Lipopolysaccharide-induced Hepatic Injury Is Enhanced by Polychlorinated Biphenyls

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After intravenous administration of bacterial lipopolysaccharide (LPS) to rats, polymorphonuclear neutrophils (PMNs) rapidly accumulate in the liver, and midzonal hepatic necrosis is prominent by 6 hr. PMNs are required for the development of hepatic injury in rats. Certain polychlorinated biphenyls (PCBs) can activate PMNs, resulting in production of superoxide anion (O2•−) and release of cytolytic factors from granules. This raises the possibility that PCB exposure might enhance PMN-mediated tissue injury, such as LPS-induced hepatotoxicity. We treated female Sprague-Dawley rats with a minimally toxic dose of LPS in saline (2 mg/kg, intravenous) and 90 min later exposed them to Aroclor 1248 (50 mg/kg, intraperitoneal), a mixture of PCBs. The animals were killed 6 hr after LPS administration, and hepatic injury was assessed. Neither LPS nor Aroclor 1248 alone produced liver injury. Co-treatment with LPS and Aroclor 1248 resulted in pronounced liver injury as demonstrated from increased activities of alanine aminotransferase and isocitrate dehydrogenase in plasma. Histological evaluation indicated increased severity of hepatic necrosis in rats receiving both LPS and Aroclor 1248. Hepatic accumulation of PMNs, normally observed after LPS, was not altered by co-exposure to PCBs. Aroclor 1248 stimulated rat PMNs in vitro to produce O2•− and to degranulate. In addition, PMN-mediated cytotoxicity to isolated rat hepatocytes in culture was increased upon addition of Aroclor 1248. PCBs activate PMNs in vitro and increase PMN-dependent hepatocellular damage in vitro and after LPS treatment in vivo. PMNs may act in vivo as an additional inflammatory stimulus to activate PMNs to become cytotoxic, resulting in increased tissue injury. Key words: Aroclor 1248, hepatotoxicity, liver, neutrophils, polychlorinated biphenyls. Environ Health Perspect 104:634–640 (1996)

Depletion of circulating PMNs before LPS exposure protects against hepatic injury, indicating a requirement for these cells (8, 10). Activation of PMNs is likely a necessary step in producing PMN-mediated tissue injury, and studies in vitro have demonstrated that activated PMNs can kill both endothelial cells and hepatocytes (11–13). Although the exact mechanisms of PMN-mediated tissue injury are unknown, they probably involve production of reactive oxygen species such as the superoxide anion (O2•−) and/or release of cytotoxic enzymes contained within granules (14).

Polychlorinated biphenyls (PCBs) are industrial chemicals that have been released into the environment. In experimental animals, these compounds produce an array of toxic responses including hepatotoxicity and alterations in the immune system (15, 16). Humans accidentally exposed to high concentrations of PCBs expressed changes in immune system parameters such as alterations in lymphocyte subpopulations and suppression of cellular immunity (17–19). In addition, monocytes and PMNs obtained from individuals exposed to PCBs had lower percentages of cells bearing immunoglobulin and complement receptors (20). Primates exposed to PCBs have exhibited a wide range of adverse responses including inflammatory lesions in the liver (21, 22), and abnormal liver function tests have been reported in exposed human populations (19).

Although immunotoxicity due to PCB exposure has been described in some detail, less is known about the interactions of these compounds with PMNs or inflammatory responses. PCBS rapidly activate PMNs in vitro to produce O2•− and to degranulate (23). PMN activation by PCBs occurs through signal transduction pathways involving phosphoinositide hydrolysis, inositol triphosphate production, phospholipase A2 activation, and Ca2+ mobilization (24–26). Activation of PMNs results in production and release of cytotoxic mediators into the extracellular environment. Moreover, exposure to PCBs in vitro alters PMN responses to subsequent stimuli (23). Enhanced PMN activation or responsiveness by PCBs could lead to increased tissue injury where PMN involvement occurs, such as during exposure to LPS resulting from bacterial infections or increased translocation of bacteria from the gut. Accordingly, since LPS-induced hepatotoxicity is PMN dependent, we hypothesized that co-exposure to PCBs would enhance LPS-induced liver injury. This hypothesis was tested by exposing rats to a minimally toxic dose of LPS followed by the PCB mixture Aroclor 1248 and assessing hepatic injury. Aroclor 1248 was chosen because this mixture of PCBs is composed predominantly of tetrachlorinated biphenyls and ortho-substituted congeners (27), which activate PMNs in vitro (26). Furthermore, ortho-substituted PCB congeners have been identified in human biological samples, and their potential health effects have yet to be determined (19).

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Materials and Methods

Animals. We used female Sprague-Dawley rats (Crl:CD BR/SD) VAF/plus, 175-220 g) for studies of LPS toxicity. Male Sprague-Dawley retired breeder rats were used as donors of isolated PMNs. Hepatocytes were isolated from male Sprague-Dawley rats (175-300 g). All animals were obtained from Charles River Laboratories (Portage, Michigan). Animals were maintained on a 12-hr light/dark cycle for at least 1 week before use. Food (rat chow, Teklad, Madison, Wisconsin) and water were provided ad libitum.

Chemicals. Acrorol 1248 was purchased from ChemService (West Chester, Pennsylvania), Lipopolysaccharide (E. coli 0128:B12 serotype), glycogen (type II from oyster), phorbol myristate acetate (PMA), superoxide dismutase (SOD), ferricytochrome C, cytochalasin B, guaiacol, dimethylformamide (DMF), N-formylmethionyl-leucyl-phenylalanine (fMLP), and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, Missouri). Histochoic fixative was purchased from Amresco (Solon, Ohio). Collagenase type A was purchased from Boehringer-Mannheim Biochemicals (Indianapolis, Indiana). Williams’ medium E and gentamicin were purchased from Gibco (Grand Island, New York). Fetal calf serum was purchased from Intergen (Purchase, New York).

Treatment protocol. Female Sprague-Dawley rats received either LPS (2 mg/kg) or sterile saline vehicle (2 ml/kg) via a tail vein. The animals then received either Acrorol 1248 (50 mg/kg) or corn oil vehicle (2 ml/kg) 90 min later by intraperitoneal injection. We used this dosing regimen to minimize potential effects on hepatocytes by the PCBs alone and to maximize exposure of both circulating and tissue-attending (in the liver) PMNs to PCBs. Rats were killed 6 or 9 hr after LPS/saline exposure. The animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), the abdominal cavity was exposed, and blood was drawn from the inferior vena cava into a syringe containing heparin. Livers were removed, and sections were prepared and immediately placed in fixative. We used an aliquot of plasma on the same day to determine enzyme activities.

Assessment of hepatic injury. We assessed hepatic injury by measuring activities of alanine aminotransferase (ALT) and aspartate dehydrogenase (ICDH) in plasma by kinetic determination using a Gilford Spectrophotometer (Sigma kits no. 59-UV and 153-UV, respectively) (28). Liver sections were fixed, embedded in paraffin, cut at 6 μm, stained with hematoxylin and eosin, and coded and evaluated without bias by a veterinary pathologist. The severity of hepatic injury was graded on a scale from 0 (no significant lesions) to 5 (severe, coalescing necrosis).

Quantification of hepatic PMN accumulation. PMNs in liver sections were visualized using an immunohistochemical technique (9). Liver sections were fixed, embedded in paraffin, and sectioned at 6 μm. Paraffin was removed from the tissue sections with xylene before staining. PMNs within the liver tissue were stained using a rabbit anti-rat PMN immunoglobulin. This anti-PMN immunoglobulin was isolated from serum of rabbits immunized with rat PMNs as described previously (8). 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. We assessed hepatic PMN accumulation by averaging the number of PMNs counted in 20 midzonal, 400× fields throughout 3 separate lobes using light microscopy. PMNs were identified by positive staining and morphology.

Isolation of PMNs. PMNs were isolated from the peritoneum of male Sprague-Dawley retired breeder rats by glycogen elicitation as described previously (29). Isolated PMNs were resuspended in Hanks’ balanced salt solution (HBSS), pH 7.4. The percentage of PMNs in the cell preparations was routinely >95%, and the viability was ≥95% as determined by the ability to exclude trypsin blue. The PMNs were suspended in borsilicate glass test tubes, 12 × 75 mm (VWR, Chicago, Illinois), at a final concentration of 2 × 10⁶ cells/ml. The isolation procedure was performed at room temperature.

Detection of superoxide anion production. PMNs (2 × 10⁶ cells) were prepared in HBSS in a final volume of 1 ml and exposed to either vehicle (1 μl DMF) or to 1 or 10 μg/ml Acrorol 1248 for 30 min at 37°C. These concentrations of PCBS were chosen for their ability to affect PMN function in the absence of cytotoxicity (23,26). PMA (0 or 20 ng/ml) was then added for an additional 10 min at 37°C. We measured O₂⁻ generated during this 40-min incubation period by the SOD-sensitive reduction of ferricytochrome C (30). In a separate series of experiments, PMNs (2 × 10⁶ cells) were incubated with LPS (10 or 100 μg/ml) or vehicle (HBSS) for 10 min, followed by exposure to Acrorol 1248 (1 or 10 μg/ml) or vehicle (DMF) for an additional 30 min. We detected O₂⁻ produced during this 40-min period as described above.

Assessment of PMN degranulation. Degranulation was assessed by the release of the enzyme myeloperoxidase (MPO), which is contained within azurophilic granules. PMNs were prepared in HBSS and pretreated with 5 μg/ml cytochalasin B for 10 min at room temperature to facilitate release of the granules into the incubation medium (31,32). PMNs (2 × 10⁶ cells) were then exposed to either vehicle (cell DMF) or to 1 or 10 μg/ml Acrorol 1248 for 15 min at 37°C. We exposed PMNs to fMLP (10 nM) for 15 min at 37°C as a positive control. fMLP is a chemotactic peptide derived from gram-negative bacteria that binds to a G-protein–coupled receptor, leading to PMN activation (33,34). The incubation was terminated by placing the cells in an ice-water bath followed by centrifugation at 4°C. The cell-free supernatant was collected, and MPO activity was measured by the H₂O₂-dependent oxidation of guaiacol (35,36). We measured the change in absorbance at 470 nm over 2 min at 25°C in a spectrophotometer and calculated MPO activity (U/l) using a molar extinction coefficient of 26,600 (30136). Lactate dehydrogenase activity (LDH) present in the cell-free supernatant was measured according to the method of Bergmeyer and Bernt (37) as a marker of cytocotoxicity.

Hepatocyte–neutrophil co-cultures. Hepatocytes were isolated according to the method of Seglen (38) as modified by Klaunig et al. (39). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg), and the portal vein was cannulated. The liver was perfused with approximately 150 ml of Mg²⁺-, Ca²⁺-free HBSS followed by perfusion with 250 ml collagenase type A (0.5 mg/ml). The resulting liver digest was filtered through gauze and spun in a centrifuge at 50g for 2 min. The hepatocytes were resuspended in Williams’ medium E containing 10% fetal calf serum and 0.1% gentamicin. Using this isolation procedure, 98% of the cells in the final preparation were hepatic parenchymal cells with viability routinely >90% (13).

The hepatocytes were plated in six-well plates at a density of 5 × 10⁵ hepatocytes/well. After an initial 3-hr attachment period, the medium and unattached cells were removed, and either Williams’ medium E containing 0.1% gentamicin or medium containing isolated PMNs was added. PMNs were isolated as previously described and plated at a density of 5 × 10⁵ PMNs/well, resulting in a ratio of 10 PMNs/hepatocyte. PMNs were allowed to attach for 30 min, then vehicle (2 μl DMF) or Acrorol 1248 (1 or 10 μg/ml)
Aroclor 1248 (µg/ml)

Figure 1. Superoxide anion (O2−) production by neutrophils during exposure to Aroclor 1248 and phorbol myristate acetate (PMA). Rat PMNs were exposed to either 0, 1, or 10 µg/ml Aroclor 1248 for 30 min at 37°C. The PMNs were then exposed to either 0 or 20 ng/ml PMA for an additional 10 min at 37°C. O2− produced during the incubation period was determined as described in Materials and Methods; N = 3–4. *Significantly different from respective value in the absence of Aroclor 1248, one-way ANOVA (p<0.05).

Table 1. Neutrophil degranulation in the presence of Aroclor 1248

| Treatment              | MPO activity (UI) |
|------------------------|-------------------|
| Vehicle control        | 5.6 ± 3.2         |
| Aroclor 1248 (1 µg/ml) | 28.2 ± 19.1       |
| Aroclor 1248 (10 µg/ml)| 87.6 ± 42.9*     |
| fMLP (10 nM)           | 291.8 ± 84.6*    |

Abbreviations: MPO, myeloperoxidase; fMLP, formyl-methionyl-leucyl-phenylalanine.

*Significantly different from vehicle control, one-way ANOVA (p<0.05).

was added to wells containing either hepatocytes alone or hepatocytes plus PMNs. After a 16-hr incubation at 37°C in 92.5% O2/7.5% CO2, the medium was collected. Previous studies have demonstrated that PMN-mediated hepatocellular toxicity in co-culture occurs by 16 hr after stimulation of PMNs (13). The cells remaining on the plate were lysed with 1% Triton X-100, followed by sonication. Both the medium and the cell lysates were spun in a centrifuge at 600g for 10 min. The activity of ALT in the cell-free supernatant fluids was determined (using Sigma kit no. 59-UV), and the activity in the medium was expressed as a percentage of total activity (activity in the medium plus activity in the cell lysates). ALT activity in PMNs is relatively small and is not changed upon exposure to Aroclor 1248 (data not shown). ALT is a sensitive and specific indicator of hepatocellular damage in the rat, and in the co-culture system release of ALT from hepatocytes correlates with cell death measured by uptake of trypan blue and electron microscopy (13). Therefore, we used ALT activity in the medium as an index of injury to the hepatocytes. To examine the mechanism by which Aroclor 1248 interacts with PMNs and kills hepatocytes, hepatocytes were cultured with cell-free conditioned medium from PMNs exposed to Aroclor 1248. PMNs in suspension (2.5 × 106 cells/ml Williams’ medium E containing 0.1% gentamicin) were pretreated with cytochalasin B (5 µg/ml) for 10 min at room temperature and then exposed to either vehicle (1 µl DMF/ml cells) or Aroclor 1248 (10 µg/ml) for 30 min at 37°C. The cells were removed by centrifugation, the cell-free supernatant was collected and added to the hepatocytes (2 ml/well), and release of ALT was determined 16 hr later as described above (13).

Statistical analysis. All results are presented as means ± standard error of the mean (SEM). For all results presented, N represents the number of individual animals and in vitro repetitions. Unless otherwise stated, data were analyzed by a two-way analysis of variance (ANOVA), and individual comparisons were performed using the least significant difference test. When variances were not homogenous, data were log-transformed before analysis. When appropriate, an outlier test (test for detection of extreme means) was applied (40). The criterion for statistical significance was p<0.05.

Results

Neither exposure of PMNs to vehicle nor to 1 µg/ml Aroclor 1248 resulted in generation of O2− (Fig. 1). However, PMNs exposed to 10 µg/ml Aroclor 1248 produced a significant amount of O2−. PMA alone caused an increase in O2− production. Exposure to 1 µg/ml Aroclor 1248 before stimulation with PMA caused significant generation of O2−. The amount of O2− produced in the presence of 1 µg/ml Aroclor 1248 and PMA was greater than the sum of O2− produced individually by these two agents. Pretreatment of PMNs with LPS did not alter O2− production in response to Aroclor 1248 (data not shown).

Exposure to 10 µg/ml, but not 1 µg/ml, Aroclor 1248 elicited a significant release of MPO from PMNs compared to vehicle control (Table 1). Release of MPO into the
Table 2. Aroclor 1248 potentiation of LPS-induced hepatotoxicity: histopathologic evaluation*

| Treatment          | N  | 0  | 1  | 2  | 3  | 4  | 5  |
|--------------------|----|----|----|----|----|----|----|
| Saline/corn oil    | 5  | 100|    |    |    |    |    |
| Saline/Aroclor     | 5  | 100|    |    |    |    |    |
| LPS/corn oil       | 11 | 27 | 18 | 36 | 18 |    |    |
| LPS/Aroclor        | 9  | 11 |    | 11 | 33 | 22 | 22 |

LPS, lipopolysaccharide.

*Rats received either LPS (2 mg/kg, intravenous) or saline and 90 min later received either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. Animals were killed 6 hr after administration of LPS or saline, and liver sections were prepared from three different lobes. Severity of hepatic injury was graded on a scale of 0–5 reflecting the frequency and size of the hepatic lesions: 0 = no significant lesions; 1 = extremely mild necrosis; 2 = mild necrosis; 3 = moderate necrosis; 4 = marked necrosis; 5 = severe, coalescing necrosis.

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Figure 4. Photomicrographs of liver sections taken 6 hr after lipopolysaccharide (LPS) administration. (A) Liver from corn oil-treated animal given LPS had sinusoidal neutrophilia and a small lesion (arrow). (B) Liver from animal treated with Aroclor 1248 and LPS had sinusoidal neutrophilia and marked midzonal necrosis. Bar = 50 μm.

Six hours after exposure to LPS alone, activities of ALT and ICDH in plasma were not significantly elevated (Fig. 2). Administration of Aroclor 1248 (50 mg/kg) 90 min after LPS exposure resulted in significant hepatic injury, as demonstrated by increased activities of ALT and ICDH (Fig. 2). Exposure to Aroclor 1248 in the absence of LPS had no effect on ALT or ICDH activities in plasma. Nine hours after administration of LPS alone, plasma ALT activity was increased compared to controls (Fig. 3). Activity of ALT in plasma of rats treated with Aroclor following LPS was significantly greater than in rats receiving LPS alone. Co-administration of Aroclor 1248 with LPS resulted in lethality in 10% of the animals. Lethality was not seen in animals treated with LPS or Aroclor 1248 alone. In a separate study, administration of a smaller dose of Aroclor 1248 (10 mg/kg) did not affect LPS-induced hepatic injury as determined by activities of ALT and ICDH in plasma (data not shown). Exposure to a larger dose of Aroclor 1248 alone (100 mg/kg) did not produce hepatic injury, as evidenced from activities of ALT and ICDH in plasma (74 ± 9 and 6 ± 1 U/l, respectively; not significantly different from vehicle controls) and histologic evaluation.

There were no significant lesions in the livers of vehicle-treated rats or rats exposed to Aroclor 1248 in the absence of LPS (Table 2). Six hours after LPS administration, livers from animals co-treated with either Aroclor 1248 or corn oil vehicle had lesions of varying degrees of severity (Fig. 4). The hepatic sinusoids of LPS-treated rats contained many PMNs, plump Kupffer cells, and small amounts of eosinophilic, proteinaceous material. There were multifocal, irregularly shaped areas of midzonal hepatocellular necrosis. These lesions were characterized by hyper-eosinophilic parenchymal cells with small, pyknotic nuclei or by swollen, pale parenchymal cells with indistinct to absent nuclei and indistinct cytoplasmic borders. The necrotic foci contained degenerate PMNs. There were also foci of single-cell necrosis scattered throughout the tissue. Aroclor 1248 administration increased the severity of the hepatic lesions, but the nature of the lesions did not change.

PMNs were observed infrequently throughout the liver tissue from animals exposed to saline, regardless of co-treatment with Aroclor 1248 (Fig. 5). A marked
accumulation of PMNs was observed at 6 hr in livers from animals exposed to LPS, and this was not affected by co-administration of Aroclor 1248.

Isolated hepatocytes, either in the presence or absence of PMNs, were exposed to various concentrations of Aroclor 1248 for 16 hr (Fig. 6). Neither 1 μg/ml nor 10 μg/ml Aroclor 1248 was toxic to the hepatocytes. Addition of PMNs to the hepatocyte cultures did not produce cytotoxicity in the absence of Aroclor 1248. However, stimulation of the PMNs with either 1 μg/ml or 10 μg/ml Aroclor 1248 resulted in a significant release of ALT into the culture medium. In a similar experiment in which hepatocytes were incubated with cell-free conditioned medium from PMNs exposed to Aroclor 1248 or its vehicle, no significant differences were observed in these two groups (data not shown).

Discussion

Under normal circumstances, the liver is exposed to small amounts of gut-derived LPS originating from gram-negative bacteria residing in the gastrointestinal tract. However, during sepsis or numerous disease states exposure may increase, and LPS may initiate a systemic inflammatory response leading to significant morbidity and mortality. PMNs have been demonstrated to play a key role in the development of organ injury during sepsis (6). In such situations, enhancement of PMN activation or responsiveness by xenobiotic agents could lead to increased tissue injury. The ability of PCBs to enhance organ injury during an inflammatory event was examined in a model of LPS-induced hepatic injury. In this model, PMNs rapidly accumulate in large numbers in the hepatic sinusoids after administration of LPS and are required for development of hepatic injury (8,10). At the dose used in this study, rats exposed to LPS alone and killed 6 or 9 hr later developed mild hepatic necrosis. Administration of Aroclor 1248 increased the severity of the hepatic injury as evidenced by elevated ALT and ICDH activities in plasma and increased severity of hepatic lesions determined histologically. The pathological changes observed are consistent with previous studies describing hepatic injury in rats treated with LPS (8,41). In addition, co-treatment with Aroclor 1248 produced lethality, whereas no mortality occurred in animals exposed to LPS alone. Exposure to Aroclor 1248 alone up to 100 mg/kg did not produce hepatoxicity.

These results are consistent with previous studies describing sensitization to LPS-induced lethality by pretreatment with PCBs. Mortality after administration of LPS was greater in mice fed Aroclor 1248 for 5 weeks compared to mice fed a control diet (42). Similarly, mice fed Aroclor 1242 for 6 weeks were more responsive to the lethal effects of LPS (43). Neither of these studies investigated damage to the liver or other organs. Other investigators have described a potentiation of LPS-mediated hepatotoxicity after exposure to compounds structurally related to some PCB congeners, i.e., 2,3,7,8-tetrachlorodibenzop-α-dioxin and polybrominated biphenyls (44–46). These studies involved treatment with halogenated biphenyls before administration of LPS so that effects on hepatic parenchymal cells, such as enzyme induction and alterations in gene expression, were likely to have occurred before LPS exposure and may have contributed to potentiation of toxicity. These effects may be related to Ah receptor-mediated events. In the present study, the direct effects of Aroclor 1248 on hepatic parenchymal cells cannot be ruled out; however, the treatment paradigm involved a short time (4.5 hr) between PCB exposure and evaluation of liver injury, so effects such as enzyme induction would be minimized.

PMNs begin accumulating in the hepatic sinusoids of rats within 30 min after LPS administration, and this event is a prerequisite for development of liver injury (9). Exposure to Aroclor 1248 did not affect hepatic PMN accumulation in LPS-treated rats; thus, potentiation of injury was not caused by increased numbers of PMNs in the liver. After absorption into the circulation, PCBs initially distribute in high concentration in the liver, which is a target organ for toxicity (15,16,47). Therefore, the presence of PCBs in the liver concurrent with PMN accumulation allows the possibility that PCBs affected the PMNs locally and that this contributed to increased hepatocellular damage. Indeed, isolated PMNs produced O₂⁻ and underwent degranulation upon exposure in vitro to Aroclor 1248, and Aroclor 1248 potentiated the production of O₂⁻ in response to PMA stimulation. PMA directly stimulates protein kinase C, leading to activation of the NADPH oxidase system and O₂⁻ production (48). These data with Aroclor 1248 are consistent with previous studies involving Aroclor 1242 and individual congeners of PCB (23,26).

PCBs initiate PMN responses within 15 min by activating the cellular signal transduction pathways responsible for these functions (24,26), thus providing a time-course for PMN activation that is consonant with the development of hepatic necrosis in vivo. This proposed mechanism for the enhancement of LPS-induced hepatic injury by Aroclor 1248 was supported by the results obtained using an in vitro co-culture system composed of isolated hepatocytes and PMNs. Aroclor 1248 was not toxic to the hepatocytes in the absence of PMNs; however, hepatocellular injury, as evidenced by increased release of ALT into the medium, occurred when hepatocytes were co-cultured with PMNs activated upon the addition of Aroclor 1248. The co-culture system is a
Aroclor 1248 stimulates endothelial cells and hepatocytes in vitro (11,13). Reactive oxygen species, such as O$_2^-$, may also play a contributing role in PMN-mediated tissue injury (14). Because Aroclor 1248 can elicit PMN O$_2^-$ production and degradation, both mechanisms may contribute to potentiating PMN-mediated tissue injury. It is also possible that PCBs may adversely affect hepatic parenchymal cells in a manner resulting in greater susceptibility to injury upon exposure to PMN-derived cytotoxic mediators.

In summary, Aroclor 1248 increased the hepatic injury that follows LPS administration. Because PMNs play a critical role in liver injury from LPS, these findings are consistent with previous studies demonstrating that PCBs can activate PMNs in vitro and sensitize animals to LPS-induced lethality. PCBs may act in a fashion analogous to inflammatory stimuli by activating PMNs to become cytotoxic, resulting in enhancement of PMN-mediated tissue injury during an inflammatory event. Studies with individual PCB congeners suggest that ortho-substituted PCBs can activate PMNs in vitro, whereas coplanar PCBs are inactive (23). Because Aroclor 1248 is a mixture of PCB congeners, the respective roles for both ortho-substituted and coplanar congeners in the mechanism for enhanced liver injury upon LPS exposure is uncertain at this time.

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