Liver cytokine production and ICAM-1 expression following bone fracture, tissue trauma, and hemorrhage in middle-aged mice

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Matsutani T, Kang SC, Miyashita M, Sasajima K, Choudhry MA, Bland KI, Chaudry IH. Liver cytokine production and ICAM-1 expression following bone fracture, tissue trauma, and hemorrhage in middle-aged mice. Am J Physiol Gastrointest Liver Physiol 292: G268–G274, 2007. First published September 7, 2006; doi:10.1152/ajpgi.00313.2006.—Although studies have indicated that hemorrhagic shock and resuscitation produces hepatic damage by mechanisms involving adhesion molecules in endothelial cells and hepatocytes, it is not known if there is any difference in the extent of hepatic damage following bone fracture, soft tissue trauma, and hemorrhage (Fx-TH) between young and middle-aged animals. To study this, young (6–8 wk) and middle-aged (~12 mo) CHS/HeN male mice were subjected to a right lower leg fracture, soft tissue trauma, (i.e., midline laparotomy), and hemorrhage (blood withdrawal to decrease the blood pressure to 35 ± 5 mmHg for 90 min) followed by resuscitation with four times the shed blood volume in the form of lactated Ringer solution. Mice were euthanized 24 h later, and liver tissues were harvested. Total bilirubin levels in the hepatocyte extract increased markedly following Fx-TH in both groups of mice; however, the increase in middle-aged mice was significantly higher compared with young mice. TNF-α and IL-6 levels in the hepatocyte extract following Fx-TH increased significantly in middle-aged mice but remained unchanged in young mice. IL-10 levels significantly decreased in middle-aged mice following Fx-TH but remained unchanged in young mice. Kupffer cells from middle-aged mice produced significantly higher IL-6 and IL-10 levels compared with young mice. Protein levels and mRNA expression of ICAM-1 in hepatocytes were also significantly higher in middle-aged mice compared with young mice following Fx-TH. These results collectively suggest that the extent of hepatic damage following Fx-TH is dependent on the age of the subject.

tumor necrosis factor-α; interleukin-10; interleukin-6; hepatocellular damage; intracellular adhesion molecule-1

MAJOR TRAUMA AND HEMORRHAGIC SHOCK are associated with the development of systemic inflammatory response syndrome, which may culminate in multiple organ dysfunction syndrome (5, 6, 9, 12, 18, 20, 29, 34, 38, 39). Although systemic inflammatory reactions by trauma-hemorrhage are characterized by elevated levels of inflammatory mediators, the exact mechanisms by which trauma-hemorrhage leads to organ dysfunction have not been fully clarified. Furthermore, the depression in hepatocellular function following trauma-hemorrhage persists despite fluid resuscitation (42). In this regard, the liver is believed to play an important role in the occurrence of multiple organ failure (42). Liver tissue has the highest population of macrophages in the body and therefore has great potential for producing the local and/or systemic inflammatory response (4, 5). Hepatocytes, Kupffer cells, and sinusoidal endothelial cells participate in host-defense responses through an orchestrated and complex intrahepatic network to respond to various stress signals such as injury and infection (10). The inflammatory responses after extrinsic stresses appear to result from an unbalanced production of pro- and anti-inflammatory mediators to maintain homeostasis. However, there are many instances in which prolonged stress leads to the overproduction of proinflammatory mediators, resulting in cellular, tissue, and organ dysfunction.

Surgical and traumatic injuries influence the function of leukocytes and endothelial cells, thus leading to various systemic responses (32). Cellular adhesion molecules are cell surface glycoproteins that are critical for leukocyte adhesion to the sinusoidal endothelium and transmigration and cytotoxicity in a variety of inflammatory liver diseases (21, 30, 48). ICAM-1 plays an important role in inflammation, and the increased expression of ICAM-1 on endothelial cells is reflected in the activation of endothelial cells (30). ICAM-1 is of particular importance since it mediates firm endothelial adhesion and facilitates leukocyte transmigration in the liver. Studies have shown that there is an upregulation of ICAM-1 on both sinusoidal cells and hepatocytes in inflammatory liver conditions such as hepatitis B viral infection, autoimmune liver disorders, alcoholic hepatitis, and liver allograft rejection (1, 2, 7, 21, 37, 41, 48). It has also been reported that during high-dose endotoxin shock, death is primarily due to liver damage caused by the accumulation of neutrophils in liver tissue (22, 24). Since the levels of ICAM-1 are elevated in the bile and serum of patients with sepsis, measurement of circulating ICAM-1 facilitates the identification of those patients with the highest risk of developing liver dysfunction (43). These data thus support the hypothesis that adhesion molecules are essential for producing inflammatory liver injury.

A potential limitation of all of the experimental studies has been the exclusive use of animals that are 6–12 wk of age. This age range in mice corresponds approximately to an age of 4–8 yr old in humans (40). Furthermore, most of the previous age-associated studies have been carried out using aged mice (18–24 mo), and no information is available concerning hepatocellular functions in middle-aged (12 mo old) mice following severe injury. Thus, although much has been learned about the mechanisms of trauma-hemorrhage, this knowledge may
have limited relevance to the adult patient population. Trauma, critical care physicians, and general surgeons have long noted anecdotal experience of a significant divergence in the response of the pediatric and adult populations to severe trauma. The age-associated experiences were recently confirmed in a retrospective analysis that showed the importance of sex and of age in the outcomes of patients following traumatic injury (16, 17). In view of this, we examined whether young (6–8 wk of age) and middle-aged (~12 mo of age) mice differed in the immune and physiologic responses following bone fracture, soft-tissue trauma, and hemorrhage (Fx-TH). The aim of this study, therefore, was to determine whether middle age has any influence on the extent of hepatic damage following Fx-TH. The degree of hepatic damage was assessed by measurements of bilirubin, TNF-α, IL-6, and IL-10 in hepatocyte extracts and ICAM-1 expression in hepatocytes following Fx-TH. The ability of Kupffer cells to produce TNF-α, IL-6, and IL-10 was also measured.

MATERIALS AND METHODS

Animals. Male young (6–8 wk old) and middle-aged (~12 mo old) C3H/HeN mice (Charles River Laboratories, Wilmington, MA) were used in the experiments. Animals were housed with free access to standard laboratory chow and water and maintained in a pathogen-free animal facility for 2 wk before the experiments. Animal experiments were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham, AL.

Experimental groups. Operated animals were randomized into four groups (with 6–8 mice/group): young mice with sham operation (group 1), young mice with Fx-TH (group 2), middle-aged mice with sham-operation (group 3), and middle-aged mice with Fx-TH (group 4).

Fracture and trauma-hemorrhage procedure. Animals in the Fx-TH groups were anesthetized with isoflurane (Minrad, Bethlehem, PA) and restrained in the supine position. Mice were subjected to a closed fracture of the right lower leg by three-point bending as described previously (45). A 2.5-cm midline laparotomy (i.e., soft tissue trauma) was performed and then closed aseptically in two layers using 6-0 (Ethilon, Ethicon, Somerville, NJ) sutures. Subsequently, both femoral arteries were aseptically catheterized with polyethylene-10 tubing (Clay-Adams, Parsippany, NJ) using a minimal dissection technique, and animals were allowed to awaken. Blood pressure was monitored continuously through one of the femoral catheters using a blood pressure analyzer (Digi-Med BPA-190, Micro-Med, Louisville, KY). Upon awakening, animals were bled through the catheter to a mean arterial blood pressure (MAP) of 35 ± 5 mmHg (preamerorhage MAP was 90 ± 5 mmHg), which was maintained for 90 min. At the end of that period, animals were resuscitated with lactated Ringer solution (4 times the shed blood volume) over 30 min. Lidocaine hydrochloride was applied to the groin incision sites, with lactated Ringer solution (4 times the shed blood volume) over 30 min. Lidocaine hydrochloride was applied to the groin incision sites, and the vessels were ligated, and the incisions were closed with 6-0 sutures. Sham-operated animals underwent the same anesthetic and surgical procedures, but neither hemorrhage nor fluid resuscitation was performed. After resuscitation, external fixation of the right lower leg by plastic tubing was performed. Buprenorphine (Reckitt & Colman, Richmond, VA) was injected subcutaneously immediately after surgery at a dosage of 0.1 mg/kg in all groups. All procedures were operated aseptically, and there was no mortality observed in this model of Fx-TH.

Preparation of hepatocytes and Kupffer cells. Animals were anesthetized by isoflurane 24 h after the operation. The circulating blood was removed by a heart puncture. Hepatocytes and Kupffer cells were isolated as described previously with some modifications (4). In brief, the peritoneal cavity was opened aseptically, the portal vein was exposed and catheterized with a 25-gauge winged infusion set, and the vena cava was nicked. Retrograde perfusion of the liver was performed with 20 ml of HBSS (GIBCO-BRL, Grand Island, NY) at 37°C through the portal vein. This was immediately followed by a perfusion with 20 ml of 0.05% collagenase type IV (162 U/mg, Sigma Chemical, St. Louis, MO) in HBSS with 0.5 mM CaCl2 (Sigma Chemical) at 37°C. The liver was then transferred to a petri dish containing warm 0.05% collagenase, minced finely, incubated at 37°C for 10 min, and passed through a sterile 150-mesh stainless steel screen into a beaker containing 10 ml of cold HBSS and 10% heat-inactivated FBS. Hepatocytes were then separated by low-speed centrifugation at 50 g at 4°C for 3 min and washed in HBSS. The residual cell suspension was then centrifuged twice at 400 g at 4°C for 3 min. The supernatant was discarded, the cell pellet was reconstituted with 10 ml complete Williams’ E medium containing 10% heat-inactivated FBS, 2 mM l-glutamine, and antibiotics of 50 U/ml penicillin, 50 μg/ml streptomycin, and 20 μg/ml gentamicin (all from GIBCO-BRL). The cell suspension was then layered over 16% metrizamide (Accurate Chemical & Scientific, Westbury, NY) in HBSS and centrifuged at 2,300 g at 4°C for 45 min to separate Kupffer cells from the remaining parenchymal cells in the pellet. Cells were washed twice with centrifugation (800 g at 4°C for 10 min) with Williams’ E medium and were incubated at 37°C with 5% CO2 and 95% humidity for 2 h; nonadherent cells were removed, and the adherent Kupffer cell population was gently removed and adjusted at a density of 1 × 106 cells/ml in complete Williams’ E medium. Cells were then cultured under the same conditions for another 24 h in the presence of 1 μg/ml LPS (from Escherichia coli, 055:B5, Sigma). After incubation, cell-free supernatants were obtained and stored at −80°C until assayed.

Hepatocyte preparation for cytokine determination. The hepatocyte suspension was centrifuged twice at 50 g at 4°C for 3 min. The supernatant was discarded, and the cell pellet was reconstituted with homogenization buffer at 4°C, the buffer contained a protease inhibitor cocktail including (in mM) 10 HEPES (pH 7.9), 10 KCl, 1 EDTA, 0.5 EGTA, 1 DTT, 0.5 PMSF, 1 sodium fluoride, 20 β-glycerophosphate, and 1 sodium vanadate in PBS (pH 7.2) containing 0.5% Triton X-100. Samples were sonicated for 1 min and incubated at 4°C for 1 h. The final homogenate was centrifuged at 120,000 g. The supernatants were used for the cytokine assay. Tissue protein content was determined by a colorimetric assay using the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA).

Analysis of bilirubin, TNF-α, IL-6, and IL-10 in hepatocyte extracts. The total bilirubin in hepatocyte extracts was measured as a marker for cholesterolosis using a total bilirubin kit from Sigma Chemical. TNF-α, IL-6, and IL-10 were measured in hepatocyte extracts using a sandwich ELISA technique as previously described by our laboratory (26). TNF-α levels were determined by the DuoSet ELISA system (R&D Research, Minneapolis, MN). IL-6 and IL-10 levels were determined by the OptEIA system (BD Bioscience, San Diego, CA).

Expression of mouse ICAM-1 mRNA in hepatocytes. Total RNA was extracted from hepatocytes using TRIzol (Life Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. Briefly, hepatocytes were homogenized in a denaturing solution, and RNA was sequentially isolated using phenol-chloroform extraction and isopropanol precipitation. To prepare cDNA, 1 μg of total RNA was reverse transcribed using murine Moloney leukemia virus reverse transcriptase (Clontech) primed with random hexamers in a final volume of 100 μl. PCR was performed using HotStarTag DNA polymerase and the Q-Solution Kit (Qiagen, Valencia, CA) in accordance with the manufacturer’s protocol. The primers used were 5'-TTCGCTAACCACCCGTGGTTATC-3' (forward) and 5'-CTG-GCCTGGAGACATTAGAAC-3' (reverse). Briefly, the PCR assay was performed using 5 μl of the cDNA mixture. RT products
were used in a total volume of 50 μl containing 5 μl of 10× PCR buffer, 10 μl of Q-solution, 0.2 mM of each dNTP, 1.5 units of HotStarTag polymerase (Qiagen), and 1 μM of each of the primers. PCRs were carried out in a gradient Mastercycler (Eppendorf, Westbury, NY). For each PCR, preheating was at 95°C for 15 min, and then annealing at 94°C for 40 s, 55°C for 45 s, and 75°C for 1 min, with a final extension phase at 72°C for 10 min. Each reaction was analyzed for amplification between 25 and 40 cycles. The amplification of β-actin (Clontech) was utilized as the housekeeping gene. PCR products were analyzed by electrophoresis on 1.5% agarose gels in 1× Tris-borate-EDTA buffer. The intensity of the bands was measured in a 500 Fluorescence ChemiImager (Alpha Diagnostics, San Leandro, CA). The relative absorbance of the PCR products was corrected against the absorbance obtained for β-actin.

Analysis of ICAM-1 in hepatocytes. The presence of ICAM-1 in hepatocytes was assessed by Western blot analysis. Briefly, hepatocytes were homogenized, the volume was adjusted to 1 ml with 1× SDS sample buffer [62.5 mM Tris·HCl (pH 6.8) at 25°C, 2% (wt/vol) SDS, 10% glycerol, 50 mM DTT, and 0.01% (wt/vol) bromophenol blue], and samples were boiled at 95°C for 5 min. An aliquot of the extract (20 μl) was loaded on a Nu-PAGE 4–12% bis-Tris gel and electrophoresed at 200 V. Proteins were electroblotted to a nitrocellulose membrane and blocked with 5% BSA in 10 mM Tris·HCl (pH 7.6) buffer containing 150 mM NaCl and 0.1% Tween 20 for 1 h at room temperature. The membrane was first incubated with goat anti-ICAM-1 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) diluted to 1:1000 with the same buffer for 2 h and later with the secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology) diluted to 1:3000 with 10 mM Tris·HCl buffer (pH 7.6) containing 150 mM NaCl, 0.1% Tween 20, and 5% nonfat dry milk. After blots had been washed three times, immunoreactive bands were visualized by chemiluminescence using Chemiflow west substrate (Alpha Diagnostics).

Statistics. Data are presented as means ± SE of 6–8 animals/group. One-way ANOVA for multiple comparison followed by Tukey’s or Dunn’s tests was employed to determine the significance of the differences between experimental means. P < 0.05 was considered significant for all statistical analysis.

RESULTS

Total bilirubin levels. We directly compared the responses to Fx-TH in young mice (6–8 wk of age) with those of middle-aged mice (~12 mo of age) to determine the manner in which these two age populations responded to the same injury. Based on the life tables, 6- to 8-wk-old mice correspond to 4- to 8-yr-old humans and 12-mo-old mice correspond to 40-yr-old humans (40).

To determine if there were any age-associated differences in the response to Fx-TH, we assessed hepatocellular damage in young and middle-aged mice at 24 h after Fx-TH. The degree of hepatocellular damage was measured by bilirubin levels in hepatocyte extracts (Fig. 1). Total bilirubin levels of young and middle-aged mice after Fx-TH increased significantly compared with sham-operated mice in both age groups; however, total bilirubin levels in middle-aged mice following Fx-TH were significantly higher (~1.5- to 2-fold) than the levels in young mice. There were no differences in bilirubin levels in young and middle-aged sham-operated mice. These findings show that hepatocellular damage occurred in our Fx-TH model and that the damage was substantially increased in middle-aged mice.

Inflammatory mediators in hepatocyte extracts. The levels of TNF-α (A), IL-6 (B), and IL-10 (C) in the hepatocyte extract of young and middle-aged mice at 24 h after Fx-TH are shown in Fig. 2. TNF-α (Fig. 2A) and IL-6 (Fig. 2B) levels in young mice did not alter following Fx-TH, whereas each of these levels in middle-aged mice following Fx-TH increased significantly compared with Fx-TH in young mice and sham-operated mice in both age groups. IL-10 (Fig. 2C) levels in young mice following Fx-TH were not different compared with IL-10 levels in sham-operated mice. IL-10 levels decreased in middle-aged mice following Fx-TH; however, the decrease in IL-10 was not evident in young mice. These results clearly demonstrate that there is an age-associated difference in the inflammatory and anti-inflammatory cytokines present in the hepatocyte extract following Fx-TH.

Kupffer cell cytokine production. As shown in Fig. 3, the production of TNF-α (A), IL-6 (B), and IL-10 (C) by Kupffer cells in young and middle-aged mice increased significantly at 24 h following Fx-TH. There were no significant differences in TNF-α production between young and middle-aged mice. In contrast, the production of IL-6 and IL-10 by Kupffer cells was significantly higher in middle-aged mice compared with young mice.

ICAM-1 mRNA by RT-PCR. To examine whether Fx-TH induces ICAM-1 mRNA in the hepatocyte, we examined mRNA levels of ICAM-1 in young and middle-aged mice 24 h after Fx-TH (Fig. 4). Fx-TH did not produce any change in ICAM-1 mRNA levels in young mice compared with sham-operated mice. ICAM-1 mRNA levels in middle-aged mice were significantly higher compared with those in sham-operated mice 24 h after Fx-TH. No significant differences in Fx-TH-mediated increases were observed in young and middle-aged mice. There were no differences in these levels in sham-operated mice in both groups. These results suggest that Fx-TH induces ICAM-1 mRNA in hepatocytes in middle-aged but not young mice.

ICAM-1 protein levels in hepatocytes. We examined ICAM-1 protein levels in hepatocyte extracts by Western blot assay following Fx-TH. Western blot analysis of hepatocyte extracts from young and middle-aged mice in sham control groups and Fx-TH groups are shown in Fig. 5. Young and middle-aged mice showed the presence of ICAM-1 protein in hepatocytes. Consistent with mRNA levels, there was a significant increase in ICAM-1 levels in hepatocytes of middle-age
mice subjected to Fx-TH compared with the levels observed in sham-operated mice. No significant differences were observed in ICAM-1 levels in young mice subjected to Fx-TH or sham operation.

**DISCUSSION**

The present study indicates that Fx-TH caused a substantial increase in the bilirubin levels in hepatocyte extracts, indicating hepatocellular damage. Our results also indicate that age-associated differences exist between tissue cytokine levels in hepatocyte extract and cytokine production from LPS-stimulated Kupffer cells. Moreover, these cytokine responses following Fx-TH were significantly influenced by age and contributed to the increased hepatocellular damage in middle-aged mice compared with young mice. The major new finding of our study is the observation that hepatocellular damage following Fx-TH was associated with increased expression of ICAM-1 in hepatocyte. In this regard, mRNA levels of ICAM-1 in middle-aged mice were significantly higher than those in young mice. Moreover, Western blot analysis demonstrated that the hepatocellular damage following Fx-TH in middle-aged mice was associated with increased ICAM-1 expression.

Liver tissue has the highest population of macrophages in the body and therefore has great potential for the production of local and/or systemic inflammatory responses. It is well known that trauma-hemorrhage activates Kupffer cells, and these cells release proinflammatory cytokines, which may increase liver damage by promoting neutrophil infiltration (4, 5). It is also known that trauma-hemorrhage decreases the cytotoxic capacity of splenic and peritoneal macrophages, but it increases the cytotoxic capacity of Kupffer cells. Previous studies from our lab...
laboratory (4, 5, 28) have demonstrated that trauma-hemorrhage also increases IL-1 and TNF-α release by Kupffer cells but not by splenic and peritoneal macrophages. As a consequence of the initial injury, hepatic inflammation develops, involving Kupffer cell activation, the release of cytokines and chemokines, and, subsequently, neutrophil recruitment to the liver. Hepatic dysfunction following ischemia-reperfusion is also believed to be the result of subsequent activation of Kupffer cells, which are the predominant source of oxygen free radicals (23). Oxygen free radicals also stimulate cytokine release and adhesion molecule expression following trauma-hemorrhage, which are events that also promote neutrophil infiltration (25). The postulated mechanism of hepatic damage is through the recruitment and activation of neutrophils and oxidative injury, all leading to cell and tissue damage (35). Our previous study (25) also demonstrated that the addition of a free radical scavenger, 2-mercaptopropionyl glycine, to the resuscitation fluid can decrease hepatocyte enzyme levels and improve hepatic recovery. With regard to ICAM-1, however, additional studies are needed to elucidate the ICAM-1-dependent events that lead to subsequent neutrophil infiltration following Fx-TH and whether such infiltration actually contributes to tissue injury.

The innate inflammatory response involved an exquisite mechanism of autoregulation in the hepatocyte. The proinflammatory response is accompanied by a compensatory anti-inflammatory reaction, as indicated by increased IL-10 production (19). IL-10 is an inhibitor of cytokine synthesis, and it inhibits the production of proinflammatory cytokines such as TNF-α and IL-6. The present results showed that Fx-TH not only significantly increased proinflammatory cytokines (TNF-α and IL-6) in hepatocyte extracts but also significantly decreased levels of the anti-inflammatory cytokine IL-10 in middle-aged mice. Decreased IL-10 levels in hepatocyte extracts of middle-aged mice were associated with increased proinflammatory cytokines. It appears that the alteration in these cytokines in hepatocyte extracts might be related to the degree of hepatocellular inflammation following Fx-TH. Furthermore, these middle age-associated alterations contributed to the increased hepatic inflammatory response following Fx-TH. Thus, it appears that aging changes the balance of the cytokine network in the liver following Fx-TH.

The posthemorrhagic inflammatory response in the liver is characterized by increased leukocyte-endothelium interactions, as observed in an intravital microscopic study (32). Neutrophil infiltration was previously implicated to play a contributory role in the later stages of liver injury in models of hemorrhage-resuscitation and hepatic ischemia-reperfusion (23). Transendothelial migration and the adherence of neutrophils to parenchymal cells requires the expression of adhesion molecules, particularly ICAM-1. In models with severe sinusoidal endothelial cell damage, e.g., ischemia-reperfusion, blockade of ICAM-1 was only moderately protective mainly because neutrophils have direct access to hepatocytes and neutrophil-hepatocyte interactions are only partially dependent on ICAM-1 (36). ICAM-1 is normally expressed at a low basal level, but its expression can be enhanced by various inflammatory mediators such as TNF-α and IL-1β (44). Furthermore, ICAM-1 can be induced by proinflammatory cytokines in endothelial cells as well as hepatocytes (37).

A clinical study (44) has indicated the strong correlation between circulating ICAM-1 levels and the development of liver dysfunction during the course of sepsis, as characterized by the highest serum bilirubin levels. In addition, the elevation of ICAM-1 precedes the increase in bilirubin in septic patients. However, the source of increased circulating ICAM-1 in producing liver damage remains unclear. Since circulating ICAM-1 levels correlated with serum bilirubin levels and the expression of ICAM-1 on hepatocytes correlates with the
degree of hepatic inflammation, hepatocytes have been suggested to be an important source of circulating ICAM-1 in producing liver damage. Upregulated expression of ICAM-1 on liver tissue could lead to an increased number of adherent neutrophils, with consecutive transmigration and the release of superoxide radicals and proteolytic enzymes. ICAM-1 can promote lymphocyte adherence and the activation of cytotoxic T cells (13). Following activation, cytotoxic T cells can cause liver damage through the CD95 receptor and ligand system (15). This hypothesis of ICAM-1-mediated liver damage is supported by studies dealing with the role of ICAM-1 in patients with acute rejection after liver transplantation. In these patients, an increased expression of ICAM-1 was found on structures that were targets for the inflammatory process (1), and circulating ICAM-1 was significantly elevated in the bile and plasma, predicting rejection of the transplant (2). In patients with resolving rejection after liver transplantation, ICAM-1 expression in the liver was greatly reduced after high-dose corticosteroid treatment (1). Furthermore, ICAM-1-deficient mice were resistant to the lethal effects of high dose of endotoxin, and this resistance was correlated with a significant decrease in neutrophil infiltration in the liver (46). In mice, anti-ICAM-1 antibodies prevented neutrophil-induced liver injury during endotoxia due to inhibition of extravasation into the parenchyma (14). It has also been shown that extravasation of neutrophils and their adherence to parenchymal cells is essential for producing hepatocellular damage (14).

Surgical and traumatic injuries induce a systemic endocrine-metabolic response that includes the stimulation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system (3, 8). Our studies (3, 11, 47) on gender dimorphism have indicated immunomodulatory effects of sex hormones as playing a critical role. It has been well documented that females have a survival advantage over males and that testosterone depletion/antagonism prevents immune suppression after trauma and shock (3). Whether there are age-associated changes in sex steroids that may contribute to the effects observed in the present study was not investigated. Since aging is associated with many endocrine alterations, it seems possible that some of these changes will have an impact on cytokine production and immune functions. Previous studies from our laboratory (26, 27) have shown that high circulating levels of estrogen in young females protect against the depression in immune functions following trauma-hemorrhage and that the process of aging is associated with decreased levels of circulating sex steroids. However, there are many factors that determine the posttraumatic response with tissue injury, such as the severity of trauma, age, gender, genetic background, and gene polymorphisms (3, 28, 31, 33). Whether these middle age-associated changes in hepatocyte extracts following trauma-hemorrhage are deleterious or not remains to be examined in further studies. Nonetheless, the observed changes in hepatocyte extracts have shown significant increases in inflammatory mediators. These, together with an increase in ICAM-1 in middle-aged mice following Fx-TH, may lead to the severe inflammation in middle-aged male mice under those conditions.

In conclusion, our present study demonstrates that middle age influences liver cytokine production and ICAM-1 expression following Fx-TH. We found strong expression of ICAM-1 in hepatocytes of middle-aged mice during the study period, suggesting that ICAM-1 may be causally involved in the development of liver dysfunction following trauma-hemorrhage. The results also indicate that there are divergent responses in mice of age groups that correspond to pediatric and adult patient populations in humans. Our data thus support the concept that the differences in gene and protein expression in pediatric and adult patient populations may be responsible for preventing or producing hepatocellular damage following Fx-TH.

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GRANTS

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EXPRESSION OF ICAM-1 IN THE LIVER FOLLOWING TRAUMA-HEMORRHAGE