels at 6 h after LPS administration. PAF-acetylhydrolase message levels appeared to maximize at 24 h. At 48 h following LPS exposure, PAF-acetylhydrolase transcripts remained elevated at 80% of the level seen at 24 h. The time course of PAF-acetylhydrolase induction in Kupffer cells isolated from LPS-exposed livers, agreed with the time course of PAF-acetylhydrolase mRNA observed in the analysis of whole liver.

PAF-Acetylhydrolase mRNA Levels in Neutropenic Rats—The previous experiments demonstrated an increase in PAF-acetylhydrolase mRNA in response to LPS administration and that the Kupffer cell was responsible for the increase in RNA detected. To exclude the possibility that an infiltration of neutrophils and mononuclear cells into the liver was responsible for the increase in PAF-acetylhydrolase mRNA, Kupffer cells were isolated from LPS-exposed neutropenic rats. Vinblastine
sulfate (0.75 mg/kg) was injected 4 days prior to LPS infusion. Twenty-four hours after LPS infusion, Kupffer cells were isolated and compared with LPS-infused rats that had not received vinblastine sulfate. A Northern blot comparison (Fig. 4) of total RNA from these Kupffer cells demonstrated no change in the amount of PAF-acetylhydrolase induction when corrected for RNA loading.

PAF-Acetylhydrolase Activity in Rat Serum—The predominant cellular source(s) of the plasma PAF-acetylhydrolase in vivo has not been determined. To investigate whether the increase in PAF-acetylhydrolase expression in endotoxin-challenged livers could result in elevated serum PAF-acetylhydrolase levels, we assayed rat serum at 1 min and 6, 12, and 24 h after saline or LPS infusion. No change in plasma PAF-acetylhydrolase activity was detected at 1 min and 12 h. A small but statistically significant increase in PAF-acetylhydrolase activity was detected at 6 h. Furthermore, a 2-fold increase in plasma PAF-acetylhydrolase activity was detected at 24 h following LPS exposure (Fig. 5). In one rat assayed 48 h after LPS administration, the serum PAF-acetylhydrolase activity remained elevated (data not shown).

Plasma-type PAF-Acetylhydrolase mRNA Levels in Cultured Kupffer Cells—To investigate the mechanism(s) responsible for the LPS-induced increase in acetylhydrolase expression, we intended to employ in vitro models. In initial experiments, we observed pronounced PAF-acetylhydrolase expression in cultured Kupffer cells from untreated rats (data not shown). To characterize this observation, Kupffer cells were isolated from untreated rats and the PAF-acetylhydrolase mRNA was assayed at different times following the establishment of the Kupffer cells in culture. Freshly isolated Kupffer cells expressed barely detectable levels of PAF-acetylhydrolase. PAF-acetylhydrolase RNA increased as early as 5.5 h after the Kupffer cells were plated (Fig. 6). Within 19 h of plating, Kupffer cell PAF-acetylhydrolase mRNA was fully induced. This level of mRNA was sustained throughout the duration of the culture interval even when extended to 7 days. When cultured Kupffer cells were treated with LPS no further increase in PAF-acetylhydrolase mRNA was detected. In fact, LPS decreased PAF-acetylhydrolase RNA in cultured Kupffer cells (data not shown).

**FIG. 3.** Time course of Kupffer cell PAF-acetylhydrolase (PAF-AH) mRNA expression in response to LPS. Kupffer cells were isolated from LPS- or saline-infused rats at 3, 6, 12, 24, or 48 h after exposure. Total RNA was prepared from liver Kupffer cells immediately after the cellular isolation procedure. Samples of total RNA (10 mg) were separated on a formaldehyde/agarose gel, transferred to a nylon membrane, and probed with a full-length rat PAF-acetylhydrolase cDNA (upper panel). Washes were performed as described under "Experimental Procedures." Molecular size markers in kilobases are shown on the right. The Northern blot was stripped and reprobed with an 18 S RNA probe (lower panel). The Northern blot shown is representative of three independent experiments.

**DISCUSSION**

Ribonuclease protection assays revealed the presence of plasma-type PAF-acetylhydrolase mRNA in rat liver. In untreated rat liver, PAF-acetylhydrolase RNA was barely detectable in 80 pg of total liver RNA. Based on the difference in the specific activity of the ribonuclease protection probes, PAF-acetylhydrolase RNA was 1000-fold less abundant than GAPDH mRNA. Thus, the PAF-acetylhydrolase mRNA is an extremely low abundance transcript in normal rat liver and is not detectable by Northern blotting of total rat liver RNA. In a Northern blot of various human tissue RNAs, Tjoelker et al. (11) detected PAF-acetylhydrolase mRNA in thymus, tonsil, and placental RNA but none in heart, kidney, or liver RNA. Although it is possible the authors' inability to detect plasma-type PAF-acetylhydrolase mRNA in human liver was a result of species differences between rat and human, the negative Northern blot for human liver most likely reflects the low abundance of PAF-acetylhydrolase RNA in normal tissue and demonstrates the necessity of detecting the acetylhydrolase transcript using the more sensitive ribonuclease protection assay. LPS infusion into
Northern blot shown is a representative blot of three independent stripped and reprobed with an 18 S RNA probe (lower panel). The Northern blot was stripped and reprobed with an 18 S RNA probe (lower panel). The Northern blot shown is a representative blot of three independent experiments.

LPS-stimulated Expression of PAF-Acetylhydrolase

PAF-AH

FIG. 6. Constitutively activated expression of PAF-acetylhydrolase mRNA in cultured Kupffer cells. Kupffer cells were isolated from untreated rats and established in culture. At various times after cell plating, total RNA was isolated. Kupffer cell RNA (10 μg)(215,120),(684,562) was separated, transferred to a nylon membrane, and probed with a full-length rat PAF-acetylhydrolase cDNA (upper panel). Washes were performed as described under “Experimental Procedures.” Molecular size markers in kilobases are shown on the right. The Northern blot was stripped and reprobed with an 18 S RNA probe (lower panel). The Northern blot shown is a representative blot of three independent experiments.

PAF-acetylhydrolase mRNA in the liver 24 h following treatment.

The low abundance of PAF-acetylhydrolase RNA suggested that the message may only be present in a specific subpopulation of liver cells. Following 24 h of in vitro LPS exposure, hepatocytes, endothelial cells and Kupffer cells were isolated and assayed for the presence of PAF-acetylhydrolase RNA. The PAF-acetylhydrolase transcript was limited to Kupffer cells in the liver. Although the 1.8-kb transcript also was detected in the Northern blot of freshly isolated sinusoidal endothelial cells, the amount of message present was consistent with a 10% contamination of the endothelial cells with Kupffer cells. This level of contamination of freshly isolated endothelial cells is routinely observed in our laboratory, and the subsequent plating and culturing of the endothelial cells removes the Kupffer cell contamination. In addition, cultured endothelial cells do not secrete PAF-acetylhydrolase into the culture medium (data not shown), whereas cultured Kupffer cells actively secrete PAF-acetylhydrolase activity into the culture medium. The increase in Kupffer cell PAF-acetylhydrolase RNA following endotoxin exposure became apparent at 6 h and reached a maximum at 24 h. Forty-eight hours after LPS treatment the PAF-acetylhydrolase remained induced. This time course of induction following LPS treatment exactly matched the time of induction seen in whole liver and paralleled the increase detected in plasma acetylhydrolase activity. Induction of neutropenia failed to alter the elevated expression of PAF-acetylhydrolase in the liver. It is our contention that Kupffer cells constitute the major source of hepatic plasma-type PAF-acetylhydrolase and that infiltrating neutrophils (and the subsequent infiltration of mononuclear cells) are not responsible for the elevated hepatic acetylhydrolase RNA detected after LPS exposure. Previously, we have demonstrated that in jaundiced rats neutropenia failed to alter the increase seen in hepatic PAF concentrations; this confirmed the Kupffer cell as the source of PAF production (30).

Plasma PAF-acetylhydrolase is considered to have an important role in controlling the pathophysiological effects of PAF. The level of PAF-acetylhydrolase in the plasma can be altered by genetic factors, since deficiency of this enzyme is transmitted as an autosomal recessive trait among five affected Japanese families (38). This inherited deficiency of PAF-acetylhydrolase is the result of a point mutation in exon 9 which abolishes enzymatic activity (44). The level of plasma PAF-acetylhydrolase activity also can be altered by acquired factors. Changes in the activity of plasma acetylhydrolase have been found in conjunction with asthma (38, 45), systemic lupus erythematosus (46), hypertension (47, 48), chronic cholestasis (49), and necrotizing enteroctis (50, 51). In the case of clinical sepsis, two conflicting reports have been published. In one instance, PAF-acetylhydrolase activity was significantly higher in 17 septic patients who died than in 13 septic patients who survived (52). However, Graham et al. (53) demonstrated that PAF-acetylhydrolase activity was approximately half of normal in patients severely ill with clinical sepsis. In the present study using an acute endotoxin exposure model to reproduce the clinical situation observed in post-surgical shock, lipopolysaccharide exposure resulted in a 2-fold increase in the plasma acetylhydrolase activity. This increase in activity was evident at 24 h following LPS exposure and remained elevated at 48 h following LPS exposure. The elevated plasma PAF-acetylhydrolase activity likely reflects a physiological response to inactive elevated levels of PAF during episodes of endotoxin exposure. Additionally, elevated levels of plasma PAF-acetylhydrolase could prevent the accumulation of oxidized phospholipids which may be problematic in the pathogenesis of endotoxia.

When Kupffer cells from untreated rats were maintained in cell culture, we observed constitutively activated expression of PAF-acetylhydrolase. This expression was apparent within 5 h of plating the cells, was maximally induced by 19 h, and persisted throughout the culture interval. The cell culture-induced expression of PAF-acetylhydrolase is in direct contrast to another LPS-stimulated response in Kupffer cells, the induction of nitric oxide synthase. Kupffer cells are known to express inducible nitric oxide synthase after exposure to LPS both in vivo (54) and in vitro. The process of Kupffer cell isolation and culturing does not induce nitric oxide synthase expression in the absence of LPS (55). Isolated Kupffer cells from untreated rats express PAF-acetylhydrolase mRNA during the culture process to the same extent as that seen in vivo following LPS treatment, this observation would argue against an increase in Kupffer cell number as being responsible for the increase of PAF-acetylhydrolase mRNA in vivo. Recent studies have demonstrated that monocytes do not secrete PAF-acetylhydrolase activity until they differentiate into macrophages (19, 20). This differentiation-dependent expression was observed at the level of RNA as there was no PAF-acetylhydrolase mRNA in freshly isolated monocytes, but expression was induced and maintained during differentiation into macrophages (11). In addition, the myelocytic leukemia cell line (HL-60) also produced and secreted PAF-acetylhydrolase when the cells were differentiated into macrophages following stimulation by 12-O-tetradecanoylphorbol-13-acetate. In the HL-60 cells, LPS inhibited the secretion of PAF-acetylhydrolase in a dose-dependent manner (20). Likewise, we have observed a decrease in acetylhydrolase mRNA in cultured Kupffer cells incubated with LPS. Although Kupffer cells are the terminally differentiated resident macrophages of the liver, they express barely detectable levels of PAF-acetylhydrolase RNA in vivo until LPS or a downstream mediator of LPS activates the macrophage. The opposite effect of LPS in cultured macrophage cells demon-

2 A. T. Eakes, personal communication.

3 S. I. Svetlov, K. M. Howard, M. S. DeBuysere, and M. S. Olson, manuscript in preparation.
strates the necessity of studying LPS effects on macrophage PAF-acetylhydrolase expression in an in vivo model of endotoxemia.

The mechanism of LPS stimulation of PAF-acetylhydrolase expression remains to be determined. LPS regulates the expression of numerous genes and can affect the rate of transcription as observed with tumor necrosis factor α (56) or can affect the stability of the message as seen with interleukin-1 (57).

PAF-acetylhydrolase activity were detected in patients with sepsis, but the stability of the message as seen with interleukin-1 (57).

Also, LPS could have an indirect effect on PAF-acetylhydrolase expression following trauma/injury episodes. Alterations in hepatic plasma-type PAF-acetylhydrolase activity were observed for tumor necrosis factor α (56) or can affect the stability of the message as seen with interleukin-1 (57).

The mechanism of LPS stimulation of PAF-acetylhydrolase expression in an in vivo model of endotoxemia.

Our evidence suggests the liver may play an important role in regulating levels of PAF-acetylhydrolase in the circulation under pathophysiological situations. Elevated levels of serum PAF-acetylhydrolase activity were detected in patients with chronic cholestasis. Normalization of liver function following liver transplantation was accompanied by a reduction to normal or near normal PAF-acetylhydrolase levels (49). We have shown in a liver-focused model of endotoxemia that plasma-type PAF-acetylhydrolase expression in Kupffer cells is induced concomitant with an increase in the circulating PAF-acetylhydrolase activity. The Kupffer cells of the liver are a likely cellular source for the increase in plasma PAF-acetylhydrolase activity; however, neutrophils, circulating macrophages, or other blood components could augment the elevation in plasma PAF-acetylhydrolase activity following trauma/injury episodes. Alterations in hepatic plasma-type PAF-acetylhydrolase expression may constitute an important mechanism for elevating PAF-acetylhydrolase levels in the blood, thus implicating the liver as an important component in minimizing PAF-mediated pathophysiologies of the liver and/or other organs exposed to endotoxic challenge.

Acknowledgments—We thank Michael DeBuysere and Lynnette Walters for their skillful technical assistance and Dr. Stephen Harvey for critical reading of the manuscript.

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