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Bovine Intestinal Alkaline Phosphatase Attenuates the Inflammatory Response in Secondary Peritonitis in Mice

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Lipopolysaccharide (LPS) contributes importantly to morbidity and mortality in sepsis. Bovine intestinal alkaline phosphatase (BIAP) was demonstrated to detoxify LPS through dephosphorylation. LPS injection combined with BIAP reduced inflammation and improved survival in various experimental settings. In this study, single-dose intravenous administration of BIAP (0.15 IU/g) was applied in a murine cecal ligation and puncture (CLP) model of polymicrobial sepsis. Saline was given as control (S group). Treatment with BIAP prior to CLP (prophylaxis; BIAP-P group) or shortly after (early treatment; BIAP-ET group) reduced cytokine concentrations in plasma and peritoneal lavage fluid (PLF). Tumor necrosis factor-alpha peak levels decreased from 170 pg/ml (S) to 57.5 (BIAP-P) and 82.5 (BIAP-ET) in plasma and in PLF from 57.5 pg/ml (S) to 35.3 (BIAP-P) and 16.8 (BIAP-ET) (all, $P < 0.05$). Peak interleukin-6 levels in plasma decreased from 19.3 ng/ml (S) to 3.4 (BIAP-P) and 11.5 (BIAP-ET) and in PLF from 32.6 ng/ml (S) to 13.4 (BIAP-P) and 10.9 (BIAP-ET) (all, $P < 0.05$). Macrophage chemoattractant protein 1 peak levels in plasma decreased from 2.0 ng/ml (S) to 1.0 (BIAP-P) and 0.7 (BIAP-ET) and in PLF from 6.4 (S) to 2.3 (BIAP-P) and 1.3 ng/ml (BIAP-ET) (all, $P < 0.05$). BIAP-treated groups showed decreased transaminase activity in plasma and decreased myeloperoxidase activity in the lung, indicating reduced associated hepatocellular and pulmonary damage. Survival was not significantly altered by BIAP in this single-dose regimen. In polymicrobial secondary peritonitis, both prophylactic and early BIAP treatment attenuates the inflammatory response both locally and systemically and reduces associated liver and lung damage.

Secondary peritonitis can ultimately lead to sepsis with shock and/or organ failure and is associated with high morbidity and mortality (30 to 40%) (5). Both secondary peritonitis and sepsis are characterized by an excessive inflammatory response (7, 28). Activation of cytokines and other inflammatory mediators in these conditions are induced by endotoxins, such as lipopolysaccharide (LPS), which is an important contributor to morbidity and mortality (28). LPS is a component of the outer leaflet of gram-negative bacteria. It is a complex and negatively charged molecule composed of a polysaccharide chain (O-specific chain) and a toxic lipid moiety (lipid A). The two phosphate groups of lipid A are essential for its immunostimulatory characteristics (2, 7). Intravenous (i.v.) injection of LPS leads to a generalized inflammatory response (29). The dephosphorylation product of lipid A, monophosphoryl lipid A, is a nontoxic derivative that does not evoke major inflammatory response (2) and is known to induce tolerance (1, 34). Therefore, LPS (and, in particular, lipid A) is a potential therapeutic target in sepsis (7, 11). Many sepsis therapies have aimed to block the effect of LPS by using antisera (6, 35) and anti-LPS antibodies (20) or by binding LPS with LPS-binding protein (8) or high-density lipoprotein (19). Although these therapeutics were quite successful in LPS injection models, they had little or no success in reducing the devastating effects of LPS during sepsis.

Alkaline phosphatase (AP) is a promising therapeutic agent and has been shown to dephosphorylate LPS in vitro and in vivo under physiological conditions. Therefore, AP effectively detoxifies LPS (16, 23, 24). In mice, mortality was reduced after lethal injection of gram-negative bacteria and administration of human placental AP (HPLAP) (2) and bovine intestinal AP (BIAP) (30). In rats, endogenous inhibition of intestinal AP led to increased and prolonged endotoxemia after oral LPS challenge compared to control animals (16). Simultaneous administration of LPS and BIAP diminished the inflammatory response compared to LPS injection alone (3). However, in all these studies, endotoxin challenge was imposed by either LPS or a single bacterial strain. The cecal ligation and puncture (CLP) model was established to induce polymicrobial abdominal sepsis, thereby mimicking the clinical situation more closely (22, 27). Using this model with mice, the present study was designed to investigate the effects of BIAP on inflammation and mortality. BIAP was used as prophylaxis by i.v. administration just prior to CLP and, as early treatment, by i.v. administration shortly after CLP. The local peritonitis and systemic inflammatory responses were investigated, as well as remote effects on liver and lungs and survival.

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MATERIALS AND METHODS

Animals. Specific-pathogen-free male C57BL/6 mice (25 to 28 g; Harlan, Zeist, The Netherlands) were acclimatized for 1 week and housed in filter-top cages under standardized laboratory conditions. After surgery, mice were maintained

in filter-top cages in a temperature-controlled room (22 to 24°C) with a 12-h light/12-h dark diurnal cycle with food and water ad libitum. Approval for the experiments was obtained from the Animal Ethics Committee of the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Clinical-grade BIAP from Biozyme (Blaenavon, United Kingdom) was donated by AM-Pharma (Bunnik, The Netherlands). BIAP was diluted with saline (Fresenius Kabi, 's-Hertogenbosch, The Netherlands) just before i.v. administration in a dose of 0.15 IU/g of body weight, which is approximately 50 to 100 times higher than plasma levels and was previously used by others as well (2, 30). BIAP activity was tested by routine laboratory testing.

Experimental design. To investigate the inflammatory parameters, mice were randomly allocated to five different groups: (i) CLP with BIAP prophylaxis (BIAP-P), (ii) CLP with BIAP early treatment (BIAP-ET), (iii) CLP with saline (S), (iv) sham with BIAP, and (v) sham treatment with S. In group 1, BIAP was given 5 min prior to puncture (prophylaxis); in groups 2 and 4, BIAP was given 15 min after puncture or sham laparotomy. Saline (0.9% sodium chloride) was given 15 min postpuncture or post sham laparotomy (groups 3 and 5). All mice were sacrificed at 30 min or 4, 6, 8, or 24 h after CLP or sham operation for peritoneal lavage and retrieval of blood and organs (8 mice/group/time point). For survival analysis up to 72 h, 83 mice were randomly allocated to the five groups mentioned above (19 mice/group for CLP and 13 mice/group for the sham condition). In these mice, peritoneal lavage fluid (PLF) was obtained to assess bacterial load, lungs were harvested for lung myeloperoxidase activity assessment, and plasma was sampled for transaminase activity assessment, all after 72 h.

CLP. Mice were given buprenorphine (Temgesic; Schering-Plough, Amstelveen, The Netherlands) 0.05 to 0.1 mg/kg subcutaneously 30 min preoperatively. All mice were anesthetized via inhalation of a mixture of N_2O:O_2 (1:1 [vol/vol]; 1 to 2 liters/min) and 2.0 to 2.5% isoflurane (Burtons, Kent, United Kingdom). During all operations, the mice were kept on a heating pad at 37°C (Animed, Barneveld, The Netherlands). After midline laparotomy, the cecum was mobilized and ligated with a 4-0 vicryl suture (Ethicon; Johnson & Johnson, Int., St-Stevens-Woluwe, Belgium) just beyond the ileocecal junction and perforated through and through with a 23-gauge needle (Becton Dickinson, Drogheda, Louth, Ireland), constituting the CLP. The abdomen was closed in two layers with a running suture using 6-0 silk (Ethicon). In sham mice, the cecum was manipulated without ligation and puncture. A penile venous injection with AP or an equal volume of saline was given.

All animals were allowed to wake up except those to be sacrificed after 30 min. Twice a day, fluid resuscitation (1 ml saline subcutaneously) was administered, and once a day, body weight and temperature were checked.

For sample harvesting, all animals were reanesthetized (2 to 2.5% isoflurane; 1:1 O_2:air mixture) at the appropriate time point. The abdomen was disinfected with iodine, and a peritoneal lavage was performed by the injection of 5 ml saline intraperitoneally. After abdominal massage, as much fluid as possible was aspirated. The abdomen was subsequently opened, and systemic blood and organs (lungs) were collected.

PLF. After aspiration of peritoneal fluids, serial dilutions were made to determine the number of CFU. One milliliter was centrifuged (1,200 × *g*; 10 min at 4°C), and the supernatant was stored at −80°C before further testing. The pellet was resuspended in 250 μl saline, and the total number of polymorphonuclear cells (PMN) was counted with a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter, Fullerton, CA; minimum diameter, 3.5 μm).

Blood sampling. Blood from the inferior caval vein was collected in a Microtainer tube containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ) and centrifuged (1,200 × *g*; 10 min at 4°C), after which plasma was collected. Activities of AP, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined by routine laboratory testing. The remaining plasma was stored at −80°C for further analysis.

CFU. PLF was plated in serial log dilutions on blood agar plates and incubated at 37°C. CFU were counted after 24 h (aerobic conditions) or 48 h (anaerobic conditions). Quantitative cultures were expressed as the number of CFU per milliliter of PLF.

Cytokines. Plasma and PLF samples of animals sacrificed up to 24 h after CLP were subjected to a cytometric bead assay (Becton Dickinson Biosciences, Alphen aan den Rijn, The Netherlands) for simultaneous measurement of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), murine chemoattractant protein 1 (MCP-1), gamma interferon, IL-12p70, and IL-10. Briefly, plasma or PLF was diluted with assay diluent and then mixed with capture bead suspension in a sample tube. Ten microliters of fluorescent-labeled detection reagent was subsequently added to the assay tubes, followed by incubation in the dark for at least 2 h. Wash buffer was added (200 μl), and the tubes were centrifuged at 200 × *g*, followed by careful aspiration of the supernatant and gentle resuspension of the bead pellet in 150 μl of wash buffer. All reagents and buffers were supplied by the manufacturer. The finished bead suspensions were analyzed on a FACSCalibur flow cytometer (Becton Dickinson Biosciences, Alphen aan den Rijn, The Netherlands) according to the instructions of the bead assay manufacturer. The detection limit of each of the cytokines was 2.5 to 5.0 pg/ml.

Myeloperoxidase (MPO). MPO activity was measured as described previously (17). Briefly, lung tissue was homogenized in potassium phosphate buffer with an ultrathorax (Heidolph, Schwabach, Germany). After being centrifuged (16,500 × *g*; 20 min at 4°C), the supernatant was discarded, and the pellet was resuspended in potassium buffer containing 10 mM EDTA and 0.5% hexadecyltrimethylammonium bromide. MPO activity was determined by measuring the H_2O_2-dependent oxidation of 3,3′5,5′tetramethylbenzidine. The reaction was stopped with glacial acetic acid. Activity was measured immediately by a spectrophotometer as the change in absorbance at 655 nm. Results were expressed as units per milligram (wet weight) of tissue.

Data analysis. The Statistical Package for the Social Sciences (SPSS 12.0.1; SPSS, Inc., Chicago, Ill.) for Windows was used for data analysis. For the time series, analysis of variance (ANOVA) for repeated measures was performed with LSD posthoc testing when appropriate. For parameters that were measured once, a Kruskal-Wallis test was done for all groups together. When appropriate, Mann-Whitney tests were performed to determine the differences between two groups. Kaplan-Meier curves in combination with log rank tests were used for survival analysis. Significance was assumed when P values were <0.05.

TABLE 1. Aerobe and anaerobe CFU[a]

Treatment group	Aerobe CFU (10^6/ml) at:		Anaerobe CFU (10^6/ml) at:	
	24 h	72 h	24 h	72 h
BIAP-P	7.5 ± 8.2*	44.3 ± 20.3*	43.0 ± 29.1*	270 ± 135*
BIAP-ET	6.3 ± 4.0*	27.9 ± 13.6*	50.9 ± 13.1*	287 ± 172*
S	7.5 ± 3.1*	37.6 ± 30.0*	146 ± 63.6*	234 ± 78.5*
Sham	0	0	0	0

[a] CFU count (mean ± SEM) was not altered by BIAP treatment (0.15 IU/g) as prophylactic (BIAP-P) or early treatment (BIAP-ET) in septic CLP mice compared to saline treatment (S); however, CLP induced a significant CFU count compared to results with a sham operation (8 to 14 mice/group). *, $P < 0.05$ versus sham (Mann-Whitney).

RESULTS

Between both sham groups (BIAP and saline), no significant differences were observed for any parameter (PMN, CFU, cytokines, transaminases, MPO, or survival). One exception was AP activity, which was increased after BIAP administration up to 30 min in the BIAP sham group compared to the saline sham group. But after 4 h, AP activities were equal in both groups. Therefore, results of these two groups have been combined into one group, named sham.

BIAP activity. To confirm efficient administration of BIAP, plasma samples were collected at different time points before and after BIAP or saline administration. Fifteen minutes after i.v. injection, plasma AP activity was 3,056 ± 621 IU/liter (25-fold increase); 30 min after injection activity dropped to 1,200 ± 417 IU/liter (10-fold increase), whereas after 4 h, normal activity was found (120 ± 25 IU/liter).

Induction of secondary peritonitis. In accordance with the model, the number of PMNs in PLF was significantly ($P < 0.05$) increased after CLP (median, 9.4×10^6; range, 3.5×10^5 to 4.8×10^7) compared to sham (median, 1.1×10^6; range, 1.0×10^4 to 1.1×10^7; $P < 0.001$). No differences between the CLP groups (BIAP-P, BIAP-ET, and S) were observed. The numbers of both aerobe and anaerobe CFU in PLF were elevated after CLP ($P < 0.05$) without significant differences among CLP groups (Table 1). Identification of cultured bac-

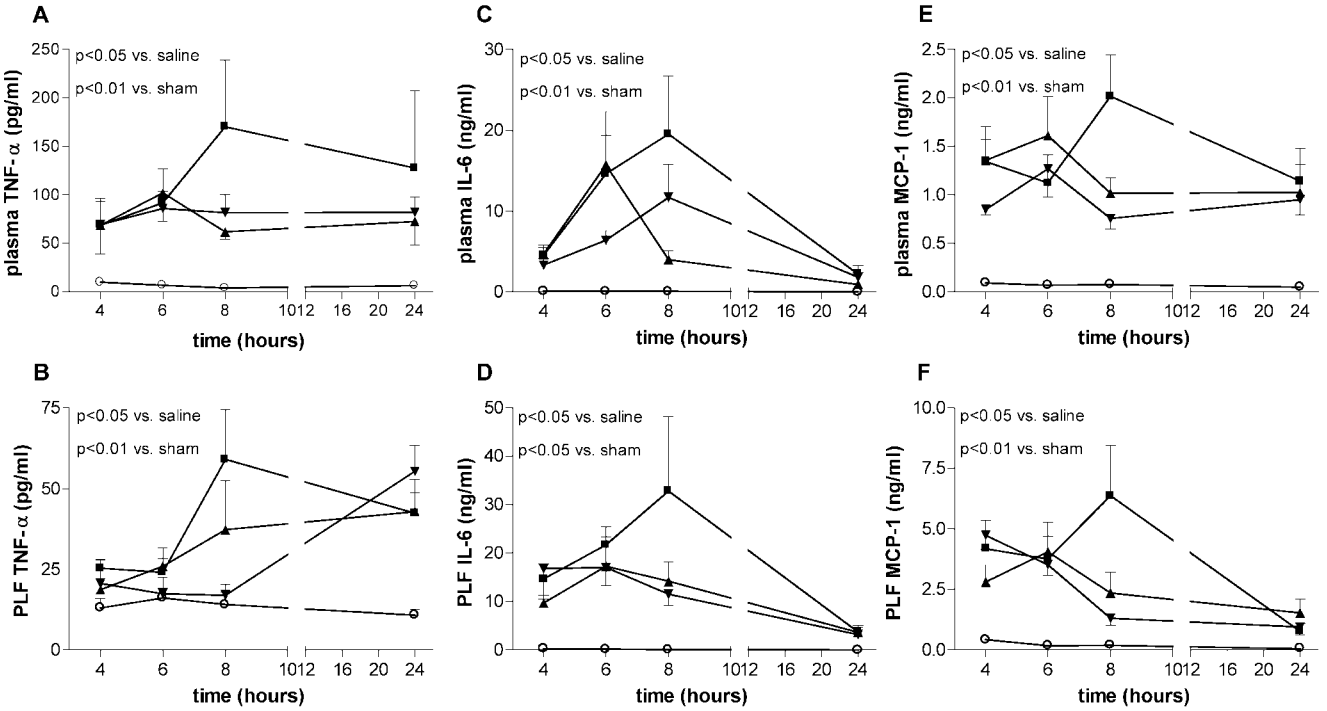

FIG. 1. Systemic and local cytokine responses during secondary peritonitis. Concentrations of TNF-α (A and B), IL-6 (C and D), and MCP-1 (E and F) were evaluated in plasma for systemic response and in peritoneal lavage fluid (PLF) for local response. Mice were subjected to secondary peritonitis by CLP and were given BIAP as prophylaxis (▲), early treatment (▼), or saline (■); sham-treated mice received saline or BIAP (○). Values represent means ± the standard error of the mean (SEM) (eight mice/group). *P* values: $P < 0.01$, all CLP groups versus sham for plasma TNF-α, IL-6, and MCP-1 and for PLF TNF-α and MCP-1 (ANOVA repeated); $P < 0.05$, CLP versus sham for PLF IL-6 (ANOVA repeated); $P < 0.05$, both BIAP groups (BIAP-P and -ET) versus saline for TNF-α, IL-6, and MCP-1 in plasma and PLF (ANOVA repeated).

teria showed fecal flora, including *Escherichia coli*, coliform rods, and *Bacterioides* and *Acinetobacter* species (data not shown).

Cytokines in plasma and PLF. After sham operation, no important changes in concentrations of TNF-α, IL-6, and MCP-1 were observed in either plasma or PLF. In contrast, these cytokines were excessively produced during secondary peritonitis (Fig. 1A to F). From 4 h continuing up to 24 h, TNF-α, IL-6, and MCP-1 concentrations in plasma and PLF were significantly elevated in all CLP groups compared to sham ($P < 0.05$, CLP versus sham for PLF IL-6; $P < 0.01$, CLP versus sham for all other parameters; ANOVA repeated). Importantly, these cytokine levels were reduced in both BIAP groups compared to the saline group. TNF-α peak levels in plasma dropped from 170 pg/ml in saline-treated mice to 57.5 and 82.5 after prophylactic and early BIAP treatment, respectively, and in PLF from 57.5 pg/ml to 35.3 and 16.8, respectively. Interleukin-6 peak levels in plasma decreased from 19.3 ng/ml in saline treated mice to 3.4 and 11.5 in BIAP-P and BIAP-ET groups, respectively. In PLF, IL-6 peak levels decreased from 32.6 ng/ml after saline treatment to 13.4 and 10.9 after BIAP-P and BIAP-ET treatment, respectively. Plasma peak levels of MCP-1 decreased from 2.0 ng/ml after saline treatment to 1.0 and 0.7 after BIAP-P and BIAP-ET, respectively, and levels in PLF decreased from 6.4 to 2.3 and 1.3 ng/ml, respectively. Besides reduced peak levels, TNF-α, IL-6, and MCP-1 concentrations in both plasma and PLF were significantly reduced after 4 up to 24 h after CLP ($P < 0.05$, BIAP versus saline for all parameters). Gamma interferon, IL-12p70, and IL-10 concentrations were found to be below detection levels (<5 pg/ml).

Distant organ damage. Hepatocellular damage was assessed by measuring AST plasma levels (Fig. 2) and ALT levels. After CLP, plasma activity of AST was increased in all groups (saline and BIAP) from 30 min until 72 h compared to the sham

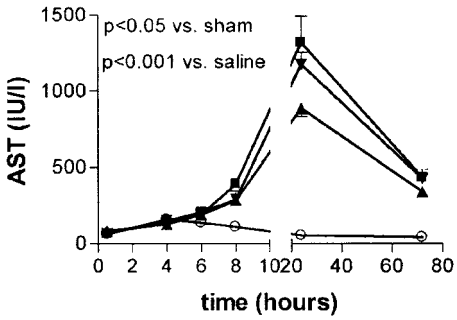

FIG. 2. BIAP attenuates increase in plasma AST activity after CLP. Mice either were subjected to CLP to induce secondary peritonitis or received a sham operation. CLP mice received BIAP as prophylaxis (▲) or early treatment (▼) or saline (■), and sham mice received BIAP or saline (○). Values represent mean ± SEM (eight mice/group). If an error bar is not visible, it coincides with the marker. $P < 0.05$, CLP for all groups versus sham (ANOVA repeated); $P < 0.05$, BIAP-P versus S and BIAP-ET (ANOVA repeated).

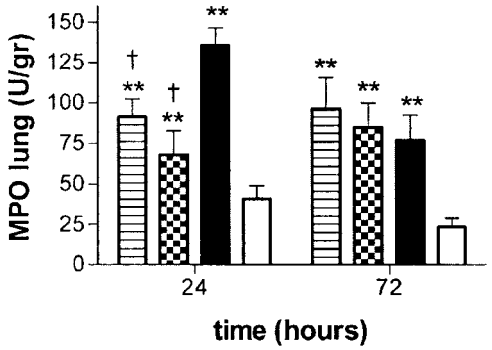

FIG. 3. BIAP attenuates MPO activity in lung homogenates the first 24 h after CLP. Mice were subjected to secondary peritonitis by CLP or sham operation. CLP mice received BIAP as prophylaxis (striped bars) or early treatment (blocked bars) or saline (black bars), and sham mice received BIAP or saline (white bars). Values represent means ± SEM for eight mice/group. **, $P < 0.01$ versus sham; †, $P < 0.05$ versus saline (Mann-Whitney).

operation ($P < 0.05$; ANOVA repeated), indicating hepatocellular damage. Prophylactic administration of BIAP, but not early treatment with BIAP, resulted in reduced release of transaminases throughout the experiments compared to saline controls ($P < 0.001$; ANOVA repeated). ALT levels (data not shown) showed a pattern similar to that seen for AST, i.e., increased activity after CLP compared to the sham operation ($P < 0.001$). ALT levels peaked at 24 h: 792 ± 142 IU/liter after saline treatment, 473 ± 33 IU/liter after BIAP prophylaxis, and 763 ± 56 IU/liter after early treatment. Again, BIAP as prophylaxis reduced ALT levels throughout the experiment when compared to saline CLP ($P < 0.001$).

Remote inflammatory response in the lung was assessed by measuring MPO activity. Myeloperoxidase is an enzyme present in the granules of neutrophils and is an indicator of tissue inflammation. Increased MPO activity (Fig. 3) in the lung was demonstrated 24 and 72 h after CLP compared to sham ($P < 0.01$; Mann-Whitney). At 24 h after CLP, both BIAP-treated groups showed less activity than in the saline group ($P < 0.05$).

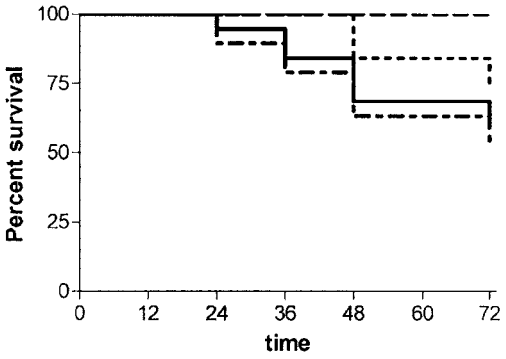

FIG. 4. BIAP did not significantly alter survival rate after CLP. Sham-treated mice (dashed line; 26 mice) and septic CLP mice (19 mice/group) treated with saline (solid black line) or with BIAP as prophylaxis (dash-dot line) or as early treatment (dotted black line) were observed for 72 h maximum. $P < 0.01$ all CLP groups versus sham (log rank).

Survival. Kaplan-Meier survival curves (Fig. 4) showed no mortality after sham operation (100% survival) and decreased survival (53 to 74%) in the CLP groups ($P < 0.01$ versus sham). Most mice died after 24 to 48 h. However, no significant differences between any of the CLP groups were observed

DISCUSSION

In this model of polymicrobial secondary peritonitis, prophylactic and early intravenous treatment with BIAP reduced the inflammatory response both locally and systemically and reduced remote liver and lung damage. A standardized fecal peritonitis was induced, encompassing high numbers of aerobic and anaerobic bacteria, whereas a sham operation had little or no effect on all negative abdominal cultures. These findings are consistent with results shown in the literature (10, 12, 27, 31). After CLP, a profound inflammatory response occurred with increased amounts of PMNs and cytokines in the peritoneal cavity (in PLF) as well as systemically (in plasma) (4, 9, 14, 18, 27). Cytokine concentrations increased up to 8 h after induction of CLP with higher levels mainly in the peritoneal cavity than in plasma, in accordance with an initially local response to a systemic response. This has been reported by others (9, 27) as well, although some noted an earlier peak of TNF-α (4). IL-6 and MCP-1 showed levels corresponding to previously reported levels in literature (9, 14, 18). Also, a 40% mortality after CLP was within the range from previous series undertaken in our laboratory using the same model and was consistent with a desired sublethal, polymicrobial model. Therefore, this study confirms that the CLP model is a reliable model for secondary peritonitis and thus an appropriate tool to investigate the effects of any intervention in abdominal sepsis.

BIAP administration reduced the inflammatory response but showed no effect on bacterial and neutrophil counts in the abdominal cavity. Since BIAP was not expected to have a direct effect on local bacterial growth or neutrophil attraction and was administered intravenously, these parameters were not expected to be different within CLP groups (BIAP groups versus saline control). However, as in other reports (3), a significant effect on the inflammatory response was observed as shown by reduced concentrations of TNF-α, IL-6, and MCP-1 in both plasma and PLF after BIAP prophylaxis, as well as after early treatment, when compared to saline controls. Decreased cytokine levels in PLF indicate a decreased local response, whereas in plasma, a reduced systemic response was demonstrated (9, 13, 21).

Although the above-mentioned parameters are all indicative of LPS detoxification by BIAP, direct effects of BIAP can only be measured through local and systemic LPS levels. However, the *Limulus* amoebocyte lysate assay—the "gold standard" for endotoxin measurement—cannot discriminate between lipid A and monophosphoryl lipid A (2) and therefore is of no use in assessing LPS detoxification in this study. Total phosphate release is another way to assess dephosphorylation of LPS by BIAP but can only be used in in vitro experiments. Therefore in this study, decreased cytokine concentrations could not be linked to decreased LPS levels, and thus the direct effect of BIAP on LPS could not be assessed. Nevertheless, the specific activity of BIAP against LPS has been demonstrated clearly in vitro (23).

After CLP, increased AST and ALT levels were demonstrated, indicating significant hepatocellular damage. Hepatocellular dysfunction and altered blood flow to the liver after CLP have accounted for increased plasma AST and ALT levels (32). Xu et al. showed increased sensitivity of the liver to LPS after inhibition of endogenous AP synthesis and release. After exposure to LPS both in vitro and in vivo, hepatocellular damage was increased when AP synthesis was inhibited. It was suggested that high levels of AP exert a protective effect against liver damage by neutralizing endotoxin (33). In the present study, this protective effect was observed in the prophylaxis group but not in the early treatment group. Prophylactic treatment with BIAP reduced transaminases to only mildly increased AST and ALT levels compared to effects from early treatment with BIAP and saline controls, indicating significantly less hepatocellular damage. A possible explanation might be that BIAP was at first taken up by the liver to be cleared from the circulation (25, 26). The subsequent LPS challenge as a result of CLP is then combated efficiently by BIAP stored in the liver. This is consistent with the observation that, particularly in the prophylaxis group, hepatocellular damage was reduced. If BIAP was injected at a later time point (e.g., early after CLP), BIAP was probably not (yet) present in the liver in high enough concentrations during the initial LPS challenge from the splanchnic area via the portal vein, thereby lacking substantial protective effects on liver damage. However, circulating BIAP in as much as a 25-fold increase in plasma AP activity 15 min after BIAP administration is available for detoxification of systemic LPS, for instance, in the lung. This study showed reduced MPO levels in the lung in the early treatment group, indicating fewer pulmonary neutrophils and hence less pulmonary inflammation and damage. In the prophylaxis group, MPO levels were also reduced, presumably because more endotoxin was already neutralized in the liver, therefore preventing a higher proportion of endotoxin from entering the systemic circulation and reaching the lungs. This resulted in a reduction of both liver and lung damage in the prophylaxis group.

In the present study, no significant effect of BIAP on survival was demonstrated. Increased rates of survival in mice were reported after LPS injection in combination with human placental AP (2, 30) and BIAP (3). However, either differences were not significant (2) or survival was analyzed only until 24 h (30). This time period is very short, since survival is appropriately analyzed over a longer time period (4, 9, 10, 12). In a study with BIAP administered as single dose in mice injected with *E. coli* (3), a statistical difference was observed only at 24 and 36 h but not when the observation period was extended after 36 h. So far, no substantial improvement on long-term survival ($\geq$72 h) has been demonstrated after endotoxin or bacterial challenge using a single dose of BIAP in mice.

It is known (3) that the half-life of BIAP is very short. In the present study, AP activity levels were normalized within 4 h. Preliminary studies with increasing doses (up to five times higher) did not alter survival rate (data not shown). Apparently, a single target intervention with BIAP might not be powerful enough to reduce mortality successfully, since the remaining mediators are still effective and LPS release may be ongoing from peritoneal bacteria. In accordance, PLF bacterial counts were elevated 24 h after CLP. Prolonged administration of AP might be more effective in this model of polymicrobial peritonitis with a continuous influx of LPS. Another factor is that patients with abdominal sepsis are usually treated with antibiotics. In this study, antibiotics were not used to assess the mere effect of BIAP. Importantly, many antibiotics are known to shed even more LPS from succumbing bacteria (15).

In conclusion, the results of this study show that in this model of polymicrobial peritonitis and sepsis, i.v. administration of BIAP had no effect on bacterial and PMN counts in the abdominal cavity but reduced the local and systemic inflammatory cytokine response, as well as distant damage in liver and lungs.

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