Effect of Thermal Injury in the Rat on Transfer of IgA Protein into Bile

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Severe thermal injury is associated with bacterial sepsis; the intestine is considered a likely source of invasive organisms. Because IgA antibody in bile accounts for much of the specific immune defense of the upper intestinal tract in the rat, the effect of thermal injury on the quantity of IgA protein in bile was examined. Sprague-Dawley rats received a 20% to 30% body surface area burn under anesthesia. Eighteen hours later the common bile duct was cannulated and bile was collected for three hours. Total IgA protein in bile decreased 90% after thermal injury. The bile volume, the concentration of bile protein, and free secretory component did not change significantly. Although blood flow to the liver 18 hours after thermal injury was not changed, there was a significant reduction in total IgA concentration in the circulation; both monomeric (m-IgA) and polymeric IgA (p-IgA) were decreased. This finding may explain, in part, the reduced concentration of IgA protein in bile. Although not examined in this study, decreased local hepatic synthesis and/or transport of p-IgA across the hepatocyte may also contribute to the reduced IgA levels in bile.

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DESPITE THE INTRODUCTION OF effective antimicrobial agents, bacterial sepsis remains an important cause of morbidity associated with severe thermal injury.[1] Although the damaged skin has been identified as the primary source of bacteremia,[2] the gastrointestinal tract has also been implicated as a potential source.[3] Translocation of bacteria across the intestine[3,4] after burn injury has been demonstrated in animals. Translocation can be enhanced by disturbance of the indigenous gastrointestinal microflora, impaired systemic immune defenses, and physical disruption of the mucosal barrier.[5-8] The normal intestinal barrier depends on specific (IgA antibody)[9,10] as well as nonspecific factors (gastric acidity, motility, mucus, digestive enzymes, and normal bacterial flora).[11] A loss of one of these components of the barrier may facilitate translocation of bacteria from the intestine into the circulation.

In the rat, IgA delivered to the intestine *via* bile accounts for 90% of the upper intestinal IgA protein.[12] The polymeric IgA protein in the blood is transported across the hepatocyte or bile duct cells *via* receptor-mediated (secretory component) endocytosis, is exocytosed as secretory IgA (s-IgA) into bile canaliculi, and carried into the upper small intestine *via* the bile duct system.[10-16] For normal transport to occur, circulating and locally synthesized p-IgA must be available[17,18] and the hepatocyte p-IgA transfer mechanism must be functional. In this study, we examined the transport of IgA protein into bile after thermal injury to assess whether this component of specific immune defense of the intestine was compromised.

Materials and Methods

Animal Model

Female CD-1 rats (Charles River Breeding Lab, Wilmington, MA) weighing 160 to 190 gm were subjected to cutaneous thermal injury by a modification[19] of the method of Walker and Mason.[20] After induction of anesthesia (Brevital, Eli Lilly, Indianapolis, IN; 50 mg/kg body weight), the backs of the rats were shaved. The rats were

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placed in a plastic mold and approximately 20% of body surface area was exposed to boiling water for 15 seconds. The rats were then given an intraperitoneal (IP) injection of saline (50 ml/kg body weight), placed in individual metal cages, and given free access to water. Control rats were anesthetized, shaved, given saline ip, and placed in metal cages.

Bile Collection

Eighteen hours after the thermal injury, rats were anesthetized with ether. A midline laparotomy was performed and the common bile duct was cannulated with PE-50 tubing (Clay Adams, Parsippany, NJ). Immediately after surgery bile was collected in one-hour samples for three hours. Bile was collected into borosilicate glass tubes placed in ice 30 cm below the level of the animal. Light ether anesthesia was maintained during the three-hour collection period. Bile was frozen at −20 C before assay.

Measurement of Total IgA Protein and Free Secretory Component (FSC)

Total IgA protein in serum and bile and free secretory component were measured by radioimmunoassay (RIA) using double antibody precipitation techniques according to the methods of Sullivan and Allansmith[21] and Sullivan and Wira.[22] Rat reference serum (Miles Laboratories, Elkhart, IN) containing both monomeric and polymeric IgA protein and purified rat SC were used as standards. Purified rat secretory IgA and purified rat SC were iodinated with $Na^{125}I$ (New England Nuclear, Boston, MA) using Iodogen (Pierce Biomedical, Rockford, IL). For the assay, "first" antibodies consisted of goat anti-rat IgA (Miles Laboratories) or rabbit anti-rat SC. The "second" antibodies, rabbit anti-goat IgG and goat anti-rabbit IgG (Miles Laboratories) were used with the appropriate first antibody. Addition of bile from burn or control animals did not interfere with the measurement of total IgA protein in serum in our assay.

Measurement of Total Protein in Serum and Bile

Protein determinations in bile and serum were performed by the Hartree assay.[23] Bovine serum albumin was used as the standard.

Characterization of IgA Protein in Serum and Bile by Bio-Gel A1.5 Gel Permeation

The molecular size of IgA protein in serum or bile was estimated by gel permeation using a Bio-Gel A1.5 (Bio-Rad, Richmond, CA) column (1.5 cm × 80 cm); the total IgA content of the eluted fractions was estimated by RIA. One milliliter of serum or bile pooled from 3 experimental or 3 control animals was applied to the column and eluted with phosphate-buffered saline (0.01M phosphate, 0.15M NaCl, 0.05% azide, pH 7.4); 2.8-ml fractions were collected. The column was calibrated with Dextran Blue 2000 (DB, M_r [relative molecular mass] $1–2 \times 10^6$, Pharmacia, Piscataway, NJ), bovine thyroglobulin Type I (TBG, M_r 6.6×10^5, Sigma, St. Louis, MO), bovine gamma globulin (BGG, M_r 1.6×10^5, Sigma, St. Louis, MO) Vitamin B_{12} (B_{12}, M_r 1.4×10^3, Sigma, St. Louis, MO). IgA concentration in serum or bile was plotted *versus* elution volume. The percentage of IgA protein in each peak was estimated by cutting out and weighing the peaks.

Assessment of Hepatic Blood Flow

Radiolabelled microspheres were used to measure cardiac output (CO) and regional distribution of blood flow. Cannulas (PE-50) were inserted surgically into the left ventricle *via* the carotid artery and into the femoral artery. Incisions were closed and the animals were allowed to recover from anesthesia. The rats were placed in a restraining cage. Microspheres (15 ± 0.1 microns in diameter), labelled with cesium-141 (^{141}Ce) (New England Nuclear) were used according to reported methods.[24–26] A 0.5 ml suspension of microspheres (400 spheres/g body weight) was injected into the left ventricle over a period of 20 seconds. Simultaneously a reference blood sample was obtained in a heparin-containing syringe from the femoral artery by a syringe pump at 0.57 ml/minute (5.7 ml total). After completing the microsphere injection, the carotid cannula was flushed with warmed normal saline at a rate equal to the reference sampling. After ten minutes, the animals were killed by an intra-arterial injection of KCl. Liver, small intestine, and colon samples were obtained, rinsed with saline, blotted, weighed, and placed in scintillation vials. Radioactivity was determined in a Compu Gamma 1282 (LKB Instruments, Gaithersburg, MD).

Calculation of the cardiac output (CO) in ml/min/kg body weight were determined as follows:

$$CO = \frac{\text{counts per minute injected}}{\text{counts per minute in reference sample}} \times \text{reference sample withdrawal rate} \times \text{body weight} \quad (1)$$

Organ blood flow (OBF) in ml/min/kg body weight was calculated from the formula

$$OBF = \frac{\text{counts per minute in organ}}{\text{counts per minute injected}} \times CO \quad (2)$$

(The liver blood flow was considered to be the sum of splanchnic blood flow and hepatic artery blood flow.)

Statistical Analysis

The specific analysis for each data set is noted in the text; analysis was performed using the Systat program (Systat, Evanston, IL).

Results

Effect of Thermal-Injury on Total IgA Protein, Free Secretory Component, and Total Protein in Bile

To evaluate the effect of thermal injury on biliary IgA protein content, bile was collected 18 hours after burn treatment in one-hour samples for three hours. As shown in Figure 1, the total IgA protein was decreased six to seven times ($p < 0.001$, ANOVA for repeated measures) in each one-hour sample. Biliary IgA protein concentration was also decreased sixfold after thermal injury; the difference in concentration of IgA in thermally injured compared to sham-treated rats was significant ($p < 0.001$) (Table 1). In contrast, no significant differences were noted in the concentration of total protein, free secretory component, or bile volume of thermally injured compared to the sham-treated groups.

The molecular size of IgA protein in bile was examined by gel permeation using a Bio-Gel A1.5 column. The IgA protein in sham-treated animals eluted predominately as a polymer with a molecular size greater than M_r 160,000 (Fig. 2). After thermal injury, the major peak was reduced ≥ 88% (results observed in two experiments each involving three thermally injured and three control rats.)

Effect of Thermal Injury on Circulating IgA Protein

Because transfer of IgA protein into bile depends, in part, on the amount of IgA protein transported to the liver *via* the circulation, the concentration of IgA protein in the circulation was measured. After thermal injury, a significant ($p < 0.001$ t-test for independent groups) decrease in total serum protein and in serum IgA concentration was noted (Table 2).

Because IgA protein transport from the circulation into bile involves primarily polymeric IgA, the molecular size of IgA protein in the circulation was examined after burn injury. A large decrease (59%) in monomeric IgA and a smaller (20%) decrease in polymeric IgA was noted (Fig. 3).

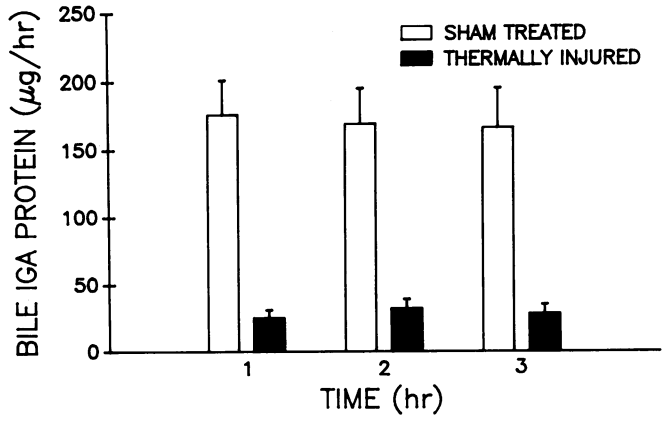

FIG. 1. IgA protein content of bile obtained 18 hours after thermal injury (n = 5) and sham treatment (n = 5). Bile was collected for three hours after common bile duct cannulation. Bars: Mean ± SEM. There is significantly less IgA protein in bile of thermally injured compared to sham-treated rats ($p < 0.001$ for treatment effect determined by ANOVA for repeated measures).

Assessment of Hepatic Blood Flow After Thermal Injury

Transport of p-IgA protein to the liver *via* the circulation is dependent on blood flow to the liver and concentration of p-IgA in the blood. Hepatic blood flow, as determined using radiolabelled microspheres, was calculated from cardiac output and the sum of radioactive microspheres trapped in the liver (representing arterial hepatic flow) and the splanchnic bed (representing portal vein flow) as described in Materials and Methods. No change was found in hepatic blood flow of thermally injured (28.2 ml/min/kg) compared to sham-treated rats (28.4 ml/min/kg) 18 hours after thermal injury.

Discussion

We have demonstrated a 75% to 90% reduction in both the amount and concentration of IgA protein secreted in bile 18 hours after thermal injury in the rat. The possibility

TABLE 1. *Characterization of Bile from Thermally Injured and Sham-Treated Sprague-Dawley Rats*

	Total IgA (μg/hr)	IgA Conc. (μg/ml)	Protein Conc.† (mg/ml)	FSC Conc.† (μg/ml)	Bile Volume (μl/hr)
Thermally injured (n = 12)	37 ± 9*	122 ± 16	5.2 ± 0.8*	74 ± 16	279 ± 37
Sham-treated (n = 11)	223 ± 26	693 ± 52	5.2 ± 0.3	61 ± 5	321 ± 28
	p < .001	p < .001	NS‡	NS‡	NS‡

* Mean ± SEM.

† FSC, Free secretory component; n = 8 in both thermally injured and sham-treated groups.

‡ Not significant.

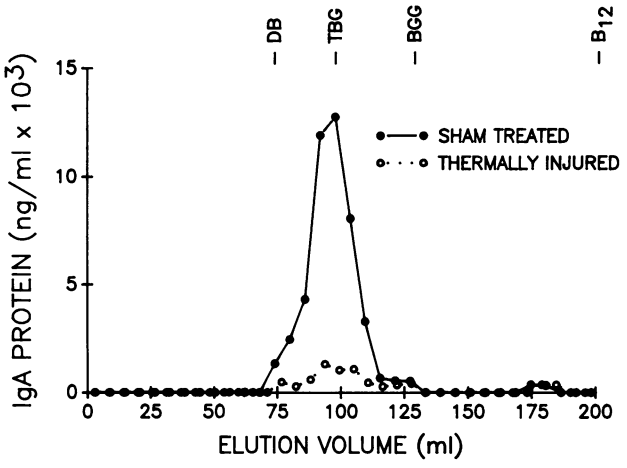

FIG. 2. Characterization of IgA protein in the bile from thermally injured and sham-treated rats by Bio-Gel A1.5 permeation. The gel column (1.5 cm × 80 cm) was eluted with PBS; 2.8-ml fractions were collected. Each fraction was examined by RIA for IgA protein. Column calibration markers included Dextran Blue 2000 (DB, M_r 1–2 × 10^6), thyroglobulin (TGB, M_r 6.6 × 10^5), bovine gamma globulin (BGG, M_r 1.6 × 10^5), and Vitamin B_{12} (B_{12}, M_r 1.4 × 10^3). The concentration of polymeric IgA protein in bile (large peak, outlined by solid dots, eluting in the position between the Dextran Blue and thyroglobulin markers) decreased ≥ 88% after thermal injury (small peak outlined by open circles).

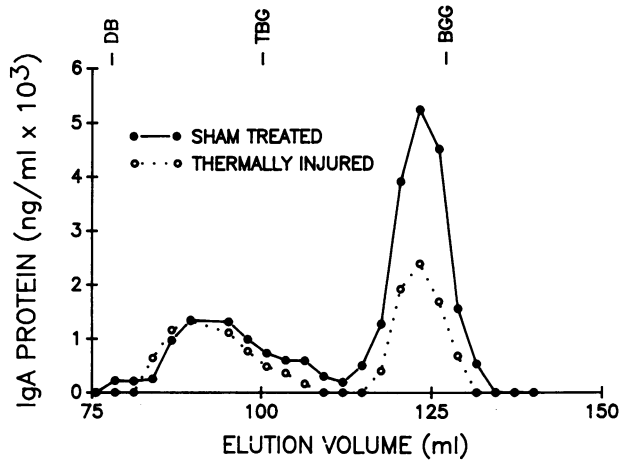

FIG. 3. Characterization of IgA protein in a serum pool from three thermally injured and three sham-treated rats. The A1.5 gel permeation column (1.5 cm × 80 cm) was eluted with PBS; 2.8-ml fractions were collected. Each fraction was examined by RIA for IgA protein. The calibration markers included Dextran Blue (DB, M_r 1–2 × 10^6), thyroglobulin (TBG, M_r 6.6 × 10^5), and bovine gamma globulin (BGG, M_r 1.6 × 10^5). Serum from both sham-treated (closed circles) and thermally injured rats (open circles) show two peaks of IgA protein; the first of these consists of polymeric IgA (eluting between the Dextran Blue and thyroglobulin markers) and the second of monomeric IgA protein (eluting near the BGG markers). After thermal injury, there was a major decrease in monomeric IgA and a minor decrease in polymeric IgA protein of serum.

that an artifact in the IgA protein assay might account for the differences observed was considered. After the addition of known amounts of IgA protein to bile from thermally-injured and normal rats, the predicted amounts of IgA protein were found in both cases (data not shown). The total protein concentration in bile from thermally injured and sham-treated rats was not different, suggesting that the mechanism(s) responsible for the large reduction in biliary IgA protein was specific for this protein. The change in biliary IgA also appears independent of the bile volume; therefore the change in IgA content of bile is unlikely to be related to factors that alter bile volume.[27–29] Kloppel[30,31] suggested that brief periods of bile duct stasis induced by collecting bile from a cannula placed above the level of the animal, or by briefly clamping the common bile duct, can disrupt normal transport of IgA protein into bile. Although the bile duct might have briefly obstructed during insertion of the cannula, both the experimental and sham-treated groups had identical surgery and bile was collected *via* a cannula placed below the level of the animals throughout the experiment.

TABLE 2. *Characterization of Serum from Thermally Injured and Sham-Treated Sprague-Dawley Rats*

	IgA (μg/ml)	Protein (mg/ml)
Thermally injured	75 ± 5*	59 ± 2
	(N = 9)	(N = 6)
Sham-treated	132 ± 8	76 ± 3
	(N = 9)	(N = 6)
	p < .001	p < .001

* Mean ± SEM.

Secretion of IgA protein into bile depends on the supply of polymeric IgA presented to the hepatocyte or bile duct cell, and the capacity of these cells to bind polymeric IgA to secretory component on the sinusoidal surface, to transport the SC-pIgA complex across the cell within a vesicle, and to release the SC-pIgA complex into bile.[32] Supply of p-IgA to the hepatocyte and bile duct cells is dependent on p-IgA delivered *via* the circulation and p-IgA synthesized locally within the liver.[17,18] In this report, we examined delivery from the circulation. Delivery of p-IgA depends on blood flow to the liver, normal perfusion of hepatocytes within the liver, and the concentration of p-IgA in the plasma. Blood flow to the liver was measured using radioactive microspheres. The total blood flow to the liver appeared normal 18 hours after injury, although we could not define the distribution of flow within the liver by this technique. Total IgA protein in serum was decreased. Because only the polymeric form of IgA is effectively transferred into bile, it was important to examine the effect of burn injury on this molecular species. The polymeric IgA was decreased approximately 20%; monomeric IgA showed a greater change (a 59% decrease). Differences in the effect of thermal injury on plasma cell populations producing p-IgA or m-IgA, or differences in the effect of thermal injury on the rate or sites of clearance

of these two molecular species may account for the observed differences in concentration of IgA molecules. The supply of p-IgA available for transport into bile may also depend on local production of p-IgA within the liver.[17] Local production may be affected by thermal injury, but this possibility was not examined in our study.

In the present study, we have defined one factor, a reduction in blood level of p-IgA, that may be responsible for the decreased IgA level of bile in thermally injured rats. It will be necessary to examine local synthesis of p-IgA within the liver and the hepatic transport of p-IgA to evaluate the contribution of these factors to the reduced p-IgA content of bile. Reduced delivery of IgA antibodies *via* the bile may be involved in the altered immune defense of the gut of the burned animal.

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