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# Pretreatment with Enteral Cholestyramine Prevents Suppression of the Cellular Immune System After Partial Hepatectomy

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## Objective
The authors tested the hypothesis that the beneficial effects of the endotoxin-binding agent cholestyramine on the postoperative course in rats that had undergone a partial hepatectomy was the result of improvement of cellular immune functions.

## Summary Background Data
Major liver resection is associated with severe postoperative complications and a high incidence of systemic infections. Gut-derived endotoxins previously were shown to be involved in the pathogenic processes after partial hepatectomy in rats. In addition, enteral cholestyramine improved postoperative survival, but how its beneficial effects are mediated is not clear.

## Methods
Rats that were force-fed for 7 days with either cholestyramine (150 mg/day) or 0.9% saline (equal volume) were randomized to undergo a partial hepatectomy or a sham operation. After 24 hours, the rats were killed and splenic mononuclear cells were tested *in vitro* for mitogenic responses and cytokine production.

## Results
Proliferative responses of splenic B and T lymphocytes and lipopolysaccharide-stimulated production of tumor necrosis factor and interleukin-1 by splenocytes were lower in rats after partial hepatectomy than in sham-operated animals. An increased concanavalin A-stimulated production of interleukin-2 also was found after partial hepatectomy compared with sham levels. Pretreatment with enteral cholestyramine preserved cellular proliferative responsiveness of both B and T cells, and restored cytokine production by splenocytes to sham levels.

## Conclusion
Prophylactic treatment with enteral cholestyramine preserved cellular immune functions after partial hepatectomy in the rat, which may explain its beneficial effects on the postoperative course. Furthermore, the authors' results are consistent with the hypothesis that endotoxemia is involved in the pathogenesis of the cellular immune derangements after partial hepatectomy.

Major liver resection is a curative treatment of some primary or secondary hepatic tumors.[1-3] However, this procedure still is associated with a considerable morbidity and mortality. The postoperative course after major resections of the liver, other than segmental or wedge resections, often is complicated by the occurrence of bleeding disorders, intra-abdominal abscesses, systemic infections, and even multiple-organ failure.[4,5] Abdominal or systemic infections contribute significantly to the postoperative mortality rate, which can be as high as 25%.[4,6,7]

Accumulating data suggest that an increased susceptibility to infectious complications after host injury may be caused by impaired cellular and humoral host defense mechanisms, as has been well documented after severe thermal and nonthermal traumatic injuries,[8-10] including major surgery.[11-13] Although the exact cause of immune defects after major injury still remains unclear, endotoxemia is suggested to play an important role. In rats with obstructive jaundice, Greve et al. have reported an impaired cellular immunity, which was abrogated when the animals were kept in germ-free conditions.[14] Experimental infusion of endotoxin in healthy humans has been shown to mimic an immunosuppressive state by altering T-cell and mononuclear phagocyte functions.[15]

After partial hepatectomy in the rat, Morimoto et al. have found reduced responses of lymphocytes to mitogenic stimuli compared with sham-operated animals, assessed in *ex vivo* experiments with splenocytes.[16] In a previous study in partially hepatectomized rats, we have demonstrated that systemic endotoxemia, hepatic failure, and mortality can be prevented by enteral administration of cholestyramine, an endotoxin binder.[17] For this study, we hypothesized that endotoxin-related changes in host defense mechanisms contribute to the development of previously observed postoperative complications. In the following experiments, we used the same rat model to investigate the effects of a major liver resection on cell-mediated immunity by evaluating the *ex vivo* proliferative responses of mononuclear cells and their cytokine production. We chose to determine immune functions of the splenic compartment because splenic macrophages do not normally encounter endotoxin. Furthermore, our aim was to investigate whether changes in cellular immunity were related to gut-derived endotoxins, and could, therefore, be prevented by enteral cholestyramine.

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## MATERIALS AND METHODS

### Animals and Treatment Protocol

This study was carried out with permission and under the guidelines of the Animal Care Committee of Harvard Medical School. Twenty-seven male outbred Wistar rats (220–230 g, Charles River Breeding Laboratories, Wilmington, MA) were allowed to acclimatize for 5 days after delivery, during which time they had free access to water and rat chow (Ralston Purina Co., St. Louis, MO). The animals were housed under standard environmental conditions, with a 12-hour light/dark cycle. After adequate acclimatization, rats were randomized to one of two treatment groups. Animals were force-fed twice daily for 7 days with either 2.5 mL of 0.9% saline or cholestyramine 150 mg/day (Bristol-Myers Squibb Company, Chicago, IL), in an equal volume. All animals had free access to chow and water during these manipulations.

### Surgical Procedures

Surgical procedures were performed on day 7 under ether anesthesia, with chow withdrawn on the evening before. Within each treatment group, the animals were randomized to receive either a two-third partial hepatectomy or a sham operation. Partial hepatectomy was performed according to the method of Higgins and Anderson.[18] Sham-operated animals underwent midline laparotomies and gentle manipulation and exteriorization of the median and left liver lobes, but no actual resections were performed. The incision was closed in a single layer by a Vicryl suture.

The following four groups of animals were studied: 1) sham-saline (sh-sal, n = 6); 2) sham-cholestyramine (sh-chol, n = 6); 3) partial hepatectomy-saline (phx-sal, n = 8); and 4) partial hepatectomy-cholestyramine (phx-chol, n = 7) (Fig. 1). After surgery, all animals continued to receive their oral gavage of saline or cholestyramine. In addition, the animals received no food, but had free access to water. Twenty-four hours after surgery, animals were re-anesthetized, and the abdomen was re-opened to remove the spleen. Afterward, the rats were killed by cervical dislocation.

### Cell Preparation

Splenocytes were isolated as described previously.[8] Briefly, splenocyte suspensions were obtained by gently teasing the freshly harvested spleens with sterile forceps and removing the coarse debris. Each spleen was assayed individually. Splenocytes were suspended in RPMI 1640 medium with 2 mM L-glutamine, 1% antibiotic antimycotic containing penicillin 10,000 units, streptomycin

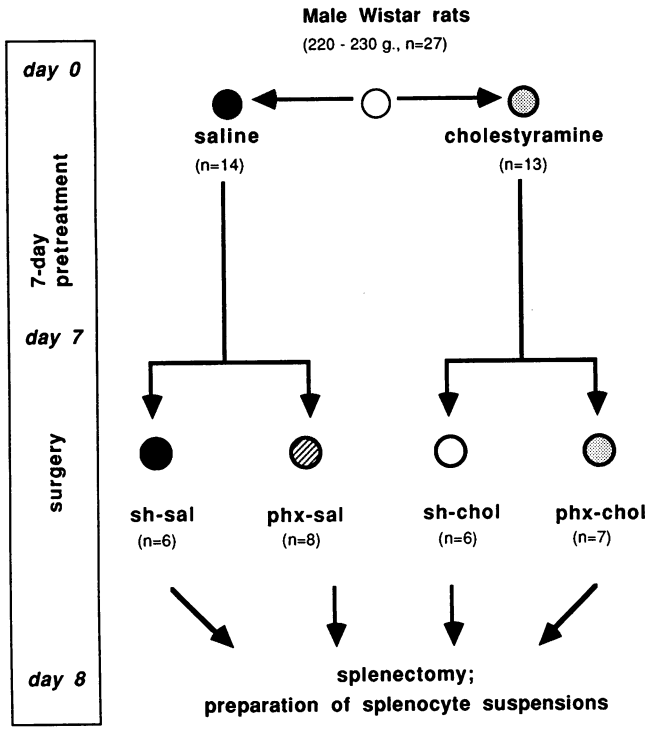


**Figure 1.** Experimental protocol: rats force-fed for 7 days with either cholestyramine or 0.9% saline, were randominzed to undergo a partial hepatectomy or a sham operation, resulting in four groups of animals. After 24 hours (day 8), the spleen was removed from each rat for *in vitro* measurements.

10,000 μg/mL, amphotericin 2.5 μg/mL, 10 mM HEPES buffer (Grand Island Biological Co. [GIBCO], Grand Island, NY) and $5 \times 10^{-5}$ beta-mercaptoethanol (Eastman Kodak, Rochester, MN). Cell suspensions were washed three times in this medium each time by centrifugation at 1500 rpm for 10 minutes and resuspension in medium. After the final wash, cells were resuspended in the same medium, with the addition of 5% heat-inactivated (30 min, 56 C) fetal calf serum (GIBCO; RPMI-FCS). Mononuclear cells were counted using Turk's solution, and viability was assessed using the trypan blue exclusion test. Cell viability was consistently >98%.

## Mitogenic Response of Lymphocytes

Mitogenic responses were assessed using the mitogens phytohemagglutinin (PHA), concanavalin A (Con A) and *Escherichia coli* 055:B5 lipopolysaccharide (LPS) (Sigma Chemical Co., St. Louis). For these assays, $2.5 \times 10^5$ splenocytes/225 μL in RPMI-FCS were incubated in either medium, with the addition of an optimally stimulating dose of 6.25 μg/well of culture of PHA or 0.4 μg/well of Con A or 0.5 μg/well of LPS for 68 hours at 37 C, followed by a 4-hour pulse with 1 μCi tritiated thymidine ($^3$HTdr) 6 Ci/mmol (New England Nuclear, Boston, MA). Uptake of $^3$HTdr was determined using a beta-plate liquid scintillation counter (Gaithersburg, MD). Assays were performed in triplicate; results are given as means of triplicate counts. Variation from the mean did not exceed 10%.

## Generation and Assay of Interleukin-2

Interleukin (IL)-2 was generated by culturing $2.5 \times 10^5$ splenocytes/well in 200 μL volume for 44 hours in RPMI-FCS with or without 0.4 μg Con A. Supernatants were collected from triplicate wells and frozen at −20 C until assayed. The assay was performed as described previously,[10,19] using the cell line CTLL-2. In our laboratory, this assay has a sensitivity of 10 pg/mL of recombinant IL-2. All supernatants were assayed twice in separate assays.

Units of IL-2 were determined by comparison with a standard rat T-cell growth factor, representing 1 unit/mL. The central part of the dilution curve was straightened by probit analysis[20] using a computer program (courtesy of Dr. Brian Davis, Immunex Corp., Seattle, WA). The dilution at which T-cell growth factor reached 50% of the maximum counts was taken as 1 unit and compared with the dilution at which the supernatants from experimental cultures gave 50% of the maximum counts.

## Generation and Assays of Tumor Necrosis Factor and IL-1

Tumor necrosis factor (TNF) and IL-1 production was determined for splenocytes as well as for isolated adherent splenocytes. Adherent splenocytes were obtained by culturing 200 μL/well of splenocytes at a concentration of $1 \times 10^7$/mL in 96 well microtiter flat-bottomed plates for 1 hour at 37 C in RPMI-FCS in the presence of 5% $CO_2$. Plates were washed three times to remove nonadherent cells and 200 μL RPMI without FCS added to all wells. Twenty-five microliters of LPS (0.5 μg/well) or medium were added and plates incubated as aforementioned. After 44 hours, supernatants were removed and stored at −20 C for subsequent cytokine analysis. Tumor necrosis factor was measured using a "sandwich" enzyme-linked immunosorbent assay technique as modified from Sheehan et al.[21] and described previously.[22] Splenocyte and adherent cell supernatants, appropriate standards, and controls were incubated in 96 well enzyme-linked immunosorbent assay plates coated with hamster monoclonal anti-murine-TNF antibody (Genzyme, Boston, MA). The plates were washed and incubated sequentially with rabbit polyclonal anti-murine TNFα antibody (Genzyme) and alkaline phosphatase-conjugated goat anti-rabbit Immunoglobulin G (Boeh-

ringer Mannheim, Indianapolis, IN). The alkaline phosphatase-linked antibody then was detected at 405 nm after incubation with 100 μL of the phosphatase substrate p-nitrophenyl phosphate disodium (1 mg/mL; Sigma, St. Louis, MO). The lower limit of sensitivity of the assay was 25 pg/mL. Supernatants were tested for IL-1 using a CTLL-2/NOB-1 bioassay.[23] Briefly, supernatants were added to 96 well plates in 100 μL serial dilutions from 1:2 to 1:128, followed by $1 \times 10^5$ NOB-1 cells and $5 \times 10^3$ CTLL-2 cells in 50 μL volume each. Plates were incubated for 20 hours at 37 C in the presence of 5% $CO_2$, pulsed with 1 μCi/well $^3$HTdr, and incorporation of $^3$HTdr was determined using a liquid scintillation counter. Interleukin-1 was then calculated using probit analysis (courtesy of Dr. Brian Davis).

### Statistical Analysis

Results are expressed as means ± standard error. Statistical analysis was performed by analysis of variance (ANOVA) for multiple comparison. The analysis was done using a commercial statistical package (Stat-View; Abacus Concepts, Inc., Berkeley, CA) on a Macintosh computer (Apple Computer, Inc., Cupertino, CA). The *post hoc* test used was the Fisher prognostic least significant difference analysis. Significance was defined as a type error of less than 0.05.

## RESULTS

### Cell Count Per Spleen

The number of cells per spleen in the phx-sal and phx-chol groups, $2.71 \times 10^8 \pm 0.38 \times 10^8$ and $1.94 \times 10^8 \pm 0.24 \times 10^8$, respectively, was significantly less than that in either the sh-sal or sh-chol group, $6.17 \times 10^8 \pm 0.65 \times 10^8$ and $5.48 \times 10^8 \pm 0.41 \times 10^8$, respectively ($p = 0.0001$; Fig. 2). This number was not significantly different between the phx-sal and the phx-chol animals or between the sh-sal and sh-chol animals. In addition, the number of splenocytes tended to be less in the cholestyramine-treated groups than those of the saline-treated groups, however, this difference was not statistically significant.

### Proliferative Response to T-Cell Mitogens After Partial Hepatectomy

In the phx-sal animals, the mean response to PHA (14,358 ± 7272 cpm) was significantly less than that of the sham-sal animals (80,141 ± 24,572 cpm, $p < 0.05$) (Fig. 3). Pretreatment with cholestyramine prevented this suppression of the splenocyte response to PHA in the rats treated with partial hepatectomy (145,314 ± 39,657 cpm; $p < 0.01$ *vs.* phx-sal). Cholestyramine-treated sham rats showed a slight but not significant ($p = 0.07$) increase in the response to PHA (148,355 ± 22,816 cpm) as compared with the sh-sal group.

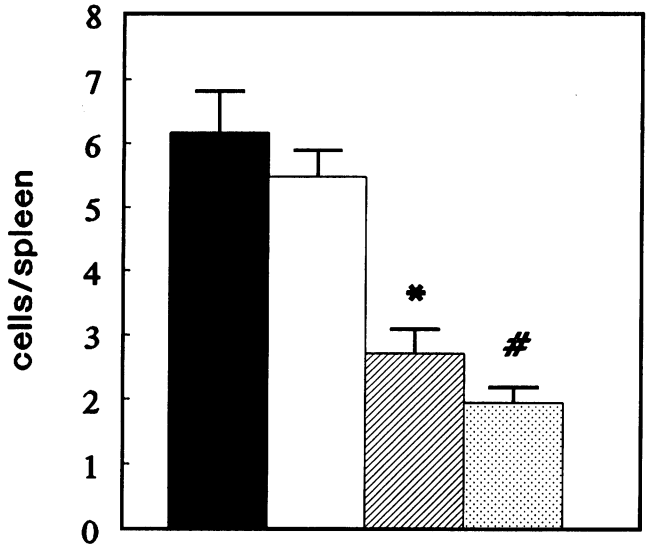


**Figure 2.** Number of cells per spleen of the treatment and control groups 24 hours after either partial hepatectomy or sham operation (closed bars, sh-shal; open bars, sh-chol; hatched bars, phx-sal; dotted bars, phx-chol). *p = 0.0001, phx-sal *vs.* sh-sal and sh-chol; #p = 0.0001, phx-chol *vs.* sh-sal and sh-chol (ANOVA).

Partial hepatectomy had no effect on the splenocyte response to Con A (phx-sal: 230,905 ± 4685 cpm; sh-sal: 242,198 ± 43,424 cpm) (Fig. 4). Cholestyramine treatment caused a slight increase of this response in both sham (sh-chol: 289,311 ± 12,607 cpm; difference with sh-sal not significant) and partial hepatectomy groups (phx-chol: 285,406 ± 25,925 cpm; $p < 0.05$ *vs.* phx-sal).

### Proliferative Response to the B-Cell Mitogen LPS After Partial Hepatectomy

The mean splenocyte response to the B-cell mitogen LPS (4118 ± 848 cpm) was significantly less after partial hepatectomy than after a sham operation (8804 ± 2439 cpm, $p < 0.05$) (Fig. 5). Cholestyramine pretreatment in phx rats prevented this decrease (8308 ± 1536 cpm, $p < 0.05$ compared with the phx-sal group). There was no difference in responsiveness to LPS between splenocytes from sh-sal (8804 ± 2439 cpm) and sh-chol animals (10,990 ± 1510 cpm).

### Splenocyte Production of IL-2

Interleukin-2 production in response to Con A was increased in splenocytes from phx-sal rats (5.16 ± 0.67 units/mL) as compared with the sh-sal group (2.30 ±

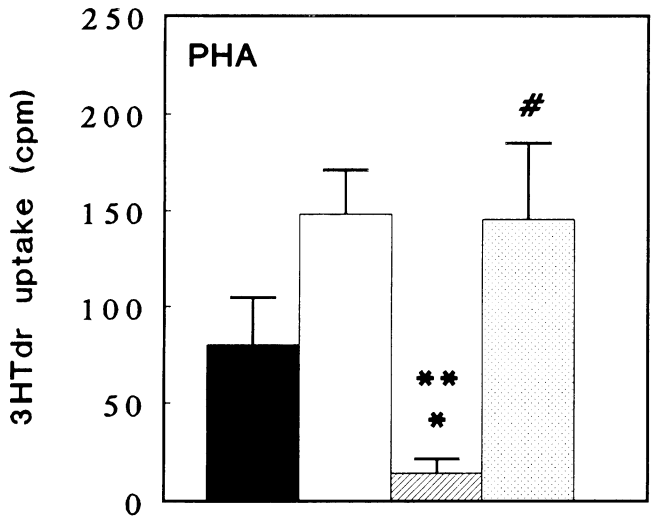


**Figure 3.** Proliferative response of splenocytes of each treatment group to phytohemagglutinin as measured by the uptake of $^3$HTdr and expressed in counts per minute (cpm) (closed bars, sh-sal; open bars, sh-chol; hatched bars, phx-sal; dotted bars phx-chol). Of each spleen, 2.5 × $10^5$ splenocytes were incubated with 6.25 μg/well of PHA. *p < 0.05, phx-sal *vs.* sh-sal; **p < 0.0001, phx-sal *vs.* sh-chol; #p < 0.01, phx-chol *vs.* phx-sal (ANOVA).

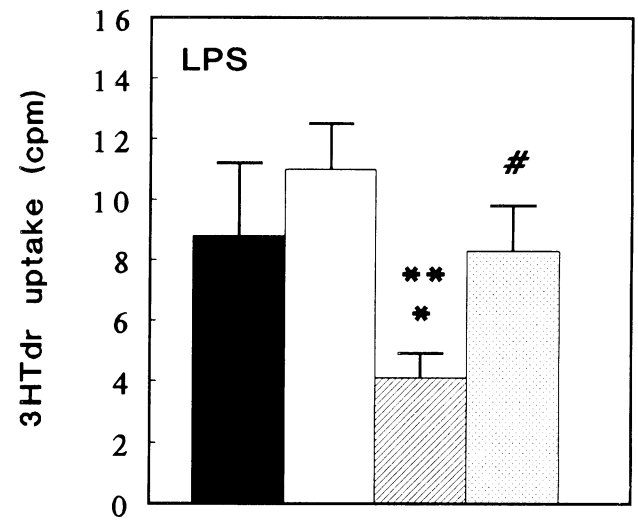


**Figure 5.** Proliferative response of splenocytes of each treatment group to lipopolysaccharide as measured by uptake of $^3$HTdr and expressed in cpm (closed bars, sh-sal; open bars, sh-chol; hatched bars, phx-sal; dotted bars, phx-chol). Of each spleen 2.5 × $10^5$ splenocytes were incubated with 0.5 μg/well LPS. *p < 0.05, phx-sal *vs.* sh-sal; **p < 0.001, phx-sal *vs.* sh-chol; #p < 0.05, phx-chol *vs.* phx-sal (ANOVA).

0.78 units/mL, p < 0.05) (Fig. 6). This increase was reversed in the phx-chol group (1.57 ± 0.35 units/mL, p < 0.001 *vs.* phx-sal), which was almost equivalent to the sh-sal group. Sham-operated rats responded to some extent to cholestyramine treatment, as demonstrated by a slightly reduced IL-2 production (0.86 ± 0.40 units/mL) compared with sh-sal rats, although this difference was not statistically significant.

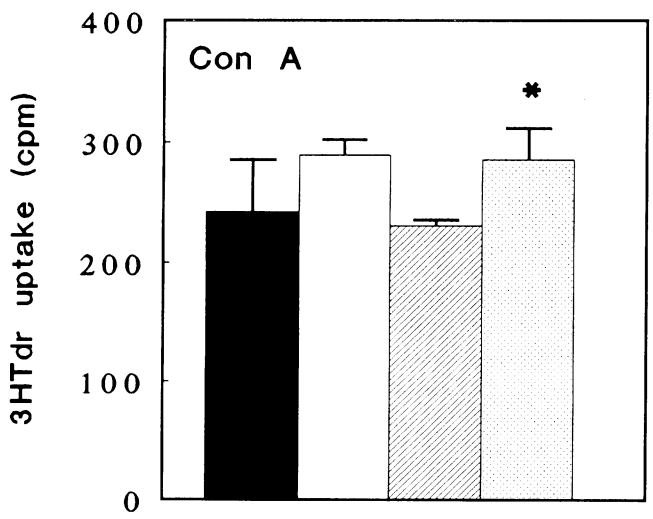


**Figure 4.** Proliferative respone of splenocytes of each treatment group to concanavalin A as measured by the uptake of $^3$HTdr and expressed in cpm (closed bars, sh-sal; open bars, sh-chol; hatched bars, phx-sal; dotted bars, phx-chol). Of each spleen, 2.5 × $10^5$ splenocytes were incubated with 0.4 μg/well Con A. *p < 0.05, phx-chol *vs.* phx-sal (ANOVA).

## IL-1 Production by LPS-Stimulated Splenocytes and Adherent Splenocytes

After partial hepatectomy, the IL-1 release from LPS-stimulated adherent splenocytes was significantly less than that after a sham procedure (p < 0.01, Table 1).

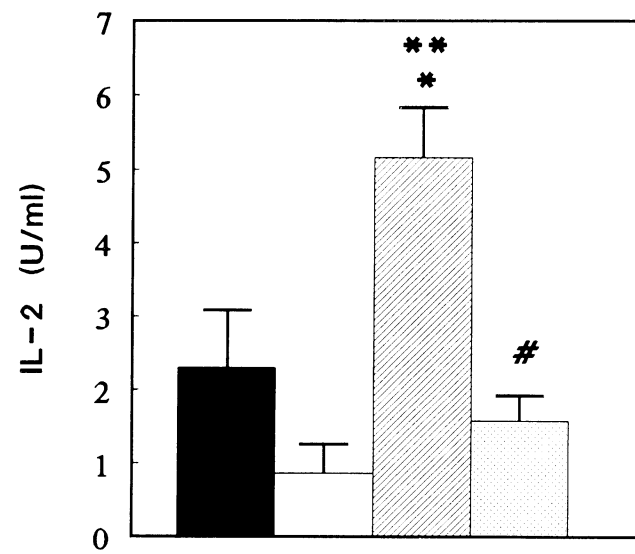


**Figure 6.** *In vitro* production of IL-2 by Con A-stimulated splenocytes of each treatment group, expressed in U/mL (closed bars, sh-sal; open bars, sh-chol; hatched bars, phx-sal; dotted bars, phx-chol). Of each spleen, 2.5 × $10^5$ splenocytes were incubated with or 0.4 μg/well Con A. *p <0.05, phx-sal *vs.* sh-sal; **p < 0.0005, phx-sal *vs.* sh-chol; #p < 0.001, phx-chol *vs.* phx-sal (ANOVA).

**Table 1. IL-1 PRODUCTION BY SPLENOCYTES AND ADHERENT CELLS**

| Groups | Splenocytes | Adherent Cells |
|---|---|---|
| Sham-saline | 2.26 (0.23) | 0.57 (0.18) |
| Sham-cholestyramine | 1.64 (0.34) | 1.06 (0.27) |
| Partial hepatectomy-saline | 1.08 (0.13)* | 0.07 (0.03)†‡ |
| Partial hepatectomy-cholestyramine | 2.19 (1.21) | 0.23 (0.07)§‖ |

Values are expressed as mean ± SEM in U/mL.
Statistical analysis (ANOVA):
* Sham-saline *vs.* partial hepatectomy-saline, $p < 0.005$;
† Sham-saline *vs.* partial hepatectomy-saline, $p < 0.01$;
‡ Sham-cholestyramine *vs.* partial hepatectomy-saline, $p < 0.005$;
§ Partial hepatectomy-cholestyramine *vs.* partial hepatectomy-saline, $p < 0.05$;
‖ Sham-cholestyramine *vs.* partial hepatectomy-cholestyramine, $p < 0.05$.

Cholestyramine pretreatment of phx rats prevented this decrease ($p < 0.05$ *vs.* phx-sal). Animals treated via partial hepatectomy-saline showed a significantly lower IL-1 production by total splenocytes compared with sh-sal animals ($p < 0.001$, Table 1). This decreased IL-1 production was restored to sham levels in the phx-chol group. However, this difference just failed to reach statistical significance because of a great variance of the measured variable in the phx-chol group ($p = 0.07$).

## TNF Production by LPS-Stimulated Splenocytes and Adherent Splenocytes

In both total splenocyte and adherent splenocyte cultures of phx-sal rats, a profoundly decreased production of TNF was found compared with that of sham-operated animals ($p < 0.0001$ and $p < 0.05$, respectively) (Table 2). This decrease in *in vitro* TNF release was not found in phx-chol rats. In this group, the production of TNF was restored to near sham levels, resulting in TNF levels in splenocyte cultures that were significantly different from phx-sal rats ($p < 0.05$). However, in adherent cell cultures, the measured variable did not reach statistical significance because of a great variance in the phx-chol group. Cholestyramine had no effect on the TNF production of sham-operated rats.

## DISCUSSION

During the past decade, many studies have shown a compromised functioning of the host immunologic defenses after trauma or major surgery.[8–13] These disturbances of the immune system may influence the outcome or postoperative course after extensive surgical procedures because an adequate resistance to infection is indispensable at the very time the patient is maximally at risk from the procedure itself. Liver surgery should be distinguished from other major surgical procedures because of the important role of this organ in the clearance of bacterial particles such as endotoxins. Up to 80% and 90% of systemic phagocytic capacity consists of the phagocytic cells lining the liver sinusoids, the Kupffer cells. In spite of a compensatory increase in splenic and lung phagocytic clearance in case of a decrease of the hepatic phagocytic function, removal of circulating particles still may be significantly decreased.[24–26] We have shown that liver resection in rats severely impairs cellular immune functions, which can be prevented by pretreating the animals with cholestyramine, an endotoxin-binding agent.

Preservation of the integrity of the hepatic phagocytic system is essential to protect the host against gut-derived endotoxins. Small amounts of these endotoxins continuously cross the intestinal barrier, and are subsequently detoxified by the Kupffer cells.[27] This phenomenon may be important for maintenance of their normal phagocytic capacity by a repetitive stimulation.[28] However, too high levels of endotoxin in the portal vein may result in a transient decrease in phagocytic capacity of the Kupffer cells, possibly mediated by a decreased activity of C3 receptors.[29] Callery et al. also emphasized the clinical importance of an intact phagocytic system of the liver, demonstrating that blockade of this system before injury was associated with systemic endotoxemia and an increased early mortality.[30] Thus, a decreased number of Kupffer cells after a major liver resection will facilitate leakage of translocated gut-derived endotoxins into the systemic circulation and will predispose patients to postoperative infectious complications and subsequent organ failure.

In a previous study, we demonstrated that enteral administration of cholestyramine prevented hepatic failure, catabolism, and even mortality after a major liver resection.[17] Cholestyramine is a potent endotoxin-binding agent.[31,32] In agreement herewith, we observed that the beneficial effects of cholestyramine coincided with

**Table 2. TNF PRODUCTION BY SPLENOCYTES AND ADHERENT CELLS**

| Groups | Splenocytes | Adherent Cells |
|---|---|---|
| Sham-saline | 472.3 (137.6) | 232.9 (118.6) |
| Sham-cholestyramine | 307.8 (24.9) | 192.2 (46.1) |
| Partial hepatectomy-saline | 27.2 (4.0)*† | 26.2 (2.0)§ |
| Partial hepatectomy-cholestyramine | 214.7 (112.2)‡ | 167.2 (110.1) |

Values are expresed as mean ± SEM in pg/mL.
Statistical analysis (ANOVA):
* Sham-saline *vs.* partial hepatectomy-saline, $p < 0.0001$;
† Sham-cholestyramine *vs.* partial hepatectomy-saline, $p < 0.0001$;
‡ Partial hepatectomy-cholestyramine *vs.* partial hepatectomy-saline, $p < 0.05$;
§ Sham-saline *vs.* partial hepatectomy-saline, $p < 0.05$.

significantly decreased endotoxin levels,[17] suggesting that endotoxins of gut origin very likely play a pivotal role in the pathogenesis of detrimental alterations after liver surgery. Systemic endotoxemia also can impair cellular immune functions.[14,15] Thus, the results of the present study showing that cholestyramine prevents the derangements of host cellular immune functions after major liver resection, most likely because of prevention of spillover of gut-derived endotoxins into the systemic circulation.

We chose to evaluate the immune state of the splenic lymphoid compartment for several reasons. First, this compartment has been shown to reflect the host immune responsiveness in different models of traumatic injury.[8–14,33] Second, splenic macrophages, in contrast to Kupffer cells, do not normally encounter endotoxin, and it is most conceivable that changes in activation state of splenic macrophages are caused by circulating endotoxins, as seen after liver resection. As we have demonstrated in this study, the number of splenocytes decreases by more than 50% in response to a partial hepatectomy, which was not influenced by cholestyramine pretreatment. Splenic monocytes and macrophages may be recruited to the liver to restore the reduced Kupffer cell population. In addition, we found a suppression of some functional properties of both B and T lymphocytes after partial hepatectomy, as demonstrated by reduced proliferative responses to the mitogenic stimuli PHA and LPS. These results are in agreement with those reported in other studies of host injury, such as multitrauma, thermal injury, endotoxemia, general surgical injury, and partial hepatectomy.[8–13,16] This suppression did not occur in rats pretreated with enteral cholestyramine, suggesting some role of endogenous endotoxins in this process. Although not statistically significant, for rats in the sham-operated group, cholestyramine also tended to have an enhancing effect on proliferative responses to PHA, Con A, and LPS. A low-grade endotoxemia evoked by a sham operation, to some extent, may have induced immunosuppression and, therefore, was influenced by cholestyramine. However, comparison of the proliferative responses of sham-operated animals with unmanipulated animals that did not receive sham or liver surgery revealed an increased responsiveness in the sham group (data not shown). It is conceivable that cholestyramine elicits an additional stimulatory effect on immunocompetent cells independent of an endotoxin-binding pathway.

In contrast to most, if not all, other models for immunosuppression after surgery, IL-2 production after partial hepatectomy was enhanced rather than diminished. Because IL-2 can be consumed by the cultured lymphocytes, IL-2 levels measured represent the balance between T-cell production and consumption by both B and T cells in this mixed culture. A decreased proliferative capacity of the added cells could shift the production-consumption balance of IL-2 to a higher net production. However, no significant differences in the proliferative responses to Con A were found between the sh-sal and phx-sal groups. Therefore, we conclude that the synthesis of IL-2 by the spleen cells was enhanced after hepatectomy. Interleukin-2 stimulates natural killer cell activity and induces the generation of lymphokine-activated killer cell activity.[34] Our data show that IL-2 induced cytotoxic activity may be upregulated in the early phase. Downregulation of IL-2 production by cholestyramine treatment may be undesirable because of inhibition of antitumor activity. However, studies on cytotoxicity and tumor growth after partial hepatectomy are contradictory; in some studies, an increased cytotoxic activity of effector lymphocytes was found, inducing protection against tumor cells,[35,36] whereas in others, this activity was found to be decreased.[16] In the latter study, the authors concluded that immunologic modification after liver resection may be responsible for the accelerated growth of liver tumor. Our data do not allow conclusions with respect to the antitumor effect because the cytotoxic activity was not examined.

We found a reduced production of TNF and IL-1 by the total splenocyte population and by isolated splenic adherent cells after major liver resection. Kupffer cells, 24 hours after a similar liver resection, also show a markedly decreased capacity to produce TNF in response to LPS stimulation, as compared with Kupffer cells from sham-operated animals.[37] Also, other immunosuppressive states induced by host injury or experimental endotoxemia are accompanied by a decreased production of IL-1.[15,19] The enhanced production of TNF in the sham-operated rats probably reflected a physiologic state of activation of mononuclear cells after a moderate surgical trauma. Cholestyramine pretreatment of rats undergoing partial hepatectomy maintained the ability of mononuclear phagocytes to produce and release TNF at a similar level as in the sham-operated animals. Thus, enteric-derived endotoxins appear to be of major importance in the pathogenesis of a diminished immune responsiveness after hepatectomy, even in distant lymphoid compartments. The great variance of LPS-induced TNF and IL-1 production that was seen in the cholestyramine-treated hepatectomized rats may reflect a variance in efficacy of the treatment. Differences in the amount of enteric endotoxin or variance in the amount of cholestyramine that reaches its target could very well influence its efficacy.

We can only speculate about the precise mechanisms that underlie the observed state of immunosuppression in this model. Evidently, an excessive amount of endotoxin that cannot be adequately detoxified by the re-

sected liver exerts several local and systemic effects through activation of various inflammatory cascade systems. These processes are known to be initiated within hours after the endotoxin challenge. After a lethal *E. coli* challenge in baboons, plasma TNF$\alpha$ and IL-1 production peaked at 45 and 90 minutes, respectively.[38,39] Recently, we measured elevated systemic TNF levels as early as 2 hours after a 70% hepatectomy in rats, suggesting a rapid activation of mediator systems (M. A. Boermeester, unpublished data). High circulating levels of cytokines induce tolerance for LPS both *in vivo* and *in vitro*.[40–43] Thus, the cellular derangements observed at 24 hours after the partial hepatectomy may be related to tolerance induced by endotoxins or cytokines during the first hours after the operation. The remaining Kupffer cells may be essential for this tolerance induction because others have demonstrated that impaired immunity can be restored by removal of macrophages.[44,45] Through what mechanism macrophages elicit an immunosuppressive effect is not fully understood at this moment, although prostaglandin $E_2$, a product of activated macrophages, is considered a responsible mediator.[46–48] Alternatively, the immunosuppressive cytokine IL-10 may add as well in this respect.[49]

Counter-reactive downregulation of cytokine production, referred to as immunosuppression or hyporesponsiveness, may exert a pathogenetic rather than a protective effect during an inflammatory response. For example, impaired cellular immune functions correlate with lethal or nonlethal complications.[8,13,50,51] Furthermore, Marshall et al. demonstrated that immunosuppression after an infusion of heat-killed bacteria correlated with the number of micro-organisms extracted by the hepatic Kupffer cells.[52,53] The preservation of immune functions we found in cholestyramine-treated rats coincided with an increased survival in this group, as described previously,[17] which demonstrates that this preservation is beneficial. In addition, we demonstrated in a previous study that cholestyramine prevents systemic endotoxemia after partial hepatectomy in rats.[17] Thus, these results stress the relation between endotoxin, immunosuppression, and a fatal outcome after liver resection. We postulate that activation of hepatic and circulating mononuclear phagocytes by gut-derived endotoxins induces an overshoot of cytokine production with subsequent downregulation of this production. It is most conceivable that this endotoxin-induced immunosuppression may play an important role in the postoperative course because potential beneficial responses also may be downregulated. Preoperative binding of endogenous endotoxins by enteral cholestyramine, before a period during which an important natural defense mechanism such as the hepatic phagocytic system is impaired, may well protect the host against the initiation of the trigger mechanism leading to an immunosuppressive state. Whether this approach can be applied to humans remains to be established.

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