The production of platelet-activating factor (PAF) and PAF-like phospholipids that also bind the PAF receptor are implicated in numerous pathological situations including bacterial endotoxemia and injury-induced oxidative damage. PAF and PAF-like phospholipids are hydrolyzed and inactivated by the enzyme PAF acetylhydrolase. In the intact rat, infusion of lipopolysaccharide (LPS) into a mesenteric vein served as an acute, liver-focused model of endotoxemia. We determined that the liver responds to LPS exposure with the production of plasma-type PAF acetylhydrolase mRNA and protein expression specifically in the resident macrophages of the liver. Liver macrophages, defined immunohistochemically using antibodies against ED1, present in livers from saline-treated animals contained no detectable PAF acetylhydrolase. Twenty-four hours following in vivo LPS administration, immunohistochemistry detected a slight increase in the number of ED1 staining cells and the ED1-positive cells now contained an abundance of PAF acetylhydrolase. The systemic administration of LPS resulted in increased expression of PAF acetylhydrolase in several tissues. Of the tissues examined, the greatest increase in PAF acetylhydrolase expression was observed in lung followed by increases in spleen, liver, kidney, and thymus. Additionally, the expression of PAF acetylhydrolase mRNA increased in circulating leukocytes and in peritoneal macrophages in response to systemic exposure to LPS. We examined the regulation of PAF acetylhydrolase expression and demonstrated the administration of the PAF receptor antagonists, BN 50739 and WEB 2170, inhibited by 50% the increase in PAF acetylhydrolase expression in response to LPS. The up-regulation of the plasma-type PAF acetylhydrolase expression constitutes an important mechanism for elevating the local and systemic ability to inactivate PAF and oxidized phospholipids in order to minimize PAF-mediated pathophysiology consequent from exposure to endotoxin. The abundance of PAF acetylhydrolase production in the liver lobule likely limits endotoxin-mediated tissue damage due to PAF synthesis.

A diverse set of pathophysiological responses accompany exposure to lipopolysaccharide (LPS), including the induction of endotoxin shock and activation of the immune system and the complement cascade. Several biological responses to LPS are thought to be mediated by the release of proinflammatory substances such as cytokines and lipid mediators. Exposure to LPS may occur during Gram-negative bacterial infections or through increased absorption of LPS from the gastrointestinal tract. In fact, increased hepatic absorption of LPS from the gastrointestinal tract has been associated with instances of chemical-induced liver injury, partial hepectectomy, and intestinal ischemia/reperfusion (1). Platelet-activating factor (PAF) is a potent pro-inflammatory phospholipid (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine), which mimics several pathophysiological responses observed after endotoxin exposure including hypotension, cardiac failure, and tissue necrosis. Moreover, PAF is involved prominently in various other pathophysiological episodes and participates as a mediator of inflammation, allergic reactions, and shock (for review, see Ref. 2).

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 is accomplished by the enzyme, PAF acetylhydrolase. There are several isoforms of this enzyme (both intracellular and extracellular) and PAF acetylhydrolase activity is present in mammalian blood, blood cells, and various tissues (3). Two independent laboratories have cloned and characterized the extracellular human plasma PAF acetylhydrolase. Tjoelker et al. isolated a 44-kDa protein from human plasma and cloned the corresponding cDNA (4). In addition, Tew et al. purified the human plasma PAF acetylhydrolase and demonstrated it was a glycosylated protein ranging in size from 43 to 67 kDa. The corresponding cloned cDNA was identical in sequence to that previously published by Tjoelker et al. (5).

Although the precise cellular/tissue source of the circulating PAF acetylhydrolase is not known, an acknowledged probable source is the macrophage. Macrophages are capable of secreting large amounts of PAF acetylhydrolase (6, 7). A recent report concluded that a majority of the PAF acetylhydrolase activity in human plasma originates from hematopoietic lineage cells such as macrophages (8). In mammals, the liver contains the largest single population of macrophages (Kupffer cells), which are crucial in the clearance and subsequent protection of an individual from LPS exposure. Evidence suggests the liver can contribute a substantial amount of PAF acetylhydrolase activity to the serum. Elevated levels of serum PAF acetylhydrolase activity were detected in patients with chronic cholestasis. Normalization of liver function following liver activating factor; PAF acetylhydrolase, platelet-activating factor acetylhydrolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; GST, glutathione S-transferase.
transplantation was accompanied by a reduction to normal or near normal PAF acetylhydrolase levels (9).

The effects of LPS on plasma PAF acetylhydrolase expression remain controversial. In the myelocytic leukemic cell line (HL-60), which produced and secreted PAF acetylhydrolase after the cells differentiated into macrophages, LPS inhibited the secretion of PAF acetylhydrolase in a dose-dependent manner (6). Likewise, we have observed a decrease in PAF acetylhydrolase mRNA in cultured Kupffer cells incubated with LPS (10). Additionally, IFN-γ and LPS decreased the human PAF acetylhydrolase promoter activity by 35% and 50% respectively in monocyte-derived macrophages and various established macrophage cell lines (11). However, changes in the in vivo activity of plasma PAF acetylhydrolase have been documented in conjunction with asthma (12), systemic lupus erythematosus (13), hypertension (14, 15), chronic cholestasis (9), and necrotizing enterocolitis (16, 17). In most cases, the levels of circulating PAF acetylhydrolase activity are increased as a physiological response to inflammatory stimuli.

To clarify the in vivo response of PAF acetylhydrolase expression to LPS, we have investigated the expression and regulation of PAF acetylhydrolase in response to LPS exposure. We have demonstrated the production of plasma-type PAF acetylhydrolase protein and its localization in the LPS-exposed liver. Additionally, we examined the expression of PAF acetylhydrolase in response to LPS exposure in several other tissues, in leukocytes, and in peritoneal macrophages. In addition, we demonstrated that the PAF receptor participates in the signaling pathway, which results in the induction of PAF acetylhydrolase expression in response to endotoxin.

EXPERIMENTAL PROCEDURES

Reagents—Collagenase (type IV from Clostridium histolyticum), protease E (type XIV from Streptomyces griseus) and bovine serum albumin (fraction V, essentially fatty acid-free) were purchased from Sigma. 2-(3-Acetamido-5-N-methylacetamido-2,4,6-triiodobenzamido)-2-deoxy-d-glucose (metrizamide) was obtained from Nyegaard and Co. (Oslo, Norway). The rat cDNA homologue of the human plasma-type PAF acetylhydrolase was kindly provided by ICOS Corp. (Bothell, WA). Unless specifically stated otherwise, all references to PAF acetylhydrolase refer to the plasma-type PAF acetylhydrolase.

Endotoxin Exposure—Male Harlan Sprague-Dawley rats weighing between 225 and 300 g were fed a standard laboratory chow ad libitum. LPS (Escherichia coli serotype 055:B5; 0.4 mg/kg; 1 × 10^8 endotoxin units) dissolved in a solution of 0.1% bovine serum albumin in saline was infused slowly through a 27-gauge needle into a distal mesenteric vein as described previously (10). In control rats, a solution of 0.1% bovine serum albumin in saline without LPS was infused into the mesenteric vein. In experiments designed to determine whether the PAF receptor was involved in the regulation of expression of the PAF acetylhydrolase, rats received the PAF receptor antagonist BN-50739 (10 mg/kg, intravenously) or WEB 2170 (5 mg/kg, intravenously) 30 min prior to LPS administration. In order to effect a systemic exposure to LPS, where specified, some rats received LPS (3 mg/kg) via a tail vein injection. Saline- and LPS-treated rats (n = 3) were used either for collection of tissues and peritoneal macrophages or for the isolation of whole blood. Whole blood was removed by inferior vena cava cannulation into 10 ml EDTA to prevent clotting. Tissues were harvested and freeze-clamped immediately in liquid nitrogen and stored at −80 °C.

Isolation of Liver Cells—Kupffer cells were isolated from rat livers using a modification of the centrifugal elutriation procedure of Knook and Sleyster (18) as described previously (19). In some experiments, total liver sinusoidal cells were isolated. In these instances, cells were sampled following digestion of the rat liver, low speed centrifugation, and the metrizamide gradient centrifugation but prior to the final centrifugal elutriation step. The viability of the liver cell preparations was greater than 95% as determined by trypan blue exclusion.

PAF Acetylhydrolase Antiserum Production—The rat cDNA encoding the plasma-type PAF acetylhydrolase was generously provided by ICOS Corp. The coding sequence was then amplified with polymerase chain reaction primers designed from the rat sequence and which incorporated BamHI and NotI restriction sites at the primer ends. This polynucleotide chain reaction fragment was subcloned into a pGEX expression plasmid (Amersham Pharmacia Biotech). The GST/PAF acetylhydrolase fusion protein was expressed in E. coli according to the manufacturer’s instructions. The recombinant PAF acetylhydrolase/GST fusion protein was purified using a glutathione-Sepharose affinity column. Recombinant PAF acetylhydrolase was eluted from the GST fusion protein using the site-specific protease thrombin. For antibody production, 100 μg of recombinant PAF acetylhydrolase was mixed with Freund’s complete adjuvant and injected subcutaneously into four sites on the rabbit. Booster injections (50 μg) were given in Freund’s incomplete adjuvant. Antibody production was monitored by enzyme-linked immunosorbent assay. An excellent antibody titer was achieved in the rabbits following the second boost. Serum was collected from antigen-exposed rabbits and the polyclonal rabbit IgG was purified over an Affi-Gel protein A column (Bio-Rad) according to the manufacturer’s instructions. For use in both Western blots and immunohistochemistry experiments, this IgG fraction was further purified using a rat recombinant PAF acetylhydrolase affinity column. One mg of recombinant PAF acetylhydrolase was coupled to 1.0 ml of Affi-Gel 10 (Bio-Rad), an activated immunoaffinity support, according to the manufacturer’s instructions. Coupling efficiency was determined to be 93%. The antibody column was washed extensively with 10 ml of 50 mM Tris (pH 7.5), 10 ml of 100 mM glycine (pH 2.5), and 10 ml of 100 mM triethylamine (pH 11.5) to remove unbound protein. One ml of purified IgG was passed through the column. The column was washed with 20 ml of 100 mM Tris (pH 7.5) and 20 ml of 500 mM NaCl, 50 mM Tris (pH 7.5). Retained anti-PAF acetylhydrolase antibodies were eluted with 10 ml of 100 mM glycine (pH 2.5) and 10 ml of 100 mM triethylamine (pH 11.5). Anti-PAF acetylhydrolase antibodies were eluted almost exclusively in the 100 mM glycine (pH 2.5) fraction as determined by enzyme-linked immunosorbent assay.

Western Blot Analyses—Freshly isolated Kupffer cells or total liver sinusoidal cells were collected by low speed centrifugation and then homogenized in 5 ml of radiomune precipitation buffer (phosphate-buffered saline containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, plus 10 mg/ml PMSF and 30 μl/m1 aprotinin). The cell lysate (20 μg) was mixed with 2× SDS sample buffer (100 mM Tris-CI, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromphenol blue, and 2% glycerol) and subjected to SDS-PAGE (10% gel) using the buffer system of Laemmli (20). The separated proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Millipore, Bedford, MA), using a semidy transfer blot system and the membranes were blocked in Tris-buffered saline (pH 7.4) containing 5% nonfat dried milk powder for 2 h at room temperature. The Western blots were incubated with the affinity-purified anti-PAF acetylhydrolase (1:5000) at 4 °C overnight with gentle shaking. The blots were rinsed extensively and then incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (Jackson Laboratories, West Grove, PA) for 2 h. Finally, the blots were rinsed extensively in 50 mM Tris/Cu, pH 7.4, 150 mM NaCl, and 0.1% Tween 20. The peroxidase-labeled proteins were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech). Western Blot Analyses—Freshly isolated Kupffer cells or total liver sinusoidal cells were isolated by low speed centrifugation and then homogenized in 5 ml of radioactive precipitation buffer (phosphate-buffered saline containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, plus 10 mg/ml PMSF and 30 μl/m1 aprotinin). The cell lysate (20 μg) was mixed with 2× SDS sample buffer (100 mM Tris-CI, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromphenol blue, and 2% glycerol) and subjected to SDS-PAGE (10% gel) using the buffer system of Laemmli (20). The separated proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Millipore, Bedford, MA), using a semidy transfer blot system and the membranes were blocked in Tris-buffered saline (pH 7.4) containing 5% nonfat dried milk powder for 2 h at room temperature. The Western blots were incubated with the affinity-purified anti-PAF acetylhydrolase (1:5000) at 4 °C overnight with gentle shaking. The blots were rinsed extensively and then incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (Jackson Laboratories, West Grove, PA) for 2 h. Finally, the blots were rinsed extensively in 50 mM Tris/Cu, pH 7.4, 150 mM NaCl, and 0.1% Tween 20. The peroxidase-labeled proteins were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech).
isolated cell types, the freshly isolated sinusoidal cells or peritoneal macrophages were homogenized immediately in TRIzol. Peripheral blood leukocyte RNA was obtained using a whole blood RNA isolation kit (Genta Systems, Inc., Minneapolis, MN).

Ribonuclease Protection Assays—For ribonuclease protection experiments, a full-length rat plasma-type PAF acetylhydrolase cDNA (ICOS Corp.) was modified to generate an appropriate antisense RNA probe and ribonuclease protection experiments were performed as described previously (10). For the analysis of whole tissues, 80 µg of total RNA was hybridized in solution with PAF acetylhydrolase and GAPDH antisense RNA probes (RPAlII kit, Ambion). For characterization of PAF acetylhydrolase RNA levels in peritoneal macrophages, peripheral blood leukocytes, and liver sinusoidal cells, 3 µg of total RNA was used in the hybridization reactions, which contained PAF acetylhydrolase and GAPDH antisense RNA probes. 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.

RESULTS

Endotoxin-induced Plasma PAF Acetylhydrolase Expression in the Liver—Previously we demonstrated a significant increase in the expression of PAF acetylhydrolase mRNA in response to LPS in total RNA isolated from rat liver (10). This up-regulation of PAF acetylhydrolase mRNA was unique to Kupffer cells, the resident macrophage of the liver. Although we demonstrated previously a 2-fold increase in circulating PAF acetylhydrolase expression in response to endotoxin in whole blood (19), we needed to verify that the increase in PAF acetylhydrolase mRNA detected in Kupffer cells resulted in an increase in PAF acetylhydrolase protein in the liver. Initial Western blots demonstrated the presence of an induced level of PAF acetylhydrolase protein present in Kupffer cells after isolation from a LPS-exposed rat liver (Fig. 1). No PAF acetylhydrolase protein was detected in 20 µg of total Kupffer cell lysate isolated from either untreated or saline-treated livers. To verify this ex vivo result and to obtain a more accurate representation of the localization of PAF acetylhydrolase in the endotoxin-exposed rat liver, immunohistochemical analyses were performed on cryopreserved liver sections (4 µm) harvested 24 h after mesenteric vein infusion of saline or LPS. In the saline-treated livers, affinity-purified rabbit anti-rat PAF acetylhydrolase detected no immunofluorescence staining above the faint nonspecific background immunofluorescence detected when using purified IgG isolated from the preimmune rabbit (Fig. 2, panel A). ED1 antibodies recognize branched, acid phosphatase-positive cells in liver sinusoidal spaces identified as the cells of Von Kupffer (22). ED1-positive Kupffer cells are clearly visible in the identical saline-treated liver section (Fig. 2, panel C). Endotoxin exposure resulted in a dramatic staining of the liver Kupffer cells for PAF acetylhydrolase (Fig. 2, panel B). An increase in the amount of background immunofluorescence also was observed in the surrounding parenchymal cells and some sinusoidal spaces. Only a slight increase in the number of ED1-positive cells was detected in the LPS-exposed liver section (Fig. 2, panel D). Immunofluorescent staining for PAF acetylhydrolase and ED1 co-localized to the same cells (Fig. 2, panels B and D) and was more apparent at higher magnifications (Fig. 2, panels E–G). Control experiments utilizing the Cy3- and FITC-conjugated secondary antibodies alone produced no detectable immunofluorescence.

PAF Acetylhydrolase Up-regulation in Other Tissues—In a Northern blot of various human tissue RNAs, Tjoelker et al. (4) detected PAF acetylhydrolase mRNA in thymus, tonsil, and placental RNA. Subsequently, Cao et al. confirmed our earlier results and reported the presence of PAF acetylhydrolase mRNA in human liver (11). To investigate the regulation of PAF acetylhydrolase expression in response to endotoxin in other tissues, 3 mg/kg LPS was injected via the tail vein to produce a more systemic endotoxemic response compared with the liver-focused model involving LPS administration into the mesenteric vein. Tissues were harvested from control and LPS-infused animals and immediately freeze-clamped for subsequent RNA isolation. Ribonuclease protection experiments demonstrated barely detectable levels of PAF acetylhydrolase

FIG. 1. Western blot detection of PAF acetylhydrolase in Kupffer cells isolated from saline- and LPS-exposed animals. Saline or LPS (0.4 mg/kg) was administered via a mesenteric vein, and 24 h later Kupffer cells were isolated from untreated (normal) and saline- and LPS-injected animals as detailed under “Experimental Procedures.” Total cell lysates (20 µg) were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. PAF acetylhydrolase protein was detected using an affinity-purified anti-PAF acetylhydrolase antibody (1/1000) and an enhanced chemiluminescent detection system. The Western blot shown is representative of the results of three independent experiments. PAF-AH, PAF acetylhydrolase.

FIG. 2. Immunohistochemical localization of liver PAF acetylhydrolase and co-localization with a macrophage-specific antibody ED1. Liver sections (4 µm) from saline-treated (A and C) and LPS-treated (B and D–G) animals 24 h after exposure were cryopreserved and fixed in a solution of methanol:acetone (1:1). The liver sections were incubated with affinity-purified rabbit anti-PAF acetylhydrolase (1/200) and mouse anti-rat ED1 (1/700) antibodies. Localization of the anti-PAF acetylhydrolase antibody was detected using a Cy3-conjugated goat anti-rabbit secondary antibody (red). Localization of the anti-rat ED1 antibody was detected using a goat anti-mouse FITC-conjugated secondary antibody (green). Scale bar represents 50 μm.

kDa
Control  Saline  LPS  LPS
104  
81  
48  
PAP-AH
35  

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message in 80 µg of total RNA from liver and kidney tissue harvested from control animals (Fig. 3). Control levels of PAF acetylhydrolase mRNA in 80 µg of total RNA from lung, thymus, and spleen tissues were slightly higher than observed in the liver and kidney tissues. Endotoxin-mediated up-regulation of the PAF acetylhydrolase mRNA was evident in all tissues examined. The increase in PAF acetylhydrolase mRNA was most pronounced in lung tissue followed by the increase seen in spleen. GAPDH was used as an internal control to account for differences in RNA loading.

Regulation of Expression in Leukocytes and Peritoneal Macrophages—In response to endotoxin administration, we have demonstrated an increased expression of PAF acetylhydrolase in resident tissue macrophages of the liver and a similar increase in PAF acetylhydrolase mRNA in the thymus, spleen, kidney, and lung, all tissues that possess large populations of macrophages. Additional experiments were conducted to examine the effects of LPS administration on total circulating leukocytes and on peritoneal macrophages. Fig. 4 illustrates the results of the ribonuclease protection analysis of 3 µg of total RNA isolated from total liver sinusoidal cells, whole blood, and peritoneal macrophages from both saline- and LPS-injected animals. Although Kupffer cells represent approximately a third of total liver sinusoidal cells, the up-regulation of PAF acetylhydrolase in the Kupffer cell component of the total sinusoidal cells remains prominent. Ribonuclease protection experiments detected a significant increase in PAF acetylhydrolase mRNA in response to LPS in both circulating leukocytes and in peritoneal macrophages.

Suppression of LPS-induced PAF Acetylhydrolase Expression by PAF Receptor Antagonists—PAF increased the secretion of PAF acetylhydrolase in cultured rat hepatocytes, whereas lysophosphatidylcholine and the nonhydrolyzable analog methylcarbamyl-PAF significantly reduced secretion (23). In addition, PAF stimulated the secretion of PAF acetylhydrolase in the human hepatoma cell line, Hep G2, in a cycloheximide-sensitive fashion (24). Liver PAF levels are elevated significantly 3 h after LPS exposure in this endotoxin exposure model. Therefore, the increase in PAF acetylhydrolase expression could be mediated through the PAF receptor by elevated levels of PAF or alternatively through a PAF-independent LPS-associated signaling pathway. Rats were treated with the non-structural PAF receptor antagonists BN 50739 or WEB 2170 30 min prior to LPS administration into the mesenteric vein. Subsequent ribonuclease protection analysis of total liver sinusoidal cell RNA from LPS-stimulated rats revealed that the PAF receptor antagonists inhibited by 50% the up-regulation of PAF acetylhydrolase mRNA normally detected in response to LPS (Fig. 5).

Western blot analyses of liver sinusoidal cell proteins isolated from 24-h exposed saline-and LPS-infused rats demonstrates the presence of PAF acetylhydrolase only in the LPS-exposed animals (Fig. 6). The antibodies raised against recombinant rat PAF acetylhydrolase react with an approximately 65-kDa protein, which corresponds to the molecular weight identified for the glycosylated form of plasma PAF acetylhydrolase (5). Treatment with the PAF receptor antagonist, BN 50739, inhibited the amount of PAF acetylhydrolase protein expressed in response to LPS. The extent of the decrease in PAF acetylhydrolase protein detected correlated with the decrease in mRNA detected. When 10 µg of isolated liver sinusoidal cell protein was analyzed, expression of PAF acetylhydrolase was apparent in the samples from LPS-treated animals. Since the liver is routinely perfused prior to enzymatic digestion and isolation of the liver cells, the liver sinusoidal cell lysate reflects only the amount of PAF acetylhydrolase present prior to secretion and/or that PAF acetylhydrolase which remains cell-associated.

**DISCUSSION**

Plasma PAF acetylhydrolase performs an essential role in controlling the pathophysiological effects of PAF and PAF-like lipid mediators. The level of PAF acetylhydrolase activity in the plasma can be altered by both genetic and acquired factors. The status of PAF acetylhydrolase expression during endotoxin exposure remains controversial. Two conflicting reports documenting PAF acetylhydrolase levels in clinical sepsis 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 (25). However, Graham et al. (26) demonstrated that plasma PAF acetylhydrolase activity was approximately half that of normal in patients severely ill with clinical sepsis. In the present study, we have examined the in vivo expression levels of PAF acetylhydrolase in normal and endotoxin-exposed rats. Although Kupffer cells are terminally differentiated resident macrophages in the liver, they express undetectable levels of PAF acetylhydrolase in vivo until LPS or a downstream mediator of LPS activates the macrophage to increase the levels of PAF acetylhydrolase (Figs. 1, 2, and 6). An increased amount of PAF acetylhydrolase appears to remain in the local environment of the liver to minimize PAF and PAF-like oxidized phospholipids generated following instigation of tissue damage (Fig. 2). The dosage of LPS used in these

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The plasma PAF acetylhydrolase cDNA was cloned from a macrophage cDNA library and initial Northern blots demonstrated the presence of PAF acetylhydrolase message in tissues that contain macrophages in abundance (4). Although initially not detected in human liver, we subsequently demonstrated the macrophage-specific expression of PAF acetylhydrolase in rat liver (10). Additionally, Cao et al. (11) investigated the tissue distribution of PAF acetylhydrolase using a human poly(A)⁺ dot blot and detected expression in brain, ovary, placenta, liver, lymph node, thyroid, spleen, and fetal spleen. Utilizing a sensitive ribonuclease protection assay, we have confirmed basal levels of expression of PAF acetylhydrolase in several tissues as well. Higher basal levels of PAF acetylhydrolase expression were detected in lung, spleen, and thymus with barely detectable levels in the liver and kidney. The in vivo systemic exposure to LPS resulted in the differential up-regulation of the expression of PAF acetylhydrolase in all the tissues examined. The greatest response to a systemic dose of LPS was detected in lung tissue followed by increases detected in spleen, liver, kidney, and thymus. In the context of total liver RNA, PAF acetylhydrolase mRNA represents a relatively low abundance message and is specific to Kupffer cells in the liver (10). The level of PAF acetylhydrolase message detected in all other tissues examined is comparable to that seen in rat liver. The level of PAF acetylhydrolase mRNA observed is consistent with PAF acetylhydrolase expression limited to a subset of cells, most likely tissue macrophages. Additional ribonuclease protection experiments demonstrated the LPS instigated up-regulation of PAF acetylhydrolase mRNA expression in peripheral blood leukocytes and peritoneal macrophages. It is apparent that PAF acetylhydrolase expression levels are controlled in a cell-specific manner and that the degree of PAF acetylhydrolase induction likely depends upon LPS as well as downstream mediators generated in response to LPS.

Recent studies have demonstrated that monocytes do not secrete PAF acetylhydrolase activity until they differentiate into macrophages (6, 7). 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 in culture (4). We have detected no appreciable in vivo expression of PAF acetylhydrolase in the resident macrophages of the liver until after LPS exposure, which suggests that PAF acetylhydrolase expression may be the result of macrophage activation rather than a monocyte to macrophage differentiation. In addition, our experiments have demonstrated conclusively that in vivo administered LPS initiates an up-regulation of both PAF acetylhydrolase mRNA and protein in numerous tissues and in peripheral blood leukocytes and peritoneal macrophages. The decreases in PAF acetylhydrolase expression in response to LPS reported in cultured macrophages can be attributed to an apparent activation of cultured macrophages, possibly in response to adhesion of cells to the tissue culture dishes. Alternatively, the in vivo environment surrounding macrophages could provide inhibitory signals that suppress PAF acetylhydrolase expression, and such signals may be lost during isolation and culturing of macrophages. Currently, we are examining the in vivo effects of other cytokines to determine whether they evoke changes in the expression of PAF acetylhydrolase.

The PAF receptor plays a fundamental role in endotoxic responses as demonstrated by experiments demonstrating that transgenic mice overexpressing PAF receptors are hypersensitive to endotoxin (27). Clearly, the PAF receptor plays a role in the in vivo response to endotoxin; however, experiments using PAF receptor knockout mice demonstrated that the PAF recep-

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**Fig. 5. PAF receptor antagonists BN 50739 and WEB 2170 inhibit LPS-induced PAF acetylhydrolase expression.** Rats received BN 50739 (10 mg/kg, intravenously), WEB 2170 (5 mg/kg, intravenously), or no receptor antagonist 30 min prior to LPS (0.4 mg/kg) infusion into a mesenteric vein. A control animal (saline) received saline via the mesenteric vein instead of LPS. After 24 h, liver sinusoidal cells were isolated and used for ribonuclease protection analysis (panel A). Densitometric analysis of the corresponding blot (panel B). The results shown are representative of the results from three independent experiments. PAF-AH, PAF acetylhydrolase.

**Fig. 6. PAF acetylhydrolase protein levels in liver sinusoidal cells.** Liver sinusoidal cell protein lysates (20 μg) isolated from 24-h saline-infused (lane 2) and LPS-infused (lanes 3 and 4) rats were separated on a 10% SDS-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and immunoblotted using an anti-rat PAF acetylhydrolase antibody (see “Experimental Procedures”). BN 50739 (lane 4) was administered 30 min prior to LPS infusion. The same membrane was subsequently stained with Coomassie Brilliant Blue and verified approximately equal total protein loading. Coomassie-stained molecular weights are shown in lane 1 (MW). The results shown are representative of the results from three independent experiments. PAF-AH, PAF acetylhydrolase.

Studies resulted in an approximately 75% survival rate at 24 h. Studies are currently being conducted to investigate whether exogenously administered PAF acetylhydrolase would increase the survival rate and/or permit the administration of higher non-lethal doses of LPS. It appears likely that an elevated PAF acetylhydrolase level generated in response to endotoxin reflects a physiological response to inactivate elevated levels of inflammatory phospholipids generated during such episodes of endotoxin exposure.
tor may not be an essential component but rather acts as an exaggerating factor in endotoxic shock (28). Elevated levels of PAF and/or other PAF-like receptor agonists acting via the PAF receptor and its signal transduction pathways may provide the signal to increase levels of the enzyme responsible for its own degradation. We demonstrated that the PAF receptor antagonists BN 50739 and WEB 2170 inhibited by 50% the in vivo LPS-induced up-regulation of PAF acetylhydrolase mRNA and the 65-kDa acetylhydrolase protein. We were unable to demonstrate any significant changes in PAF acetylhydrolase expression after a single bolus injection of PAF into the mesenteric vein. However, PAF acting via the PAF receptor stimulated by 52% the human PAF acetylhydrolase promoter in RAW264.7 macrophages (11). Failure to observe PAF acetylhydrolase regulation in response to PAF infusion in the intact rat may be a technical problem related to the inherent difficulty in maintaining a significant local PAF concentration in the appropriate cell type necessary to activate the transcriptional event(s) required for the observed response without significant lethality. Further study of the role of PAF and LPS in the in vivo regulation of PAF acetylhydrolase gene expression is clearly necessary to understand the dynamics of PAF and oxidized phospholipid degradation.

In summary, rats exposed to LPS up-regulate PAF acetylhydrolase expression via a PAF receptor-dependent mechanism in numerous macrophage-containing tissues, in blood leukocytes, and in peritoneal macrophages. This increased expression of a secreted PAF acetylhydrolase in numerous tissues and in cells in the circulation constitutes an important host defense mechanism for elevating the local and systemic ability to inactivate PAF and oxidized phospholipids. The induction of plasma PAF acetylhydrolase expression is a crucial event in controlling PAF-mediated tissue damage in response to endotoxin.

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