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THE SEPTIC ABSCESS WALL: A CYTOKINE-GENERATING ORGAN ASSOCIATED WITH PORTAL VENOUS CYTOKINEMIA, HEPATIC OUTFLOW FIBROSIS, SINUSOIDAL CONGESTION, INFLAMMATORY CELL SEQUESTRATION, HEPATOCELLULAR LIPID DEPOSITION, AND FOCAL CELL DEATH

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ABSTRACT—An acute septic inflammatory response with access to the portal circulation was created in a rat model using an intra-abdominal abscess composed of a sterile agar pellet, or one contaminated with 10^2 *Escherichia coli (E. coli)* and 10^9 *Bacteriodes fragilis (B. fragilis).* After 3 days postimplantation, a well-formed intra-abdominal abscess occurred whose wall showed IL-6 DNA by PCR and IL-6 mRNA by *in situ* hybridization. Portal venous blood draining into the liver from the intra-abdominal abscess had increased levels of TNF-α, IL-1β, and IL-6 in both sterile and septic groups compared with a control normal animal group. Increased levels of these cytokines were also found in suprahepatic inferior vena caval blood, but were correlated with the higher portal vein levels, suggesting a gradient from abscess wall to portal vein into the systemic circulation via the liver. Liver histology demonstrated sinusoidal congestion centering on the central vein, growing worse with progression from normal in control, to sterile, to septic. Similarly, the degree of intrahepatic myeloperoxidase-positive inflammatory cell infiltration and hepatocellular lipid deposition and apoptosis also increased from control, to sterile, to septic. Gene expression by *in situ* hybridization demonstrated a significant increase in IL-6 and fibrinogen mRNAs in cells surrounding the central vein in sterile and septic animals, being greatest in animals with sepsis, associated with an increased deposition of collagen in the central vein area, most prominent in the septic liver. The pericentral vein cells with IL-6 and fibrinogen mRNA increases paralleled the increases in cells containing IL-6 and fibrinogen mRNAs in the abscess walls of sterile and septic animals, respectively. The data suggest that an intra-abdominal abscess, especially when contaminated with gram-negative bacteria, induces mRNA-generated cytokine responses in the abscess wall that are related to increased portal venous levels of the inflammatory cytokines TNF-α, IL-1β, and IL-6 perfusing the liver. These, in turn, induce localized production of IL-6 and fibrinogen mRNAs in cells at the central vein area with resultant outflow fibrosis and increased inflammatory cell sequestration within the liver lobular sinuses. This is associated with a generalized inflammatory response and intrahepatic portal sinusoid congestion. There is also increased hepatocellular lipid deposition and apoptosis. Thus, the cytokine production of the abscess wall itself appears to be a major mediator of the septic hepatic response.

KEYWORDS—Sepsis, systemic inflammatory response syndrome, hepatic organ failure, intra-abdominal abscess, chronic inflammation, tumor necrosis factor, interleukin-1, interleukin-6, acute phase protein, fibrinogen, hepatocellular apoptosis

INTRODUCTION

Severe injury occurring in the absence of infection produces a systemic inflammatory response syndrome (SIRS) (1). Sepsis is the systemic inflammatory response of the host to infection, and may occur after major trauma or surgery. It is not a disease but a clinical syndrome, and hepatic failure is a common manifestation of septic decompensation and the resultant multiple organ failure syndrome (2).

The local inflammatory response to an initially localized intra-abdominal infectious process in the normal sterile peritoneum is called intra-abdominal sepsis, in which endotoxin containing gram-negative bacilli, *Escherichia coli*, and anaerobes, *B. fragilis*, are among the most frequent causes (2, 3). Proinflammatory cells, mainly activated macrophages, are among the prime organizers of SIRS and are responsible for most of the cellular and molecular pathophysiology of sepsis/SIRS by producing cytokines and other proinflammatory molecules (4, 5). Studies have shown that the peritoneal levels of cytokines are higher than the levels measured systemically during an intra-abdominal septic process (5), which suggests that the peripheral blood cytokine levels represent only a portion of the host's cytokine response to a localized intra-abdominal process.

The liver is the central organ in whole-body metabolism, and is also an essential organ of natural immunity response through its production of acute-phase proteins, complement, and cytokines. It has a rich blood supply consistent with its important metabolic and immunomodulatory functions. The acute-phase response is one of the most important defense mechanisms of the organism, which tends to control a sterile or septic process introduced into the body. It controls these by focusing the local inflammatory host defense to eliminate or isolate the insult to avoid systemic dissemination. The acute-phase response also plays a key role in the alteration of the microvascular and metabolic physiology of the body to fight against various

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insults. Cytokines such as TNF-α, IL-1β, IL-6, IFN-γ, TGF-β, and IL-8 are all proinflammatory cytokines that activate the host defense response, but among them, IL-6 is the chief stimulator of the hepatic acute-phase protein response (6). Fibrinogen is one of the major acute-phase proteins produced in response to intra-abdominal sepsis and it contributes to coagulation, isolation of a septic focus, and supports the lattice functions needed for entrapment of foreign material thus enhancing macrophage ingestion and structure for collagen deposition and wound healing (5–7).

Although there are a large number of studies investigating sepsis in the literature, there is no specific treatment for the prevention or management of septic hepatic failure other than nonspecific supportive care, and sepsis is still the leading cause of death in intensive care units. The future treatment modalities of sepsis will depend on our understanding of the underlying pathophysiological mechanisms. To study and define the septic process and to develop effective treatment modalities, we need animal models that mimic the course of sepsis in patients. Most of the current animal models give information only about the acute-phase of sepsis and are incapable of inducing the entire process, including the later chronic metabolic phase of the host defense response that we usually see in patients (4–7).

For this reason, we used a well-established, reliable, and highly reproducible chronic septic rat fecal-agar pellet model (8–15). This model has been previously shown to follow a similar course of sepsis as seen in patients (16), and is able to give information about not only the acute hyperdynamic phase (8), but also the chronic hypercatabolic phase (12–15) and metabolic fuel-energy shift, as well (9, 14, 15). By using a sterile abscess as one type of control, this rat model has the advantage of allowing the study of SIRS with and without the addition of a septic process, and also enables these states to be compared with a noninflammatory control condition. The aim of this study was to characterize the immunopathophysiologic and cytohistologic mechanisms of the hepatic inflammatory response to a sterile versus a septic intra-abdominal abscess.

MATERIALS AND METHODS

Experimental design and the chronic septic rat fecal-agar pellet model

The chronic septic rat fecal-agar pellet model is a well established, highly reproducible, and reliable animal model (8–15) that mimics the clinical picture of sepsis and SIRS seen in patients (7, 16).

Sprague-Dawley rats were randomly assigned into three groups, namely; control, sterile abscess, and septic abscess groups. The control group was anesthetized but did not undergo laparotomy, whereas sterile and septic fecal-agar pellets were implanted into the abdominal cavity of the sterile and septic abscess groups, respectively. A 98-animal trial using a range of *E. coli* concentrations was done to establish the initial concentration:mortality relationship. From this trial, a study group of 30 septic animals in the 65% to 75% mortality range was established in which the septic fecal-agar pellets had been inoculated with 10^2 CFU/pellet of *E. coli* and 10^9 CFU/pellet of *B. fragilis* before implantation. All surviving animals (sterile and septic) were sacrificed 72 h after pellet implantation, when the intra-abdominal abscess wall was fully formed and when our previous studies in this model have shown that the IL-6 levels in the right heart mixed venous blood are at their highest levels (17, 18). Animal procedures were conducted in accordance with National Institutes of Health guidelines and were approved by the New Jersey Medical School Institutional Animal Care Committee.

Tissue morphology

Tissue sections of abscess wall, liver, and small intestine were prepared from three animals in each group (control, sterile abscess, and septic abscess) and the histology slides were examined under the microscope. Positive cells were counted in hematoxylin and eosin (H&E), myeloperoxidase, Oil Red O for lipids, and *in situ* hybridization slides in nine different 100× power fields per slide for each animal (i.e., 27 fields per determination for each type of staining; biochemical, enzymatic, and *in situ* hybridization) in each experimental group. All slides for a given histochemical or *in situ* hybridization determination were done as a single batch and were examined in a blinded fashion. The results were statistically analyzed using one-way analysis of variance (ANOVA) for multiple groups as described in below.

H&E stain was used to evaluate tissue morphology and to obtain information about the apoptosis and overall tissue structure allowing the various cell types and tissue components to be distinguished. The technique of Guan (19) was used to identify apoptotic cells. Myeloperoxidase stain (Sigma Diagnostics Peroxidase kit) was used (procedure no. 391) to measure inflammatory cell infiltration of tissues and to differentiate the various inflammatory cells in cryosectioned tissue samples. The Oil Red O method for neutral fats was used to show the location and magnitude of lipid deposition in the liver. Gomori's one-step trichrome stain was used to identify an increase in collagenous connective tissue fibers in the abscess wall and in liver.

In situ hybridization

Ambion's mRNA *locator*-Hybridization kit and mRNA *locator*-Biotin Detection kit for *in situ* hybridization (catalog no. 1805) was used for the analysis and localization of specific mRNA expression within cells or tissues. IL-6 and fibrinogen oligo-RNA probes were used to localize positive cells in the abscess wall, liver, and small intestine sections.

Polymerase chain reaction (PCR)

The 3′-RACE System GIBCO BRL (Life Technologies, Rockville, MD) was used to amplify the number of copies of IL-6 DNA in the abscess wall to produce enough DNA to be adequately detected.

Enzyme-linked immunosorbent assay (ELISA)

The BioSource Cytoscreen rat tumor necrosis factor-α (raTNF-α), raIL-1β, and raIL-6 solid phase sandwich kits were used for *in vitro* quantitative determination of TNF-α, IL-1β, and IL-6 in rat serum.

Statistical analyses

All data was analyzed using one-way ANOVA, and the means of the experimental groups were compared using Tukey's honest significant difference test (Tukey-Kramer HSD) using the JMP version 3.2 statistical software (SAS Institute Inc., Cary, NC). An alpha value of 0.05 was used in all statistical analyses, and P values were considered as being statistically significant at or below the alpha value of 0.05.

RESULTS

The effects of intra-abdominal septic and sterile abscesses on mortality rate and morbidity

Control, sterile, and septic abscess rat groups were observed during the postoperative period in terms of mortality and weight changes. There was no mortality in the control or sterile abscess groups. In the seven batches of animals (total 98 rats) used to establish the relation of the initial *E. coli* count to the early mortality, all of the mortality was seen in the first 36 h (acute phase). The septic mortality rate was directly and significantly correlated with the initial *E. coli* count in the abscess pellet implantation (septic mortality rate = 10.5 + 0.43 *E. coli* count, $P < 0.0004$; Fig. 1A), whereas *B. fragilis* had no role in the mortality. Surviving animals from the three batches of septic abscess animals (total of 30 rats), which had 60% to 75% early mortality rate (average 70%), were sacrificed at 3 days postabscess pellet implantation. Their blood cytokines were assayed and the abscess wall tissues and organs were analyzed by histologic, cytochemical, and *in situ* hybridization studies, which compared the responses of the control (n = 3), sterile (n = 7), and surviving septic (n = 9) abscess animals. Both the sterile and the septic abscess groups lost statistically significant amounts of weight after the pellet implantation

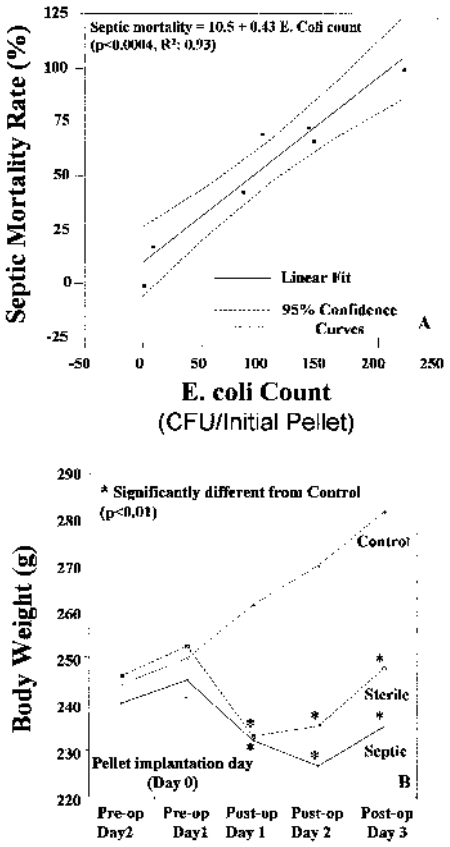

FIG. 1. **(A) Linear regression analysis of septic mortality rates by *E. coli* count (each point represents a batch of 10 to 17 animals with sepsis).** (B) Weight changes of the control, sterile, and surviving 70% mortality septic abscess groups after pellet implantation surgery. A total of 98 animals with sepsis divided into seven groups based on the concentration of *E. coli* in implanted pellet. There were 30 animals total in the three groups with sepsis with an average of 70% mortality that were used in this study.

surgery when compared with the anesthesia-only control group, but there was no significant difference in the weight loss or weight recovery response between the sterile and the septic groups in any given postoperative period (Fig. 1B). These data are consistent with those previously reported for this preparation (8, 9, 20, 21), demonstrating the reproducibility of the model over a 17-year period of study.

The intra-abdominal septic and sterile abscesses: the anatomy, histology, and pathophysiology of abscess development

The gross anatomy of the intra-abdominal abscess development—The gross anatomy of the intraperitoneal cavity of the control (Fig. 2A), the sterile abscess (Fig. 2B), and the septic abscess (Fig. 2C) animals on postoperative day 3 (chronic phase) are shown. Both the sterile and the septic abscess groups had signs of diffuse peritonitis (vasodilation, edema, and adhesions) and well-formed abscesses when compared with the control group. The only gross difference between the sterile and the septic groups was that the abscess wall was much thicker in the septic group.

H&E stain of the intra-abdominal abscess wall—H&E stain of the sterile (Fig. 2D) and the septic abscess (Fig. 2E) walls showed an increased amount of inflammatory cell infiltration surrounding the necrotic abscess cavity, mainly polymorpholeukocytes. There was no marked difference in the quantity of the inflammatory cells between the two groups.

Detection of IL-6 DNA and mRNA gene expression in the intra-abdominal abscess wall—The presence of IL-6 gene DNA in the sterile and septic abscess walls was confirmed by the PCR technique, which amplifies the small quantities of DNA to achieve detectable levels. The specific locations of

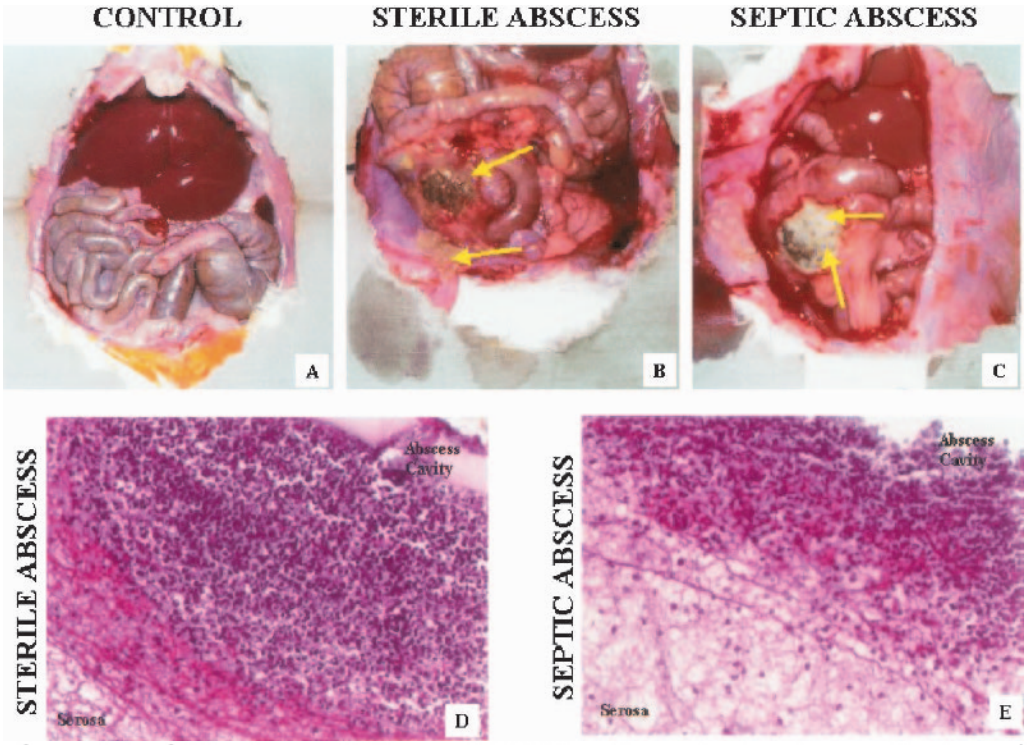

FIG. 2. **The gross anatomy of the intraperitoneal cavity of the control (A), the sterile abscess (B), and the septic abscess (C) groups on postoperative day 3 (chronic phase).** Abscess walls are shown by arrows. Figure also shows the H&E stain of the sterile (D) and the septic (E) abscess walls in 200× magnification. Abscess cavities and serosal layers are shown.

IL-6-producing cells in the sterile and septic abscess walls were delineated by the *in situ* hybridization technique, which detects the sites of mRNA gene expression (Fig. 3). The IL-6 mRNA-positive cells were found in discrete clusters in specific locations in the sterile and septic abscess walls. However, there was no significant difference in the quantity of IL-6 mRNA positive cells between the two abscess groups (Table 1).

The IL-6 mRNA-positive cells in the sterile abscess tended to lie more toward the serosal surface of the abscess near the adherent bowel (Fig. 3A), whereas in the septic intra-abdominal abscess, the IL-6 mRNA cells were congregated about the necrotic abscess cavity (Fig. 3C). As a result, the morphology of the IL-6 mRNA-containing cells is more distorted by the compression of the collagen fibers of the sterile

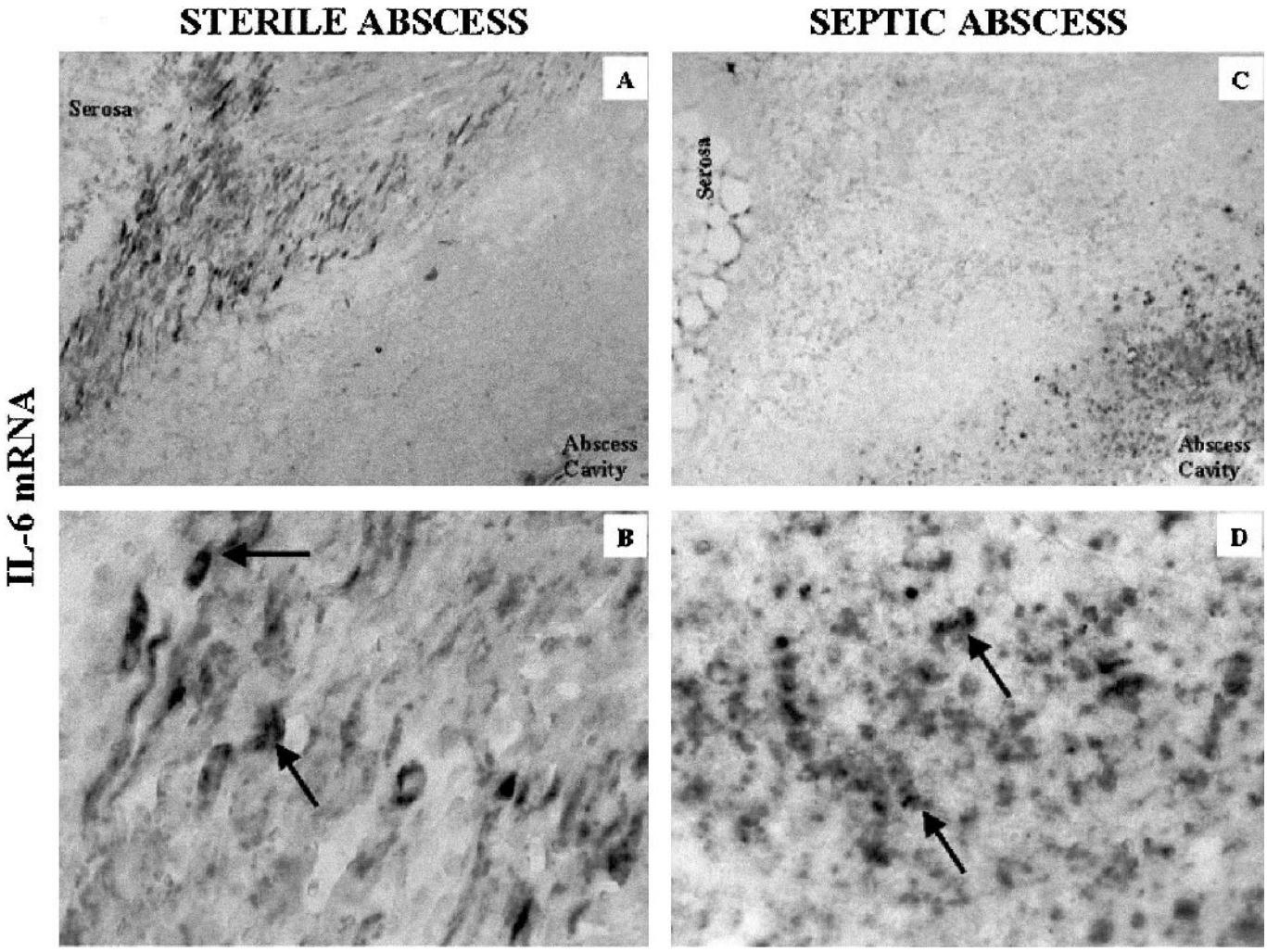

FIG. 3. ***In situ* hybridization of IL-6 mRNA in the sterile (A and B) and the abscess walls with sepsis (C and D).** Abscess cavities and serosal layers are marked, and IL-6 mRNA-positive cells are shown by arrows. A and C: 100×, B and D: 400× magnifications.

TABLE 1. **Abscess wall, intestine and liver. Mean number of IL-6 and fibrinogen mRNA, positive, myeloperoxidase, lipid-filled, and apoptotic cells per 100× power field**

Groups n = 3	Abs-IL-6 RNA	Abs-fibrinogen-RNA	Small intestine-IL-6 RNA	Liver-IL-6 RNA	Liver-fibrinogen RNA	Liver-lipid	Liver-myeloperoxidase	Liver-apoptosis
Control	No abscess	No abscess	14 ± 3	8 ± 2	4 ± 1	34 ± 6	51 ± 4	14 ± 1
Sterile	120 ± 9[#]	45 ± 7	48 ± 3*	47 ± 3*	18 ± 2*	223 ± 22*	119 ± 14*	59 ± 2*
Septic	140 ± 8[@]	65 ± 4	56 ± 4*	57 ± 3*	25 ± 3*	242 ± 21*	178 ± 5*[†]	66 ± 2*[†]
P value	Not significant	Not significant	0.0002	0.0001	0.001	0.004	0.0002	0.0001

P value shows significance of ANOVA simultaneously comparing differences between all three groups (control, sterile, and septic) by Tukey-Kramer honest significant difference test. *P* values that are less than the 0.05 threshold for significance demonstrate the additional degree of increased confidence that the difference shown is significant.
*Sterile and septic cell numbers significantly different from control cells.
[†]Septic cell numbers significantly different from sterile cells.
[#]IL-6, Sterile abscess mRNA cells greater than small intestine mRNA cells, $P < 0.02$.
[@]IL-6, Septic abscess mRNA cells greater than small intestine mRNA cells, $P < 0.003$.

abscess wall (Fig. 3B) than in those nearer the fluid filled necrotic center of the septic abscess (Fig. 3D).

Detection of IL-6 mRNA gene expression in the small intestine remote from intra-abdominal abscess—Examination of representative sections of small bowel near the jejunal-ileal junction, but remote from the abscess, revealed small numbers of IL-6 mRNA-positive cells in the Paneth cell areas of the crypts at the base of the intestinal villi in the sterile (Fig. 4B) and septic abscess animals (Fig. 4C). In contrast, the control animals showed only an occasional IL-6 mRNA-positive cell in these areas (Fig. 4A). The difference between control and the sterile or septic animals' small intestinal IL-6 mRNA-containing cells was significant ($P < 0.0002$, Table 1). However, using the method of randomly selected 100× power field analyses, the number of IL-6 mRNA-positive cells in the walls of the sterile ($P < 0.02$) and septic ($P < 0.003$) abscesses was significantly greater than the number of IL-6 mRNA-positive cells found in the intestinal crypts taken from the same group of animals. Because, as can be seen in Figure 2, the average size of an intra-abdominal abscess (about 3 cm in diameter) fills a very large portion of the peritoneal cavity, the total quantity of IL-6 mRNA-positive cells (120–140 per 100× power field) times the number of fields contained in the total volume of the abscess wall, which is 1- to 3-mm thick, would appear to be very large. They may even equal or exceed the magnitude of IL-6 mRNA-positive cells contained in the total number of crypt areas of the small intestine, considering that the IL-6 mRNA-positive crypt cells (48–56 per 100× power field) appear to be only 2% to 5% of the total number of cells in the single layer of mucosal cells of a given villus.

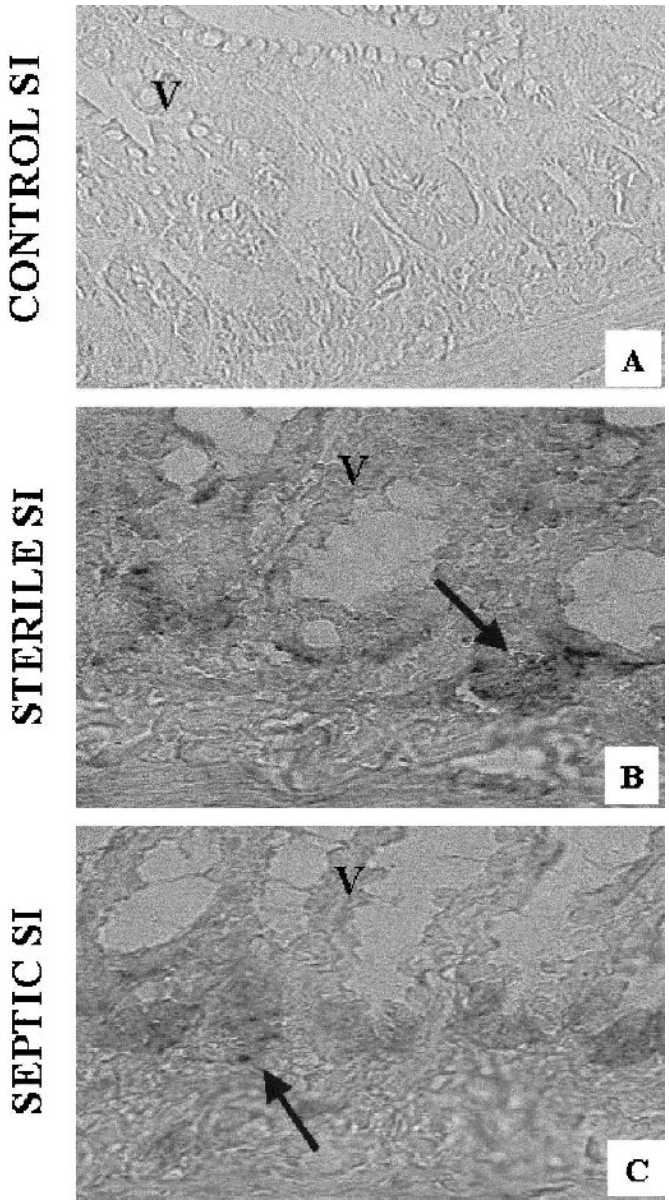

FIG. 4. ***In situ*** **hybridization of IL-6mRNA in the control (A), sterile (B), and the septic (C) small intestine remote from the abscess.** IL-6 mRNA-positive cells are shown by arrows. V, Villus. 100x magnification.

Detection of fibrinogen mRNA gene expression in the intra-abdominal abscess wall—Figure 5 shows the localization of fibrinogen gene mRNA expression in the sterile and the septic abscess walls, detected by *in situ* hybridization. There was no significant difference (Table 1) in terms of the quantity of fibrinogen-positive cells between the two groups, but in the septic abscess wall, the fibrinogen mRNA-positive cells were more concentrated than in the sterile abscess wall. However, the location of the fibrinogen mRNA-expressing cells showed a major difference between the two groups. The fibrinogen mRNA-expressing cells were located at the intermediate layer of the sterile abscess wall (Fig. 5, A and B), where the extracellular matrix and fibrous tissue were prominent, whereas in the septic animals, the fibrinogen mRNA-positive cells were concentrated in the inner part of the septic abscess wall (Fig. 5, C and D), where the inflammatory cell infiltration was most dominant. The location of fibrinogen mRNA-producing cells showed a close proximity to the IL-6 mRNA-positive cells in both the sterile and the septic abscess walls (Fig. 3).

Collagen deposition in the intra-abdominal abscess wall—Figure 6 shows the Gomori's One-Step Trichrome Stain of the abscess walls of the sterile and the septic groups, indicating the deposition of the collagenous connective tissue fibers in the abscess wall. The sterile and septic abscess walls showed a marked difference in the magnitude and localization of collagen deposition. The collagen fibers were deposited in a scattered pattern in the sterile abscess wall (Fig. 6A) and did not take much space compared with those in the septic abscess wall (Fig. 6B) where the collagen was deposited in a dense, concentrated pattern, taking up almost all of the space of the intermediate layer (extracellular matrix). The collagen in the septic abscess wall produced a thick layer starting from the intermediate layer and extending through to the serosal layer (Fig. 6B), which was consistent with the gross picture of the septic abscess wall (Fig. 2).

The effects of intra-abdominal septic and sterile abscesses on the portal vein and the suprahepatic inferior vena cava plasma cytokine (TNF-α, IL-1β, and IL-6) levels

ELISA was used to detect the circulating plasma TNF-α, IL-1β, and IL-6 cytokine levels in both the portal vein and the inferior vena cava for the control, sterile, and septic abscess groups. The sterile and the septic abscess groups had statistically higher amounts of the IL-6 cytokine both in the portal

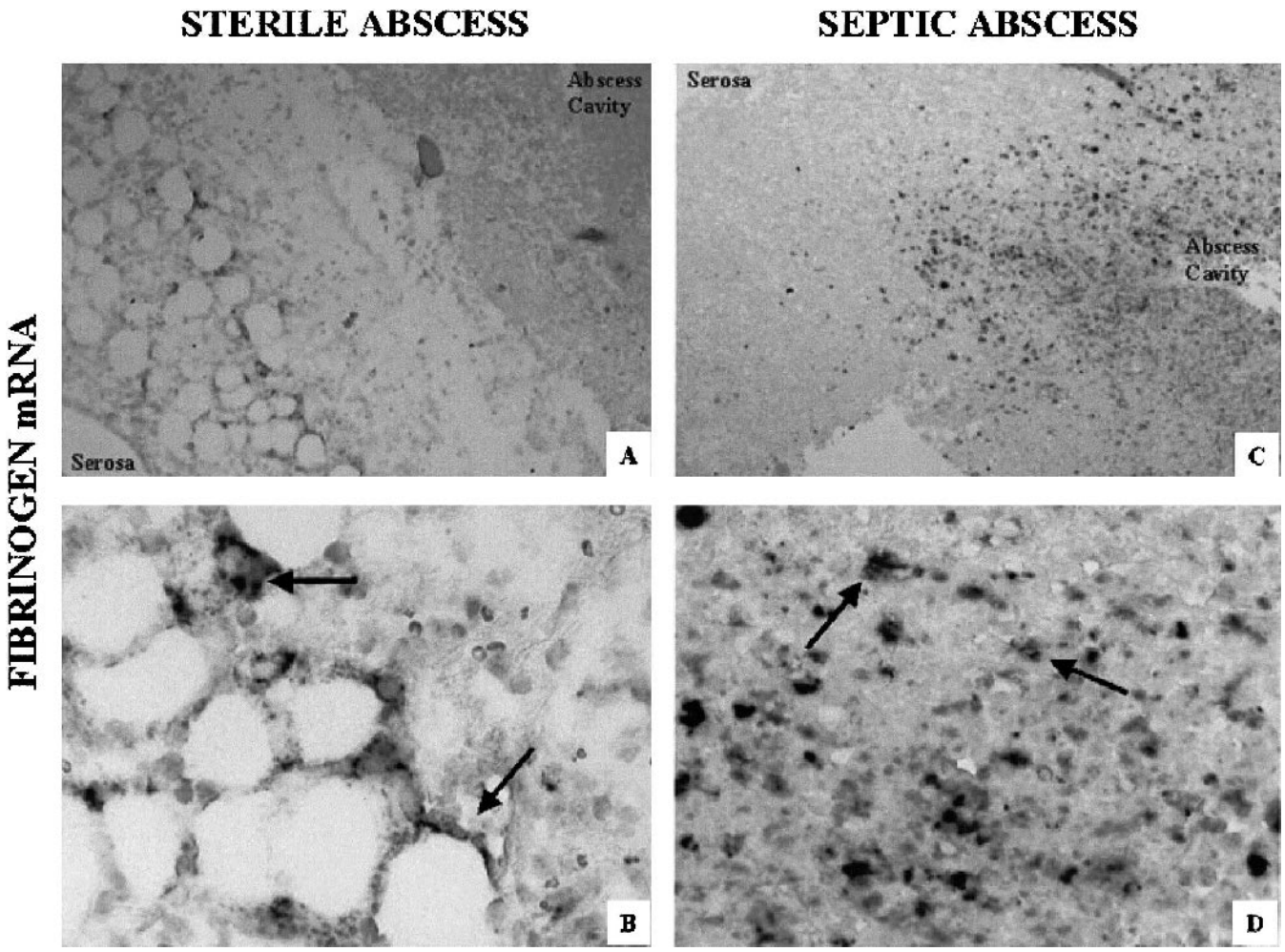

FIG. 5. ***In situ*** **hybridization of fibrinogen mRNA in the sterile (A and B) and the abscess walls with sepsis (C and C).** Abscess cavities and serosal layers are marked, and fibrinogen-positive cells are shown by arrows. A and C: 100×, B and D: 400× magnifications.

vein (Fig. 7A) and inferior vena cava (Fig. 7B) when compared with the control group ($P < 0.01$) at postimplantation day 3 when the intra-abdominal abscess was fully formed. There was no significant difference between the mean levels of IL-6 in the sterile and the septic abscess groups in either venous circulation. However, the linear regression analysis of the simultaneously obtained pairs of samples from the portal vein and the suprahepatic vena cava, which included the data from all of the groups, showed a statistically significant direct correlation between the IL-6 levels of the portal vein and the inferior vena cava (–0.053 + 0.95 PV, $P < 0.0001$), which explained 99% of the variability in the data ($R^2 = 0.99$; Fig. 7C). Moreover, it is of considerable importance with respect to the site of origin of this cytokine consequent to the formation of an intra-abdominal abscess that this regression also showed that as a function of the higher levels found in the sterile and septic abscess animals the portal vein IL-6 levels were approximately 10% greater than the corresponding suprahepatic inferior vena cava values, and this difference was highly significant ($P < 0.0001$).

Evaluation of TNF-α and IL-1β levels in the portal vein on day 3 showed that there were also elevated levels of both cytokines in the sterile and septic groups, but they were only significantly different from control in the sterile group (Table 2).

The effects of the intra-abdominal septic and sterile abscesses on the liver

Sinusoidal architecture in control, sterile, and septic livers—H&E stain of the liver (Fig. 8) showed an increased amount of sinusoidal dilatation and inflammatory cell infiltration in the sterile (Fig. 8B) and the septic (Fig. 8C) livers when compared with the control liver (Fig. 8A), being somewhat greater in the septic liver. This sinusoidal dilatation appeared to be radiating from the hepatic lobular central vein.

Evidence of hepatocyte necrosis and apoptosis was determined by examining both 100× and 400× power fields in histologic sections of control, sterile, and septic livers as described by Guan et al. (19). The number of pycnotic nuclei consistent with apoptosis was significantly ($P < 0.0001$) higher in sterile and septic livers than in control livers, but the magnitude of the degree of cell death was significantly ($P < 0.05$) greater in the septic abscess livers than in the sterile abscess ones. (Table 1).

Inflammatory cell concentrations in control, sterile, and septic livers—Myeloperoxidase stain of the liver (Fig. 9, A-C) confirmed the significant ($P < 0.0002$) increase in the inflammatory white cell infiltration in the sterile (Fig. 9B) and the

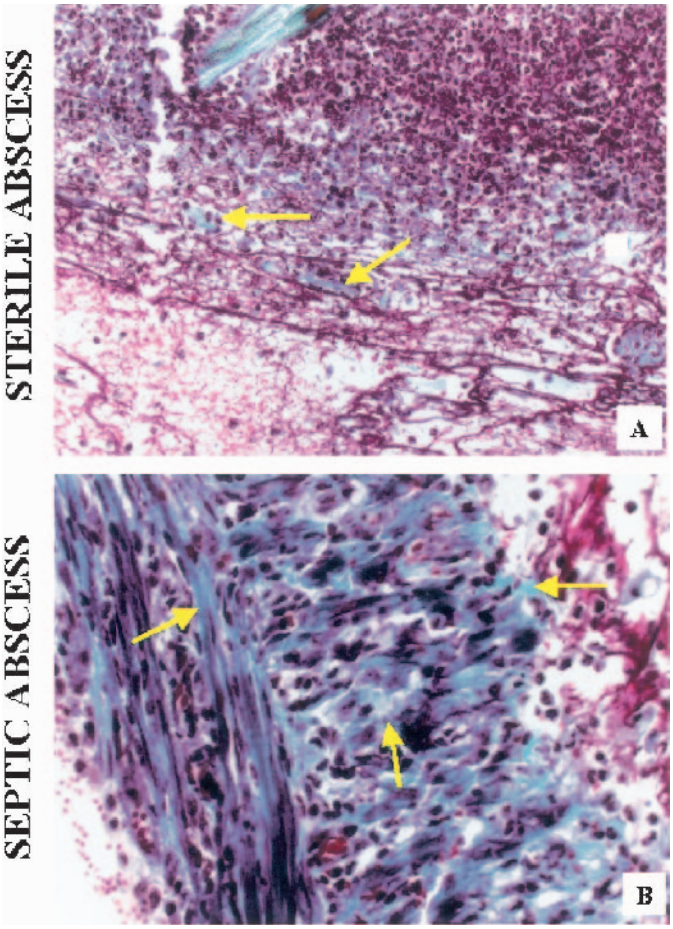

FIG. 6. **Gomori's trichrome stain for collagen in the sterile (A) and the septic (B) abscess walls at 200× magnification.** The collagen deposition (greenish blue) is shown by arrows.

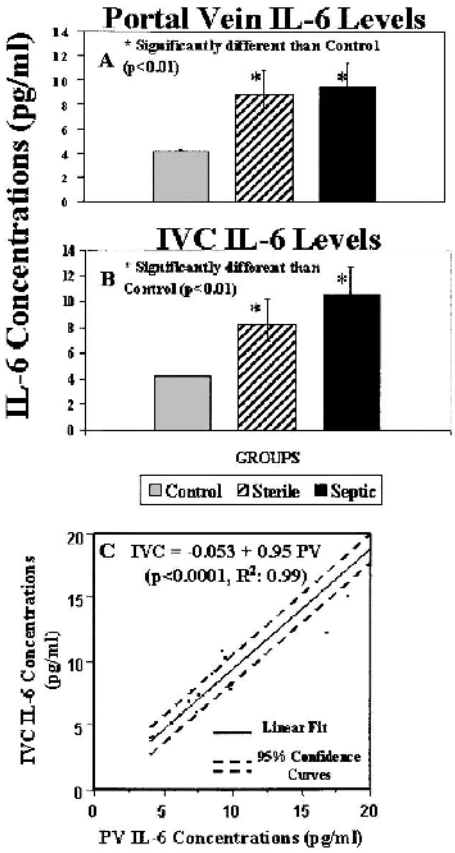

FIG. 7. **The portal vein (A) and the inferior vena cava (B) circulating IL-6 levels in control, sterile, and septic abscess groups, and the linear regression analysis of the portal vein and inferior vena cava IL-6 levels (C).** Two high values not shown in the linear regression model, but included in statistical analyses. PV, portal vein; IVC, inferior vena cava. A and B, the error bars represent the SEM.

septic livers (Fig. 9C) when compared with the control liver (Fig. 9A). This infiltration was significantly ($P < 0.01$) greater in the septic liver compared with the sterile abscess liver and appeared to involve the entire sinusoidal system, whereas in the sterile abscess liver it was most concentrated in the sinusoids adjacent to the portal triad.

Lipid deposition in control, sterile, and septic livers—Figure 9D–F shows the Oil Red O lipid stain of control, sterile, and septic livers, indicating the deposition of lipids in the liver parenchyma. Control livers (Fig. 9D) showed very few lipid droplets, whereas sterile (Fig. 9E) and septic livers (Fig. 9F) showed a significantly ($P < 0.004$) increased number of cells with lipid droplets when compared with control liver. However, the size of the hepatocyte lipid droplets was generally larger in the septic liver than in the sterile liver. Although the lipid deposition in the liver samples was diffusely distributed throughout the liver parenchyma, the lipid droplet concentration appeared higher in that portion of the individual hepatocytes, which was adjacent to the sinusoids in both the sterile and septic livers compared with that seen in the controls.

Detection of IL-6 mRNA gene expression in control, sterile, and septic livers—In situ hybridization of the liver for IL-6 mRNA is shown in Figure 10 (A-C). When compared with the control liver (Fig. 10A), there was a significantly ($P < 0.0001$) increased amount of cells demonstrating IL-6 gene expression (mRNA) in both the sterile (Fig. 10B) and the septic livers (Fig. 10C), the number of positive cells being greatest in the septic liver (Table 1). The vast majority of the IL-6 mRNA-positive cells in the sterile and the septic livers were uniquely located around the central vein, with the density of staining

TABLE 2. **Means ± SEM and 95% confidence intervals of the portal vein and suprahepatic inferior vena cava TNF-α and IL-1β concentrations**

	Groups	Mean ± SEM (pg/mL)	95% Confidence intervals (pg/mL)
Portal Vein			
TNF-α	Control	12.98 ± 0.20	12.78, 13.19
	Sterile	18.36 ± 1.68*	16.68, 21.03
	Septic	15.04 ± 0.96	14.08, 16.33
IL-1β	Control	32.59 ± 0.82	31.77, 33.45
	Sterile	57.39 ± 7.50*	49.89, 67.55
	Septic	40.98 ± 3.02	37.96, 44.52
Inferior vena cava			
TNF-α	Control	12.32 ± 0.31	12.01, 12.66
	Sterile	17.21 ± 1.56*	15.65, 19.67
	Septic	14.75 ± 0.89	13.86, 15.94
IL-1β	Control	32.64 ± 0.54	32.10, 33.19
	Sterile	50.63 ± 6.46*	44.17, 59.31
	Septic	42.07 ± 3.31	38.76, 45.99

*Indicates significantly different from control ($P < 0.05$).

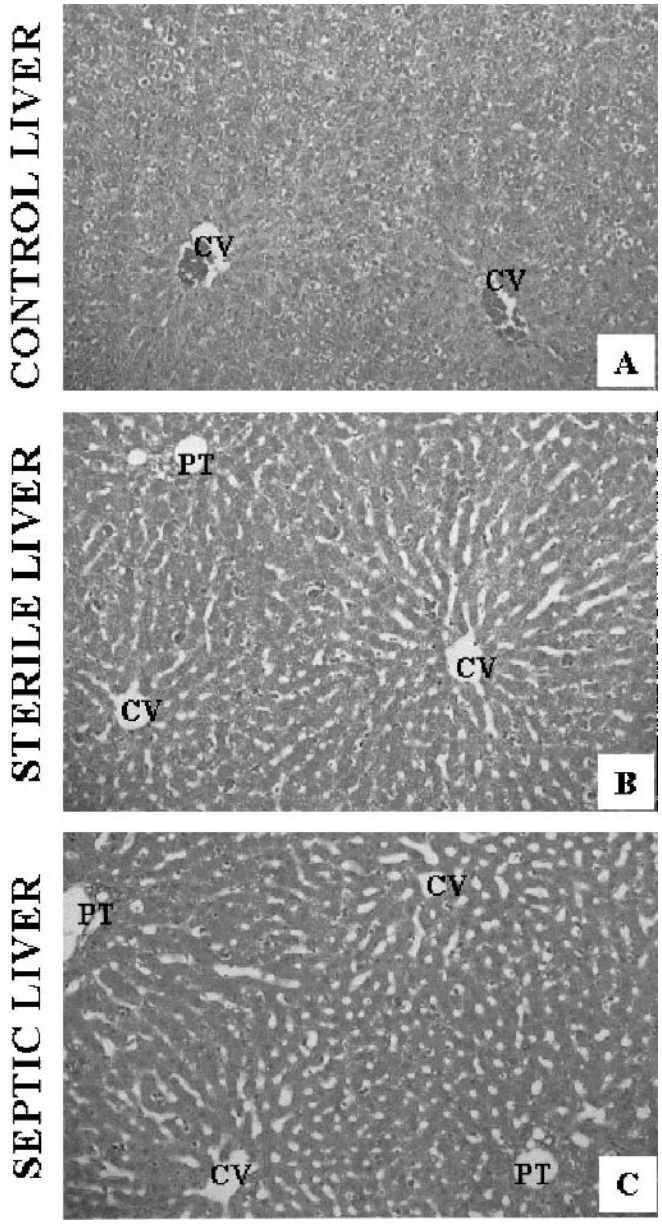

FIG. 8. **The H&E stain of the control (A), sterile (B), and septic (C) livers in 100× magnification.** CV, central vein; PT, portal triad.

being most prominent in the pericentral vein location in the septic livers (Fig. 10C).

Detection of fibrinogen mRNA gene expression in control, sterile, and septic livers—In situ hybridization of the liver for fibrinogen mRNA (Fig. 10, D–F) depicted a significantly ($P < 0.001$) increased amount of fibrinogen gene expression (mRNA) in both the sterile (Fig. 10E) and the septic livers (Fig. 10F) when compared with control liver (Fig. 10D). As with IL-6 mRNA, the number of cells with fibrinogen mRNA expression was also greatest in the septic liver. All of the fibrinogen mRNA-expressing cells were located around the central vein, close to the IL-6 mRNA-expressing cells, in both the sterile and the septic livers. The density of staining for fibrinogen mRNA was also greatest in the septic livers (Fig. 10F).

Collagen deposition in control, sterile, and septic livers— Gomori's Trichrome stain of the liver for collagen (Fig. 11) showed an increased amount (quantity and density of the blue stain) of collagen deposition around the central vein of both the sterile (Fig. 11B) and the septic livers (Fig. 11C) when compared with control liver (Fig. 11A). However, this collagen fibrosis around the central hepatic vein was the greatest in the septic liver where it appeared to partially obstruct the exit of nearly every sinusoid into the hepatic lobular central vein.

DISCUSSION

The chronic septic rat fecal-agar pellet model is a reliable, highly reproducible, and well-established animal model (8–15) that reproduces the cardiovascular and metabolic alterations seen in chronic hyperdynamic septic patients, and is consistent with the clinical progress of sepsis (4, 7, 16). It allows not only a study of the local effects of an intra-abdominal septic process, but also permits investigation of the differences between the septic and sterile systemic inflammatory responses and the role of specific types and strains of organisms on this inflammatory process (8, 20, 21).

All of the mortality in this model is seen during the first 36-h postseptic pellet implantation due to acute disseminated *E. coli* bacterial peritonitis (8, 20, 21). Sterile pellet implantation does not produce death. All animals that were inoculated with a sterile or septic pellet and that survived the initial peritonitis stage formed an intra-abdominal abscess and survived until sampling at day 3. By definition, an abscess is a localized inflammatory process with a necrotic center, and the abscess wall plays a unique role in our model by limiting the invasion of the abscess bacteria to the peritoneum, thus preventing the high mortality rate due to the peritonitis acute phase seen in the septic group. The initial *E. coli* inoculum of the pellet had a statistically significant direct correlation with the septic mortality rate, whereas *B. fragilis* has been previously shown to contribute to the abscess formation process, to modulate skeletal muscle metabolism, to induce muscle glucose uptake, and to influence the systemic lactate concentrations in response to sepsis by lowering the proportion of active pyruvate dehydrogenase complex (21). However, intraperitoneal pellet insertions containing *B. fragilis* alone produced well-defined abscesses at 3 days, but were not associated with any acute or chronic mortality (20, 21).

Because even a sterile formal laparotomy produces some intraperitoneal inflammatory response, it was felt that a sterile abscess pellet of the same size and composition was the correct control for the septic abscess and that the "true control" for both of these inflammatory processes was the nonlaparotomy anesthesia control used here. The morbidity of the sterile and the septic groups seen during the first 48-h period is due to the anesthesia and the inflammatory effect of the intraperitoneal sterile pellet insertion, but the mortality was due only to the acute bacterial peritonitis in the septic pellet animals. Both groups of pellet-inserted animals have been shown to have diarrhea, crusted eyes, a lack of mobility, and a reduced appetite in the first postpellet insertion day. However, while they regain their appetite and eat a normal or increased quantity of food, they remain at a statistically significant lower weight over the initial 7 days postpellet implantation (8). Moreover,

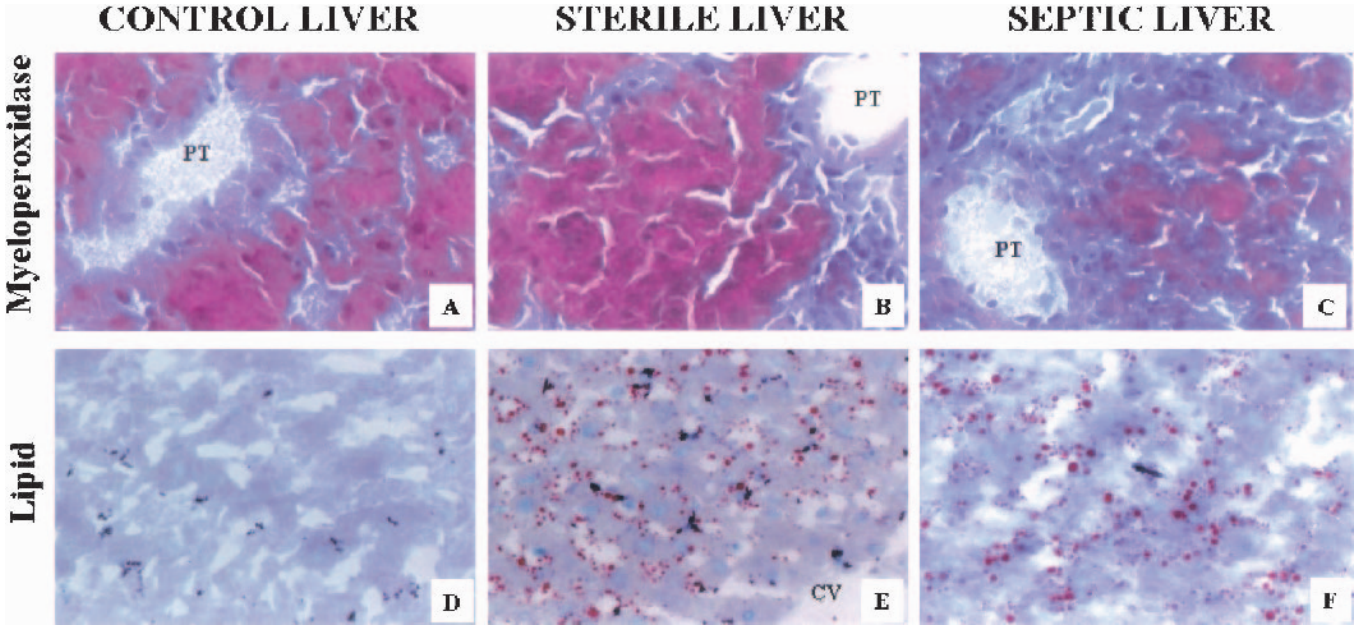

FIG. 9. **Myeloperoxidase (A through C) and Oil Red lipid (D through F) stains of control (A and D), sterile (B and E), and septic (C and F) livers at 200× magnification.** CV, central vein; PT, portal triad.

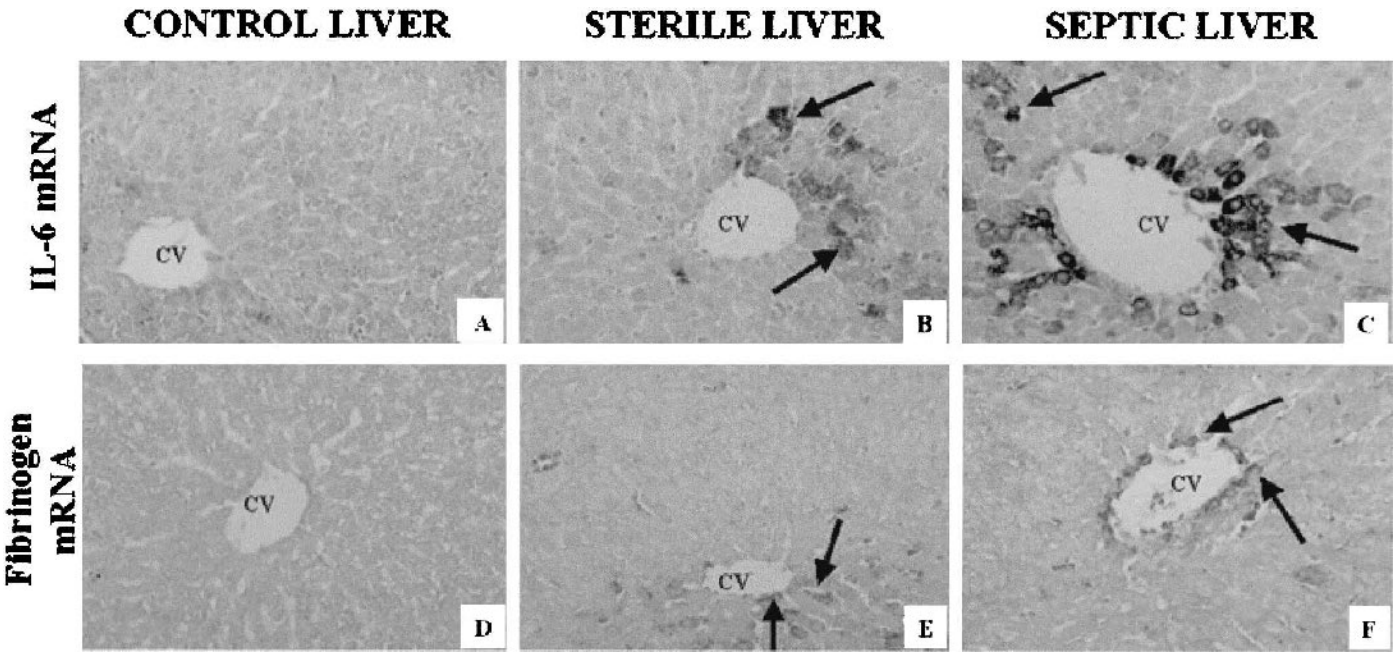

FIG. 10. ***In situ*** **hybridization of IL-6 mRNA (A through C) and fibrinogen mRNA (D through F) in the control (A and D), sterile (B and E), and septic (C and F) livers at 200× magnification.** Arrows: IL-6- (B and C) and fibrinogen- (E and F) positive cells. CV, central vein.

the septic abscess animals show inhibition of skeletal muscle protein synthesis and have evidence of increased proteolysis and accelerated gluconeogenesis secondary to the metabolic responses of the inflammatory process when compared with the sterile abscess and control groups (9–10, 13–15, 20, 21).

It has been previously shown by Vary, et al. (9–15), as well as in this study, that both sterile and septic pellets are totally walled off by postoperative day 3 and that the intra-abdominal abscess formation has a distinct effect on the morbidity and mortality of the chronic septic rat fecal-agar pellet model. Although a walled-off intra-abdominal abscess formation decreases the mortality rate by encapsulation of the bacterial source, it does not prevent the systemic clinical course of sepsis/SIRS as shown in the present study. This course appears to be the direct effect of the presence of inflammatory cells in the abscess wall as the source of proinflammatory mediators, such as cytokines and acute-phase proteins. Moreover, the production of these abscess wall inflammatory mediators appears to be a major cause of the chronic systemic effects of sepsis and of SIRS by effecting an altered cellular function in organs distant to the abscess, such as the liver and the lung (18).

The earlier observations of Quinn, et al. (17) and by Lussier (18), as well as those contained in this study, have shown that

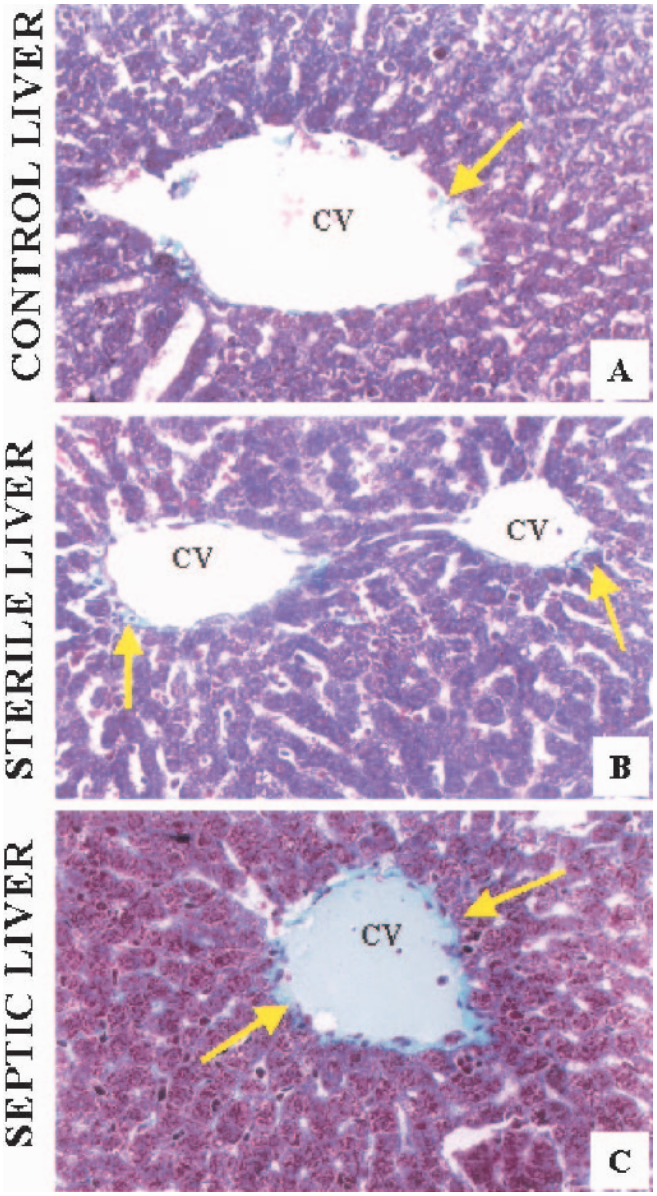

FIG. 11. **The trichrome stain of the collagen in the control (A), sterile (B), and septic (C) livers in 200× magnification.** Arrows: collagen deposition. CV, central vein.

the circulating levels of the acute proinflammatory cytokines TNF-α, IL-1β, and IL–6 are elevated in response to injury in the acute phase of chronic septic rat fecal-agar pellet model. In their studies (17, 18), TNF-α and IL-1β peaked in the mixed central venous blood at day 2, but were still elevated at day 3 postabscess pellet insertion. In contrast, IL-6, which is known to be a more chronic or late phase proinflammatory cytokine, peaked at day 3 in the mixed central venous blood (17, 18). In the present study done at day 3 postpellet insertion, IL-6 as well as the other inflammatory cytokines were shown to have levels that were significantly higher in the portal circulation of the sterile and the septic intra-abdominal abscess animal groups when compared with the control group. Moreover, the portal vein IL-6 levels were significantly higher and the TNFα and IL-1β levels were equal to, or higher than the corresponding simultaneously obtained suprahepatic inferior vena cava levels, suggesting that the abscess wall cytokine-positive mRNA cells are a major contributor to the elevated systemic IL-6 shown here, as well as to other inflammatory cytokinemias (18).

The intestinal response to both a sterile and a septic abscess also includes an increase in IL-6 mRNA in the Paneth cells of the intestinal villus crypts, which has also been noted in association with increased defensin production after hemorrhagic shock (22). Also, both Paneth cell TNFα and defensin production has been found in response to inflammatory bowel disease (23). Undoubtedly, the sepsis-induced IL-6 secretion from the intestinal Paneth cells contributes to the total circulating level of this cytokine. However, the studies of Magnotti (24) and of Deitch (25) suggest that the pathway for entry of all of the intestinal generated cytokines into the general circulation is via the intestinal lymph channels, which enter into the general circulation not through the portal venous system, but rather via the thoracic duct into the superior vena cava. Indeed, they showed no elevation in portal venous blood inflammatory mediators in posthemorrhagic shock rats (23). In contrast, as shown here in the sterile and septic intra-abdominal abscess animals, an additional major source of TNFα, IL-1β, and IL-6 entry into the general circulation is via the portal vein. Moreover, this abscess wall generated portal cytokinemia appears to induce a direct influence on the hepatic cells as its first organ effect before contributing to an increase in systemic cytokine levels.

When fully formed by the 3rd postimplantation day, the intra-abdominal abscess, regardless of being sterile or septic, acts as a primitive, temporary host defense organ that meets Webster's (26) definition of an organ as "a differentiated structure consisting of cells and tissues and performing some specific function in an organism." In this capacity, the abscess effects the liver as a remote new endocrine organ by secreting substances (cytokines) that initiate and activate an intrahepatic inflammatory cell infiltration and sequestration. It also appears to induce an increase in hepatocellular apoptosis, which appears greater in the septic abscess animals. The viable hepatocytes respond to the mediators released from an intra-abdominal abscess by increased lipogenesis and intracellular lipid deposition, secondary to the changes in the hepatic lipid, glucose, and ketone metabolism previously described in this model (9, 11) and by the expression of mRNA for the elaboration of the acute phase protein fibrinogen.

The present study shows that the liver also has an important role as a response organ in terms of an intrinsic focal IL-6 mRNA gene expression in reaction to the cytokines released by an intra-abdominal abscess. This response may be a critical one in response to abscess formation, even though the hepatic IL-6 elaborated focally at the hepatic lobular central veins does not appear to significantly contribute further to increasing the systemic circulating levels of this proinflammatory cytokine because the suprahepatic inferior vena cava blood does not show higher levels of IL-6 than the portal vein blood. The unique location and close proximity of the cells expressing IL-6 and fibrinogen mRNAs around the hepatic lobular central vein confirms the heterogeneity of the liver parenchyma and suggests a direct paracrine effect of the hepatic central vein cells producing the IL-6 on those producing the fibrinogen.

Fibrinogen activation produces fibrin deposition, which acts as a temporary lattice for collagen formation (6, 7). As a result, the consequent laying down of collagen in the lobular pericentral vein location in close proximity to the fibrinogen-producing cells appears to produce some degree of functional obstruction of the hepatic sinusoidal outflow. The functional obstruction of the hepatic lobular portal outflow into the hepatic lobular venules in turn induces hepatic sinusoidal congestion, and may thus reduce or slow the hepatic blood flow.

This relative stasis appears to be responsible for the sequestration of the portal venous cytokine-activated inflammatory cells seen in the hepatic sinusoids, which extends the effect of the local production of superoxide radicals by inflammatory mediator upregulation of the myeloperoxidase-containing white blood cells. This in turn may be an additional stimulus to the altered hepatic cellular response inducing increased gluconeogenesis (9–12) and lipogenesis (11), with the increased lipid deposition seen in this study, as well as to the chronic perisinusoidal inflammatory changes seen in response to the intra-abdominal sterile and septic processes. A consequence of this influx of inflammatory mediators and the evidence of increased superoxide-generating enzyme upregulation in the sequestered leukocytes appears to be an increase in hepatocyte apoptosis in the sterile and septic abscess animal's livers. This would appear to be an indication of early organ failure of the liver.

Even though the liver of animals with a septic abscess showed a more intense inflammatory response and greater fibrotic changes than that seen in the sterile abscess animals, the data supports the contention that the hepatic response is primarily caused by the basic role of the sterile inflammatory induction of abscess wall-produced cytokines, which contribute to remote organ injury independent of the septic contamination. However, the inflammatory response as evidenced by the significantly increased sequestration of myeloperoxidase-containing white cells is further enhanced by the associated presence of gram-negative bacteria, such as *E. coli* and *B. fragilis*, in the abscess. This enhanced leukosequestration appears to be the result of the gram-negative bacterial endotoxin mediator effect that also greatly amplifies the basic inflammatory cytokine effects of the forming abscess wall host defense organ on the hepatic cells because the major alterations in hepatic glucose, ketone, and lipid metabolism are seen in the septic abscess animals (9–11, 14), as is the greater incidence of late hepatocellular death and of animal mortality.

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