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Lipopolysaccharide Augments Aflatoxin B_1-Induced Liver Injury through Neutrophil-Dependent and -Independent Mechanisms

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Exposure to small, noninjurious doses of the inflammagen, bacterial endotoxin (lipopolysaccharide, LPS) augments the toxicity of certain hepatotoxicants including aflatoxin B_1 (AFB_1). Mediators of inflammation, in particular neutrophils (PMNs), are responsible for tissue injury in a variety of animal models. This study was conducted to examine the role of PMNs in the pathogenesis of hepatic injury after AFB_1/LPS cotreatment. Male, Sprague-Dawley rats (250–350 g) were treated with either 1 mg AFB_1/kg, ip or its vehicle (0.5% DMSO/saline), and 4 h later with either *E. coli* LPS (7.4×10^6 EU/kg, iv) or its saline vehicle. Over a course of 6 to 96 h after AFB_1 administration, rats were killed and livers were stained immunohistochemically for PMNs. LPS resulted in an increase in PMN accumulation in the liver that preceded the onset of liver injury. To assess if PMNs contributed to the pathogenesis, an anti-PMN antibody was administered to reduce PMN numbers in blood and liver, and injury was evaluated. Hepatic parenchymal cell injury was evaluated as increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum and from histologic examination of liver sections. Biliary tract alterations were evaluated as increased concentration of serum bile acids and activities of γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), and 5′-nucleotidase (5′-ND) in serum. Neutrophil depletion protected against hepatic parenchymal cell injury caused by AFB_1/LPS cotreatment but not against markers of biliary tract injury. This suggests that LPS augments AFB_1 hepatotoxicity through two mechanisms: one of which is PMN-dependent, and another that is not.

Key Words: **lipopolysaccharide; LPS; endotoxin; aflatoxin B_1; sepsis; liver injury; sensitivity to intoxication; inflammation; neutrophil; PMN; hepatotoxicity.**

Aflatoxin B_1 (AFB_1) is a metabolite produced by the fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, which are contaminants of grain foods. Treatment of animals with AFB_1 causes hepatic injury in the periportal regions of liver lobules. This damage is evident acutely as hemorrhage, parenchymal cell necrosis, and injury to intrahepatic bile ducts.

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Lipopolysaccharide (LPS, endotoxin) is a constituent of the outer membrane of the cell walls of Gram-negative bacteria. It has been extensively studied as a major contributing factor to the pathogenesis of bacterial infection. Although the mechanisms contributing to tissue injury from LPS are many and may vary among tissues, a commonality appears to be the involvement of host-derived, soluble, and cellular mediators of inflammation (Molvig *et al.*, 1988). Interactions among several of these appear to be necessary for full manifestation of tissue injury during LPS exposure (Hewett and Roth, 1993). For example, at large doses LPS produces midzonal liver injury in rats, and this requires inflammatory mediators such as neutrophils (Hewett *et al.*, 1992; Jaeschke *et al.*, 1993), Kupffer cells (Arthur *et al.*, 1985; Brown *et al.*, 1997), tumor necrosis factor-alpha (TNF-α) (Hewett *et al.*, 1993), platelets (Pearson *et al.*, 1995) and thrombin (Hewett and Roth, 1995; Moulin *et al.*, 1996; Pearson *et al.*, 1996).

Exposure to smaller doses of LPS initiates a more modest and noninjurious inflammatory response. However, exposure to such small doses of LPS can render the liver more sensitive to injury from hepatotoxic chemicals (reviewed by Roth *et al.*, 1997), including AFB_1 (Barton *et al.*, 2000b). A small dose of LPS given to rats converted an otherwise nontoxic dose of AFB_1 into one that is markedly hepatotoxic. In this model, both the periportal hepatocellular and bile-duct epithelial cell (BDEC) injuries induced by AFB_1 were markedly enhanced by administration of LPS (Barton *et al.*, 2000b). The mechanism behind this increased sensitivity has yet to be determined, but it seems likely that aspects of the inflammatory response initiated by exposure to small amounts of LPS may be responsible (Roth *et al.*, 1997).

Neutrophils (polymorphonuclear leukocytes, PMNs) contribute to tissue damage in a number of disease models, including reperfusion injury following ischemia in the heart (Romson *et al.*, 1983) or liver (Bautista *et al.*, 1993; Langdale *et al.*, 1993), and immune complex-mediated injury to lung (Johnson and Ward, 1981) or kidney (Johnson and Ward, 1982). Moreover, liver injury from large doses of bacteria (Arthur *et al.*, 1986), alpha-naphthylisothiocyanate (ANIT) (Dahm *et al.*, 1991) or LPS (Hewett *et al.*, 1992) is prevented

by prior depletion of PMNs, suggesting a causal role for PMNs in the pathogenesis. In a cell coculture system, activated rat PMNs injure hepatic parenchymal cells (Ganey *et al.*, 1994; Mavier *et al.*, 1988). Thus, evidence exists that PMNs can play a causal role in hepatic injury.

The involvement of PMNs in other liver injury models and the capacity of LPS to cause tissue PMN accumulation led us to hypothesize that the LPS-induced enhancement of AFB_1 hepatotoxicity is dependent on PMNs. We tested this hypothesis by determining whether PMNs accumulate in liver before the onset of liver injury and whether prior depletion of PMNs prevents the augmentation of AFB_1 hepatotoxicity by LPS.

MATERIALS AND METHODS

Animals and Materials

Male Sprague-Dawley rats (CD-Crl: CD-(SD)BR VAF/Plus; Charles River, Portage, MI) weighing 250–350 g were used in these studies. The reagent kits used for measuring serum markers of liver injury (ALT, 59-UV; AST, 58-UV; GGT, 419; bile acids, 450; ALP, 245; and 5′-ND, 265-UV) were purchased from Sigma Chemical Co. (St. Louis, MO). LPS derived from *E. coli* serotype 0128:B12 with an activity of 1.7×10^6 EU/mg was purchased from Sigma Chemical Co. A colorimetric, kinetic Limulus Amebocyte Lysate (LAL) assay was employed to estimate LPS concentration, using a kit (#50–650U) purchased from BioWhittaker (Walkersville, MD). Unless stated otherwise, all chemicals were purchased from Sigma.

Treatment Protocol

Rats, fasted for 24 h, were given 1 mg AFB_1/kg or vehicle (0.5% DMSO in 0.9% sterile saline), ip, followed 4 h later by 7.4×10^6 EU LPS/kg or sterile saline via the tail vein. Doses of AFB_1 and LPS used in this investigation caused minimal to no injury by themselves; however, as we have previously reported (Barton *et al.*, 2000b), given together via this treatment regimen they caused pronounced liver injury. At 24 h after AFB_1 administration, the rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and blood was drawn from the dorsal aorta, allowed to clot, and centrifuged to separate serum. Before the liver samples were placed in neutral buffered formalin, a mid-lobe radial section of the right anterior lobe was removed and frozen in liquid nitrogen. The 24 h time-point was chosen because the injury is maximal at this time (Barton *et al.*, 2000b).

Determination of Hepatotoxicity

Serum markers of liver injury. Reagent kits (see Animals and Materials) were used to measure serum markers of liver injury. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured spectrophotometrically by the methods of Wroblewski and LaDue (1956) and Karmen (1955), respectively. Serum gamma-glutamyltransferase (GGT), 5′-nucleotidase (5′-ND), and alkaline phosphatase (ALP) activities were measured by the methods of Szasz and Persijn (1974), Arkesteijn (1976), and Bowers and McComb (1966), respectively. Serum bile acids concentration was measured by the method of Mashige and colleagues (Mashige *et al.*, 1981).

Histopathologic evaluation. A mid-lobe, radial section of the right anterior lobe of the liver from each rat was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 5-micron thickness and stained with hematoxylin and eosin (H&E). Slides were coded, randomized, and evaluated with light microscopy.

TdT-mediated dUTP nick end labeling. Apoptotic cells were detected with the procedure of Sgonc and co-workers (Sgonc *et al.*, 1994) and from morphologic evaluation of H&E stained tissue. The *in situ* cell death detection reagent kit (POD) was purchased from Boehringer Mannheim (Indianapolis, IN; Cat. No. 1–684–817). In this method, formalin-fixed, paraffin-embedded liver sections were used for *in situ* TdT-mediated dUTP nick-end labeling (TUNEL) of 3′-hydroxy-DNA strand breaks. Briefly, 3′-hydroxy-DNA strand breaks were labeled with fluorescein-tagged nucleotides via terminal deoxynucleotidyl transferase and subsequently exposed to horseradish peroxidase-conjugated antifluorescein antibody. Staining was developed with diaminobenzidine (DAB), and sections were counterstained with methyl green. Between 2000 and 2500 hepatocytes per slide were counted in 12–20 randomly selected fields at 400× using a light microscope (Olympus BX50; Lake Success, N.Y.), and the percentage (labeling index) of cells that were stained and had apoptotic morphology was determined. Apoptotic morphology was defined by the morphological characteristics detailed by Kerr and co-workers (Kerr *et al.*, 1972), i.e., (1) marked condensation of chromatin and cytoplasm; (2) cytoplasmic fragments with or without condensed chromatin; and (3) intra- and extracellular chromatin fragments.

Proliferating Cell Nuclear Antigen (PCNA) Immunohistochemistry

PCNA immunohistochemistry was conducted as described by Greenwell and colleagues (Greenwell *et al.*, 1991). Briefly, the liver sections mounted on slides were first blocked with casein and then reacted with monoclonal antibody to PCNA (Dako Corporation, Carpentaria, CA). The antibody was then linked with biotinylated goat anti-mouse IgG antibody (Boehringer Mannheim, Indianapolis, IN), then labeled with streptavidin-conjugated peroxidase (Jackson Immunoresearch, West Grove, PA). Color was developed by exposing the peroxidase-labeled streptavidin to DAB, which forms a brown reaction product. The sections were then counterstained with Gill's hematoxylin. Each slide contained a section of duodenum as a positive control. G_0 cells were blue and did not take the PCNA stain, whereas cells in the active stages of the cell cycle stained brown. Three cell types (1) parenchymal, (2) bile duct epithelial (BDEC), and (3) sinusoidal were examined per liver section for hyperplasia, and were assigned a score of 1–5. For parenchymal and sinusoidal cells, the following scores were given, based upon the percentage of cells stained: 1 = less than 5%; 2 = 5 to 10%; 3 = 11 to 15%; 4 = 16 to 20%; 5 = >20%. For BDECs, the following scores were given based upon the percentage of bile ducts which contained stained cells: 1 = less than 5% of the bile ducts; 2 = 5 to 25%; 3 = 26 to 50%; 4 = 51 to 75%; and 5 = >75%.

Neutrophil Depletion Protocol

Rabbit anti-rat neutrophil immunoglobulin (Ig) serum fraction was prepared by the method of Hewett and colleagues (Hewett *et al.*, 1992) as modified by Bailie and colleagues (Bailie *et al.*, 1994). Rats received anti-neutrophil Ig (NAb) or control Ig (CAb) (0.5 ml via the tail vein) at 16 and 8 h before AFB_1 treatment. Four h after AFB_1 administration, LPS was administered. Twenty-four h after AFB_1 administration, the rats were anesthetized and killed, and injury was assessed. Blood PMN concentration was determined from total blood leukocyte numbers, assessed using an automated cell counter (Serono-Baker Diagnostics, Model System 9000, Allentown, PA) and differential counting of cells in modified Wrights-stained blood smears. Depletion of hepatic PMNs was assessed by enumeration of PMNs in liver sections.

Enumeration of Hepatic PMN

PMNs in liver sections were visualized using an immunohistochemical technique (Pearson *et al.*, 1995). Liver sections were fixed, embedded in paraffin, and sectioned at 6 microns. Paraffin was removed from the tissue sections with xylene before staining. PMNs within the liver tissue were stained using a rabbit anti-PMN Ig. This anti-PMN Ig was isolated from serum of rabbits immunized with rat PMNs, as described previously (Hewett *et al.*, 1992). After incubation with the primary antibody, the tissue sections were incubated with biotinylated goat anti-rabbit IgG, avidin-conjugated alkaline phosphatase, and Vector Red substrate to stain the PMNs within the tissue. PMNs in each section were enumerated in 20 evenly distributed, randomly selected, high-power (400×) fields (HPFs).

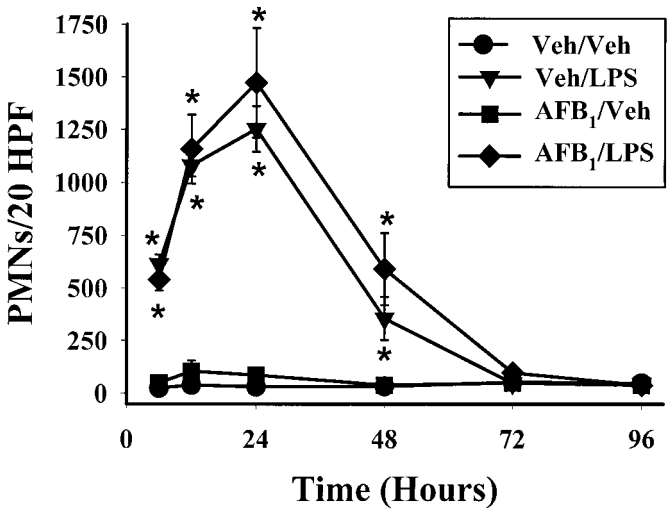

FIG. 1. Effect of AFB_1/LPS administration on hepatic PMN accumulation. One mg AFB_1/kg, ip, or vehicle (0.5% DMSO/saline) was administered, and this was followed 4 h later by 7.4×10^6 EU LPS/kg or saline vehicle, via the tail vein. Rats were killed, the livers removed, fixed in formalin, sectioned, and immunohistochemical staining for neutrophils performed. Hepatic neutrophils were estimated by counting the numbers of neutrophils present in 20 high-power (400×) microscopic fields (HPFs). *Significantly different from the Veh/Veh group.

Statistical Analysis

Results are expressed as mean ± SE of groups of 5–25 rats. Homogeneity of variance was tested using the F-max test. If the variances were homogenous, data were analyzed using a completely randomized, factorial ANOVA. Individual comparisons were made with Tukey's test. For data sets with nonhomogenous variances, Kruskal-Wallis′s nonparametric ANOVA was used; individual comparisons were made with Dunn's multiple comparisons test. The criterion for significance was $p < 0.05$ for all comparisons.

RESULTS

Hepatic PMN Accumulation after AFB_1/LPS Cotreatment

We reported previously that, in rats treated with AFB_1/LPS, serum markers of hepatocellular injury and cholestasis were unaffected 6 h after AFB_1 but were markedly elevated by 24 h (Barton *et al.*, 2000b). To characterize the development of hepatic PMN accumulation, liver tissue was assessed immunohistochemically at various times after the injection of AFB_1 and/or LPS (Fig. 1). An increase in hepatic PMNs was not observed after administration of AFB_1 alone. In contrast, LPS treatment resulted in a significant increase within 6 h that peaked by 24 h, and returned to normal by 72 h. The distribution of these cells was panlobular; however, midzonal accumulation was more pronounced. This increase was unaffected by cotreatment with AFB_1.

Effect of NAb on PMN Numbers

AFB_1/LPS cotreated rats that received control antibody from non-immunized rabbits (CAb) had a large increase in blood PMNs 24 h after AFB_1 administration as compared to Veh/Veh-treated rats that received CAb (Fig. 2A). Administration of PMN antibody from immunized rabbits (NAb) markedly attenuated this increase. There was also a pronounced accumulation of PMNs in liver tissue from AFB_1/LPS cotreated rats that received Cab (Fig. 2B). This increase was greatly diminished in the rats that received NAb.

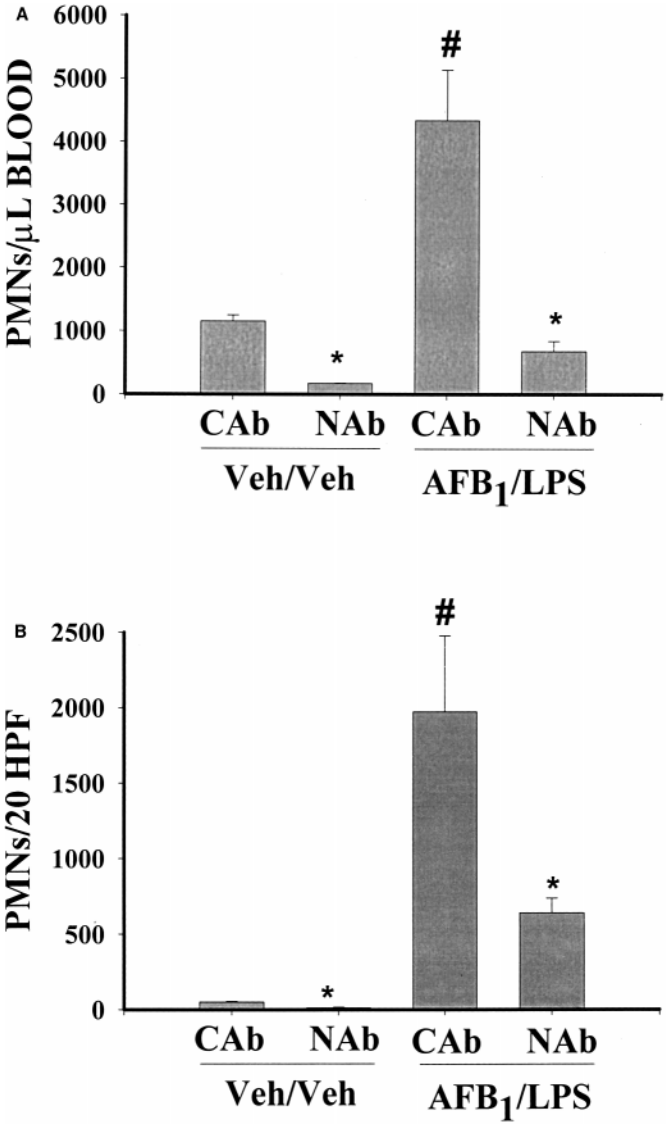

FIG. 2. Effect of NAb on PMN numbers after AFB_1/LPS administration. Rats were treated with antibody to rat neutrophils (Nab) or control antibody (Cab), as described in Materials and Methods, 16 and 8 h before administration of AFB_1. One mg AFB_1/kg, ip, or vehicle (Veh, 0.5% DMSO/saline) was administered, and this was followed 4 h later by 7.4×10^6 EU/kg or saline via the tail vein. Rats were killed 20 h after the last treatment, and blood (A) and hepatic (B) PMN numbers were determined. *Significant difference between NAb and CAb groups that received the same cotreatment. #Significant difference compared to CAb/Veh/Veh.

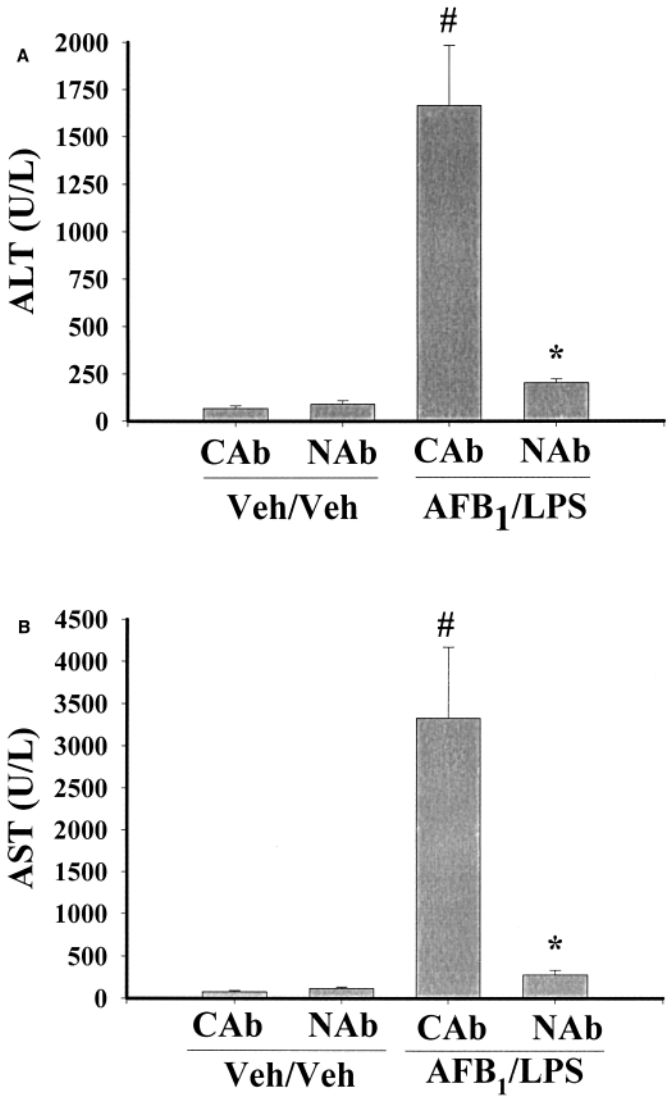

FIG. 3. Effect of PMN depletion on markers of hepatic parenchymal cell injury after treatment of rats with AFB_1/LPS. Rats were treated with NAb or CAb (0.5 ml via the tail vein) 16 and 8 h before administration of AFB_1. One mg AFB_1/kg, ip, or vehicle (0.5% DMSO/saline), was administered, and this was followed 4 h later by 7.4×10^6 EU LPS/kg or saline, via the tail vein. Hepatocellular injury was assessed as increases in serum ALT (A) and AST (B) activities. *Significant difference between NAb and CAb groups that received the same cotreatment. #Significant difference from CAb/Veh/Veh.

Effect of PMN Depletion on AFB_1/LPS-Induced Hepatic Parenchymal Cell Injury

Hepatic parenchymal cell injury was estimated by measuring serum ALT and AST activities 24 h after the injection of AFB_1. AFB_1/LPS cotreated rats receiving CAb had a large increase in serum ALT (Fig. 3A). Administration of NAb prevented this increase. Similar results were observed for serum AST activity (Fig. 3B).

These findings were supported by histological examination of liver sections for necrotic or swollen parenchymal cells. Necrotic cells were identified by pyknotic nuclei, indistinct cell borders, and vacuolization. Swollen cells were identified by cell enlargement and eosinophilic staining of the cytoplasm. The lesions found in AFB_1/LPS-treated rats have been described in detail elsewhere (Barton *et al.*, 2000b). Livers of AFB_1/LPS cotreated rats given CAb had widespread areas of single-cell or foci of oncotic necrosis characterized by hypereosinophilic cytoplasm and darkly stained nuclear fragments. These were frequent in the periportal areas, but they occurred to a lesser extent in midzonal regions and were absent in centrilobular regions. By contrast, livers from AFB_1/LPS-cotreated rats given NAb had occasional single-cell necrosis only in periportal areas.

Effect of PMN Depletion on AFB_1/LPS-Induced Biliary Injury

Cholangiodestructive cholestasis was estimated through examination of biochemical markers in serum and by histology. Increased 5′-ND, ALP, and GGT activities and bile acid concentration in the serum were observed in AFB_1/LPS cotreated rats that were given CAb (Fig. 4). Administration of NAb did not diminish these increases.

Effect of PMN Depletion on AFB_1/LPS-Induced Apoptosis

Previously, we reported that LPS treatment resulted in a small yet significant increase in apoptosis associated with single, hepatic parenchymal cells scattered throughout the lobule (Barton *et al.*, 2000b). Furthermore, this increase was unaffected by cotreatment with AFB_1. To examine if neutrophil depletion altered apoptosis, TUNEL assay was conducted. Neutrophil depletion did not alter TUNEL staining (Fig. 5). These findings were further corroborated through the observation of cytoplasmic cell fragments and cells with condensed nuclear chromatin or chromatin fragmentation indicative of apoptosis in H&E sections.

Effect of PMN Depletion on AFB_1/LPS-Induced Hepatocellular Hyperplasia

Previously, we reported (Barton *et al.*, 2000b) that LPS given alone stimulated hyperplasia of both sinusoidal and parenchymal cells, and AFB_1 given alone stimulated hyperplasia of BDECs. Furthermore, this latter effect was enhanced with the co-administration of the 2 compounds. To examine if neutrophil depletion altered cellular hyperplasia, PCNA immunohistochemistry was conducted. This assay was chosen because it allows identification of all cells that are in any of the active stages of the cell cycle (i.e., not in G_0). Neutrophil depletion attenuated sinusoidal and parenchymal cell PCNA staining; however, staining of BDECs remained unaltered (Table 1).

DISCUSSION

We reported recently that in rats treated with AFB_1/LPS, serum markers of hepatocellular injury and cholestasis were unaffected 6 h after AFB_1 treatment but were markedly ele-

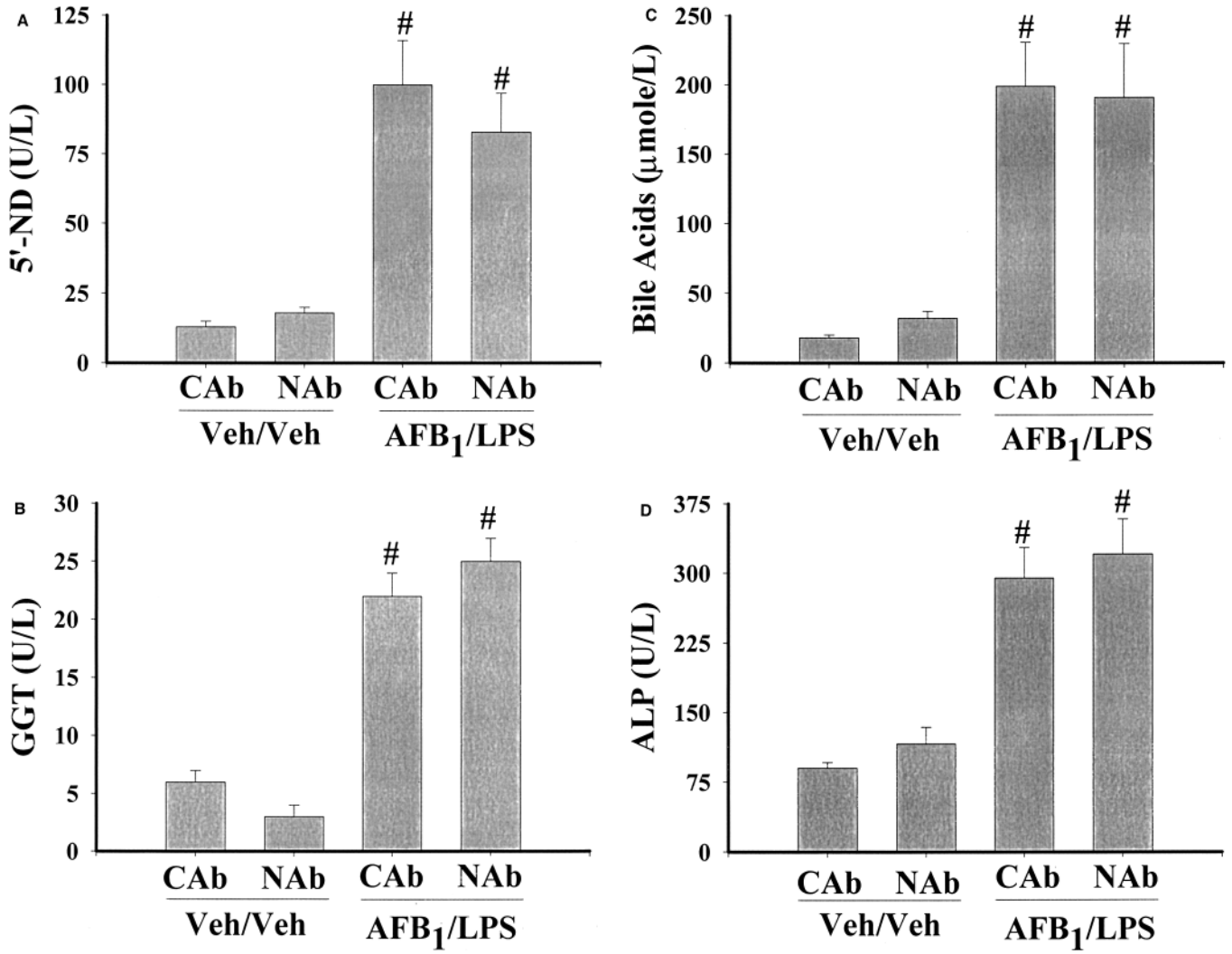

FIG. 4. Effect of PMN depletion on markers of cholestasis after treatment of rats with AFB_1 and/or LPS. Rats were treated with NAb or CAb (0.5 ml via the tail vein) 16 and 8 h before administration of AFB_1. One mg AFB_1/kg, ip, or vehicle (0.5% DMSO/saline) was administered, and this was followed 4 h later by 7.4×10^6 EU LPS/kg or saline, via the tail vein. Cholestasis was assessed from serum 5′-ND (A), GGT (B), bile acids (C), and ALP (D). #Significantly different from Veh/Veh group receiving the same antibody treatment.

vated by 24 h (Barton *et al.*, 2000b). PMNs were more noticeable in H&E-stained liver sections from the groups that received LPS. To verify that there was an increase in PMNs, these cells were stained immunohistochemically and quantified 6 to 96 h after AFB_1 administration. LPS administration resulted in a significant increase in PMNs in the liver within 2 h after its administration, irrespective of AFB_1 co-administration. The numbers continued to increase until 24 h. By 48 h, the numbers of PMNs had declined, and by 72 h there was no longer an elevation. Previously, we reported that we did not observe injury to parenchymal cells until 24 h in this model (Barton *et al.*, 2000b). Therefore, the accumulation of PMNs in the liver preceded the onset of liver injury. This suggested that the PMNs did not arrive in response to dead cells and cellular debris.

To evaluate if the PMNs contributed to the pathogenesis in this model, an anti-neutrophil antibody (NAb) was administered to decrease the PMN numbers prior to AFB_1/LPS treatment. The NAb treatment regimen decreased blood PMN concentration by approximately 85% and markedly reduced the hepatic PMN accumulation that followed AFB_1/LPS treatment. This reduction in PMNs was associated with prevention of oncotic necrosis to hepatic parenchymal cells.

LPS at this dosage produced a modest increase in apoptosis, irrespective of AFB_1 treatment (Barton *et al.*, 2000b). Interestingly, although PMN-depletion prevented oncotic necrosis of parenchymal cells in this model, it did not alter the apoptotic response. This suggests that oncotic necrosis is PMN-dependent, whereas apoptosis results from a different mediator.

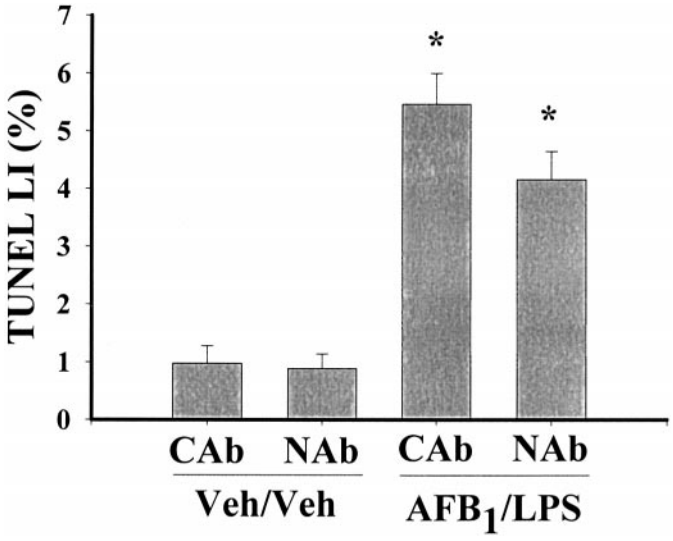

FIG. 5. Effect of PMN depletion on TUNEL staining in liver. A labeling index (LI) was determined by counting between 2000 and 2500 cells per slide and calculating the percent that were labeled positive for TUNEL. *Significantly different from the respective Veh/Veh group.

Increases in circulating endotoxin trigger a systemic inflammatory response in a variety of clinical conditions (Bone, 1992; Deitch, 1992; Kelly *et al.*, 1997). In animal models, PMNs have been implicated as contributors to tissue damage. These cells also contribute to chemically induced liver injury in several models, including retinol potentiation of carbon tetrachloride hepatotoxicity (Badger *et al.*, 1996), large dose of endotoxin (Hewett *et al.*, 1992), and ANIT hepatotoxicity (Dahm *et al.*, 1991). It is clear, however, that the mere presence of PMNs is not sufficient to cause damage, since hepatic PMN accumulation was similar in rats treated with LPS or the AFB_1/LPS combination, yet only the latter treatment caused liver injury (Barton *et al.*, 2000b).

PMNs release not only reactive oxygen species but also serine proteases such as cathepsin G and elastase, which can injure hepatic parenchymal cells of liver (Hill and Roth, 1998; Ho *et al.*, 1996). PMN-derived proteases are important mediators of hepatocellular injury in rats cotreated with LPS and galactosamine (Okabe *et al.*, 1993). The mechanism(s) by which PMN-derived proteases cause hepatocellular damage has not been completely characterized, but it is known that they can induce death of other cells by rupturing the plasma membrane (Varani *et al.*, 1989). It is possible that PMN-induced hepatocellular injury in the AFB_1/LPS model is mediated by this action; however, other mechanisms cannot presently be ruled out.

PMN-depletion did not afford protection against injury to BDECs and markers of cholestasis after AFB_1/LPS treatment. This suggests that LPS augments AFB_1-induced cholestatic injury through a mechanism independent of PMNs.

In contrast to observations here with AFB_1/LPS, biliary injury in response to ANIT is PMN-dependent (Dahm *et al.*, 1991). A hallmark of hepatotoxicity from ANIT is an early and marked accumulation of PMNs (Goldfarb *et al.*, 1962) next to periportal hepatocytes as well as adjacent to BDECs within portal triads (Dahm *et al.*, 1991). This accumulation precedes cellular injury (Goldfarb *et al.*, 1962; McLean and Rees, 1958). Like AFB_1, ANIT administration to rats results in periportal lesions characterized by injury to parenchymal cells as well as BDECs. Prior neutrophil depletion protects against injury to both cell types, suggesting a causal role for PMNs in ANIT pathogenesis (Dahm *et al.*, 1991). Why injury to BDECs is PMN-dependent in the ANIT model but not in the AFB_1/LPS model remains unclear.

It is reasonable to hypothesize that an LPS-induced inflammatory mediator other than PMNs may be responsible for the increased susceptibility of BDECs to AFB_1 toxicity. One candidate is tumor necrosis factor-alpha (TNFα), which is a critical mediator of injury in other models. In a preliminary study, we found that elimination of the LPS-induced increase in serum TNF concentration by neutralizing antibody or pentoxifylline treatment attenuated injury to both BDECs and parenchymal cells after AFB_1/LPS cotreatment (Barton *et al.*, 2000a). TNF has numerous actions that may render it a critical mediator in this model, including effects on inflammatory cells and on xenobiotic metabolism. Although it is possible that TNF might affect toxicity by altering the metabolism of AFB_1, it is unlikely that this cytokine increases AFB_1 bioactivation, since it decreases the synthesis of responsible P-450 isoforms (Warren *et al*., 1999; Pous *et al*., 1990; Bertini *et al*., 1988). In another model of inflammatory liver injury (Hewett *et al*., 1993), evidence suggested that interaction occurs between TNF and PMNs; whether or not interaction among these factors is important in augmentation of AFB_1 hepatotoxicity by LPS remains to be determined.

Cell division and tissue repair occur in response to tissue injury (Mehendale, 1991). Parenchymal cell hyperplasia was attenuated after PMN-depletion in rats given AFB_1/LPS. However, PMN-depletion was not associated with attenuation of BDEC hyperplasia. Since injury to BDECs is independent of

TABLE 1
Effect of PMN Depletion on AFB_1/LPS-Induced Hepatic Hyperplasia

Treatment	Parenchymal	BDEC	Sinusoidal
CAb/Veh/Veh	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
NAb/Veh/Veh	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
CAb/AFB_1/LPS	3.3 ± 0.2*,#	3.0 ± 0.3#	2.8 ± 0.3*,#
NAb/AFB_1/LPS	2.0 ± 0.2	3.3 ± 0.3#	1.8 ± 0.2

Note. Liver samples were examined for hyperplasia and assigned a score according to the criteria described in Materials and Methods. Mean ± SE of 4–10 rats.

*Significant difference between NAb and CAb groups that received the same cotreatment.

#Significant difference from CAb/Veh/Veh.

PMNs, these results suggest that the hyperplasia occurred in response to injury.

In summary, the results of this study demonstrate that LPS-induced inflammation makes rats more susceptible to AFB_1-induced injury to hepatic parenchymal cells by a mechanism that involves PMNs. However, potentiation of AFB_1-induced injury to BDECs appears to be independent of these inflammatory cells. The mechanism by which BDECs are injured in this model requires further study.

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