Impact of bile duct obstruction on hepatic *E. coli* infection: role of IL-10

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**MATERIALS AND METHODS**

**Animals.** Six- to eight-week-old male wild-type C57BL/6 and IL-10 knockout B6.129IL10tm1Cgn/J (1) mice were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were handled and housed according to National Institutes of Health and institutional guidelines, and the study was approved by the Institutional Animal Care and Use Committee.

**Bile duct ligation.** Mice were anesthetized using inhaled isoﬂurane. The midline laparotomy was performed under sterile conditions. Bile duct ligation (BDL) was performed by dissecting the common bile duct (CBD) below the entrance of the cystic duct. Care was taken to stay above the level of the entrance of the pancreatic duct to avoid ligation of the pancreatic duct and pancreatitis. Lack of pancreatitis was conﬁrmed by measuring serum amylase levels. The CBD was ligated at this level using 6–0 silk suture. BDL was performed at day −1, 0, or +1, in relationship to PV injection with *E. coli*; see PV injection. The animals were closed in two layers using 6–0 silk suture.

**PV injection.** The PV was isolated by reflecting intestinal contents to the left of the animal. Initial experiments were performed using PV methylene blue injections and demonstrated hepatic, but not splenic or mesenteric, perfusion of blue. Free-hand injection of *E. coli*, LPS, or saline was performed using a 0.25-ml volume and 30-gauge needle. Hemostasis was achieved by direct compression. The day of PV injection was deﬁned as day 0.

**Bacteria growth and preparation.** *E. coli* (ATCC #25922; known to be pathogenic in rodents) was grown in broth overnight. Turbidity was measured, and dilutions were prepared from previously delineated growth curves. The number of viable bacteria, which were injected, was deﬁned as plating the inoculum on soy agar plates and determining the colony-forming units (cfu).

**Survival studies.** Animals were injected with *E. coli* into the PV as outlined above. BDL was performed at days −1, 0, and +1, and PV injection performed at day 0. These groups were designated BDL−/PV0, BDL0/PV0, and BDL+1/PV0. Animals were followed for survival. Control animals were injected with saline solution. All further experiments were performed with the BDL0/PV0 protocol.

**Bacterial growth in infected livers.** To study the kinetics of bacterial growth, the left lobe of the liver was harvested at 4 and 24 h after PV injection of $5 \times 10^4$ *E. coli* (BDL0/PV0). The liver was homogenized and plated on soy agar plates (Remel, Lenexa, KS) and the colony-forming units recovered were counted after overnight incubation. The total colony-forming units recovered from each liver were calculated. The animals that were injected with saline in the presence or absence of BDL were used as controls to ensure sterility.

**Histology.** Liver tissues from animals receiving $5 \times 10^4$ cfu *E. coli* into the PV in the absence or presence of BDL were harvested 24 h after injection, placed in formalin, and subjected to standard hematoxylin and eosin staining. Slides were examined with the assistance of an experienced hepatopathologist.

**Cytokine protein.** Serum was obtained by retroorbital bleeding from animals that had been injected with $5 \times 10^4$ cfu *E. coli* into the liver.

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PV in the presence of BDL (BDL0/PV0) 24 h before. IL-10 protein levels were measured by ELISA (Endogen).

**Infection of transgenic knockout mice.** To examine the role of IL-10 production in our model, 6-wk-old B6.129IL10tm1Cgn/J mice, deficient in IL-10, were injected with $5 \times 10^4$ cfu *E. coli* into the PV with concurrent BDL (+BDL). To assess whether there was a baseline alteration in the IL-10 knockout mouse’s ability to handle *E. coli* even in the absence of BDL, another group of IL-10 knockout animals were subjected to PV injection of $5 \times 10^4$ cfu *E. coli* without BDL (−BDL). Survival was monitored. Six-week-old C57BL/6J mice were used as controls.

**Statistical analysis.** Log-rank test was used to assess significance for survival experiments. Student’s t-test was used to assess differences in cytokine production.

**RESULTS**

**BDL impairs survival after PV *E. coli* infection.** Figure 1 is representative of three experiments and demonstrates that BDL exacerbated PV *E. coli* infection. All mice received a PV injection of *E. coli* on day 0 (D0PV). In addition, control mice had sham surgery on day −1, 0, or +1 relative to the *E. coli* injection; hepatic bacterial growth in all of these mice was similar, and they are shown as a single group designated as sham/PV. In addition to PV *E. coli* injections, experimental mice also had BDL on day −1 (D−1 BDL/D0PV), day 0 (D0 BDL/D0PV), or day +1 (D+1 BDL/D0PV) relative to these injections. The only deaths occurred in animals with both PV *E. coli* and BDL. Among the BDL mice, the greatest mortality occurred when the bile ducts were either ligated on the day of PV *E. coli* injection (D0BDL/D0PV) or ligated the day after injection (D0BDL/D+1PV). In the remainder of this manuscript, the focus is on the D0BDL/D0PV group.

Figure 2 demonstrates that BDL impairs hepatic clearance of PV *E. coli* infections. The initial cfu of hepatic *E. coli* were similar in animals that had or had not received BDL. This result confirms equivalent initial delivery of pathogen to the liver. However, by 24 h postinfection, significantly greater cfu were recovered in animals that had BDL compared with those that had no BDL. To confirm that the *E. coli* recovered from the infected livers was derived from those injected, we compared the antibiotic sensitivities of the initial inoculum and the *E. coli* recovered at 24 h. All *E. coli* were resistant to ampicillin and chloramphenicol but sensitive to penicillin.

**BDL results in greater histological injury after PV infection.** Histology confirmed increased hepatic injury when PV infections occurred in the presence of BDL compared with infection in the absence of BDL. Figure 3 shows the massive geographic necrosis and exuberant sinusoidal bacterial growth in the livers that were infected in the presence of BDL. There was much less infection and injury in the livers that were infected in the absence of BDL.

**BDL and PV *E. coli* infection increased serum IL-10.** Figure 4 demonstrates that IL-10 serum levels were significantly increased in animals that had BDL compared with those that had none at the time of PV injection with bacteria. Lower levels of IL-10 were found in animals that received PV saline, instead of *E. coli*, in the presence or absence of BDL. Unmanipulated animals had levels of IL-10 that were <200 pg/ml.

**Transgenic knockout of IL-10 increased late mortality after PV infection plus BDL.** The previous data show that BDL exacerbates hepatic infection after PV injections of *E. coli*. The increased serum levels of IL-10 in infected mice with BDL compared with the sham-operated infected mice suggested a role for this cytokine because of its known immunosuppressive effects. To test this idea, we compared PV infections in wild-type vs. IL-10 knockout mice in the experiment shown in Fig. 5. We found that, in contrast to our expectation, IL-10 knockout increased late mortality after PV injection of *E. coli* plus BDL.

Note that transgenic knockout of IL-10 resulted in late death of mice (days 3–5). In contrast, death in wild-type mice typically occurred at days 1–3 (see Figs. 1 and 5). This result suggests that the presence of IL-10 in the late phase of infection was beneficial. Consistent with this idea, Fig. 6 shows a trend to increased hepatic bacterial growth in the transgenic knockout animals.

To rule out an abnormal response to PV injection in the IL-10 knockout mice, we injected the PV of these mice with saline instead of *E. coli*. The mice displayed no symptoms and survived for >10 days.

**Fig. 1.** Bile duct ligation (BDL) impairs survival after portal venous (PV) *Eschericia coli* infection. Six- to eight-week-old C57BL/6 mice received a PV injection of $5 \times 10^4$ colony-forming units (cfu) *E. coli* on day 0 (D0PV); in addition, each of these injected mice also had a sham operation or BDL at days −1 (D−1 BDL/D0PV), 0 (D0BDL/D0PV), or +1 (D+1BDL/D0PV). Survival was monitored (n = 7/group).

**Fig. 2.** BDL impairs hepatic clearance of PV *E. coli* injections. Six- to eight-week-old C57BL/6 mice received PV *E. coli* ($5 \times 10^4$ cfu) and, at the same time, also had either sham surgery or BDL. The left lobe of the liver was harvested and cultured at either 4 or 24 h after infection, as outlined in MATERIALS AND METHODS. Significantly greater *E. coli* were recovered from the BDL group at 24 h after infection (n = 4 animals per group; P < 0.05 by t-test).
DISCUSSION

Our data show that BDL dramatically increases the mortality of hepatic infections introduced via the PV (Fig. 1), increases hepatic bacterial growth (Fig. 2), and increases hepatic pathol-

Fig. 3. BDL results in greater histological injury after PV infection. Animals received either PV injection with E. coli alone (A: ×200; B: ×400) or PV injection with concurrent BDL (C: ×40; D: ×100). Livers were examined by hematoxylin and eosin staining at 24 h after infection for A–C and at 48 h after infection for D. PV infection in the absence of BDL resulted in small areas of necrosis (*) with infiltrating neutrophils. The presence of BDL in addition to PV E. coli resulted in more geographic necrosis (*) with subsequent growth of E. coli in the sinusoid. Filled arrow, central vein; open arrow, portal tract. Images are representative of 4 animals per group per time point.

Fig. 4. Serum levels of IL-10 are increased in animals that have biliary obstruction at the time of hepatic infection. Animals received 5 × 10⁶ cfu of PV E. coli and BDL on the same day (E. coli BDL0/PV0), PV E. coli without BDL (E. coli PV), PV saline and BDL (saline PV/BDL), or PV saline without BDL (saline PV). Serum was harvested at 24 h after these injections, and IL-10 levels were measured by ELISA (n = 3/group). Significantly greater IL-10 was detected in the presence of infection and BDL (P < 0.05 by t-test, E. coli PV/BDL vs. E. coli PV).

Fig. 5. Transgenic knock-out of IL-10 increased late mortality after PV infection + BDL. Wild-type (WT) mice received 5 × 10⁶ cfu PV E. coli with concurrent BDL. IL-10 knockout (IL10KO) mice received PV E. coli in the presence (+BDL) or absence (−BDL) of BDL. P < 0.02 between WT (+BDL) and IL10KO (+BDL) (n = 10 mice per group). d, Day.
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increased their production of IL-10, this does not account for bacterial growth, and pathology. Although the BDL mice

published observations are consistent with the possibility that inflammatory response (2, 3, 5, 10, 12, 20, 22, 23). Some

One possibility is that biliary obstruction alters the hepatic infections have a high mortality (6, 9, 13, 17, 24).

Because of the known immunosuppressive effects of IL-10, we investigated its production in our model. We found increased IL-10 in mice with PV infection plus BDL compared with mice with PV infection without BDL (Fig. 4). However, transgenic knockout of IL-10 impairs survival after BDL and PV infection (Fig. 5). In addition, there was a trend toward increased bacterial growth in the transgenic knockout mice (Fig. 6).

Our results illustrate the complex roles of IL-10 in infection. In some situations, IL-10 may inhibit inflammation, decrease host defenses, and thus increase mortality (8). In other situations, IL-10 may prevent “collateral” damage by excessive inflammation (14). Furthermore, IL-10 also may have proinflammatory functions (7). Our results are similar to previous data showing increased mortality in transgenic IL-10 knockout mice after E. coli peritonitis except that we did not find increased bacterial clearance in these mice (15, 21).

In summary, we have established a model of murine hepatic infection and shown that BDL has a dramatic detrimental effect on PV E. coli infection as assessed by mortality, hepatic bacterial growth, and pathology. Although the BDL mice increased their production of IL-10, this does not account for the detrimental effect of BDL.

REFERENCES


