Complement Depletion Enhances Pulmonary Inflammatory Response After Liver Injury

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Hepatic cryoablation can produce acute lung injury, with activation of nuclear factor (NF)-κB in the remnant liver and lungs, production of C-X-C chemokines, and neutrophil infiltration of the lungs. Activated complement stimulates NF-κB and cytokine secretion from Kupffer cells. The role of complement in the development of acute lung injury after cryoablation was examined using HLL transgenic mice (5′ *H*IV-*L*TR-*L*uciferase gene; 5′ HIV-LTR is an NF-κB–dependent promoter). Total complement depletion was achieved with preoperative administration of cobra venom factor (CVF). After hepatic cryoablation, bioluminescent NF-κB activity increased in the nonablated liver remnant by 4 hours in both control (119,093 ± 22,808 net RLU/mg protein) and CVF-treated mice (117,722 ± 14,932) from cumulative baseline (657 ± 90, $P < 0.0001$). In the lung, complement-depletion induced significantly greater increases in NF-κB activation at both early and later times. Likewise, chemokines were higher in complement-depleted mice relative to controls (KC: 493 ± 43 versus 269 ± 29 pg/mg protein, $P < 0.001$; MIP-2: 171 ± 29 versus 64 ± 13 pg/mg protein, $P < 0.0001$). Pulmonary myeloperoxidase activity was equivalent at 24 hours, but complement-depletion caused a significantly more rapid influx of neutrophils. Complement depletion results in increased pulmonary inflammation following liver cryo injury via relative upregulation of NF-κB activity. Activated complement is not the initiator of the systemic inflammatory response; in fact, downstream components of the complement cascade may diminish subsequent inflammation. (J GASTROINTEST SURG 2006;10:357–364)

KEY WORDS: Cryoablation, acute lung injury, complement, C3, CVF

Local ablative techniques for treating hepatic tumors, both primary and metastatic, are increasingly used as adjuncts to surgery, particularly in patients who are not candidates for standard oncologic resection. Cryoablation is one such method that has been used extensively to ablate liver masses.[1–3] This technique relies on the reduction of local temperatures to at least −35° C during a rapid freezing cycle, and it produces cell death through a variety of mechanisms, including (1) internal freezing with ice crystal disruption of cellular membranes, (2) solute/solvent shifts, and (3) hypoxia-induced programmed cell death from small vessel obliteration. [4–6] Initial clinical experience with hepatic cryoablation revealed the possibility of inducing a systemic inflammatory response, with the lungs seeming particularly susceptible to injury.[7] Acute lung injury tended to occur more frequently following larger ablations.[8] For unknown reasons, this acute inflammatory response is not observed as frequently following surgical resection of similar volumes of liver or when alternative methods of ablation are used.[4]

Prior research has demonstrated that the "cryoshock" phenomenon is mediated, at least in part, by activation of the transcription factor nuclear factor kappa-β (NF-κB) in the liver.[9–11] Binding of NF-κB to DNA leads to the production and elaboration of various cytokines (tumor necrosis factor α, interleukin [IL]-1β) and chemokines (IL-8, CINC), which then initiate a similar proinflammatory response in the lung, ultimately producing a mixed-cell pulmonary infiltrate and the resultant acute

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lung injury.[12] Presumably, the initial activation of NF-κB occurs in the hepatic Kupffer cells, which comprise over 80% of the body's tissue macrophages. However, why systemic inflammation occurs with large-volume cryoablation and not other hepatic ablative techniques is uncertain, and the initial stimulus of these NF-κB–dependent mechanisms remains unknown.

The complement cascade is a central component of innate immunity. The principal biological functions of the complement system include cell lysis, opsonization, potentiation of humoral responses, and activation of the inflammation cascade (via the anaphylotoxins C3a, C4a, and C5a).[13] Both C3a and C5a are known to stimulate NF-κB in cells of monocytic origin.[14] C3a modulates Kupffer cell prostaglandin synthesis and C5a induces secretion of C-X-C chemokines from alveolar macrophages.[15,16] Furthermore, upregulation of anaphylotoxin receptors in the liver and lung has been observed in other systemic proinflammatory states such as sepsis.[17]

The current study examined whether complement is the initial trigger of the NF-κB–mediated inflammation that follows large-volume hepatic cryoablation. An established experimental method of total complement depletion was used in combination with a model of cryoablation in HLL transgenic mice. Subsequently, we quantified the activation of NF-κB in both liver and lungs using bioluminescence, measured the generation of proinflammtory chemokines, and determined the degree of acute neutrophilic pulmonary infiltration. Our findings suggest that complement is not the initial proinflammatory stimulus within the liver and that downstream components of the complement cascade may actually diminish the systemic inflammatory response.

MATERIAL AND METHODS

Animal Model

All experiment were performed using transgenic mice expressing *photinus* luciferase cDNA under the control of the 5′ human immunodeficiency virus long terminal repeat promoter. The 5′-HIV-LTR is a known binding sequence for NF-κB, and the construct is termed HLL (5′-*H*IV-*L*TR-*L*uciferase gene). We have demonstrated successful quantification of NF-κB activation following hepatic cryoablation with this model using ex vivo measurement of luminescence.[10] Animals were housed under pathogen-free barrier conditions, acclimated to 12-hour light cycles, and provided access to food and water ad libitum throughout the experiments. All procedures were approved by the Washington University Animal Studies Committee.

Total complement depletion was achieved using serial doses of cobra venom factor (CVF) from the species *Naja kaouthai* as previously described.[18–20] This 149,000-kDa glycoprotein is a structural and functional C3 homolog that binds avidly to factor B. The CVF-Bb complex acts as a C3/C5 convertase, cleaving C3 and C5 to produce consumptive depletion of circulating complement through activation of both the classical and alternative pathways.[21] CVF was administered intraperitoneally in sterile phosphate-buffered physiologic saline (PBS, 200 μl) at 24 and 1 hour prior to hepatic cryoablation. Pilot studies were performed to determine the appropriate dose (15, 30, or 60 μg) necessary to achieve complete complement depletion (data not shown); subsequently, the 30-μg dose was used for all studies as this was the minimal dose that achieved near-total complement depletion. Mice receiving PBS only were used as controls.

Surgical anesthesia was established using ketamine (87 mg/kg) and xylazine 1% (13 mg/kg). Mice were then prepped and placed on a heating place to maintain systemic normothermia. Following midline laparotomy and limited hepatic mobilization, a commercial cryoablation device (Candela, Wayland, MA) that uses recirculating liquid N_2 through a 3-mm-diameter metallic probe was used to perform a 35% (by mass) ablation of the left lateral and median hepatic lobes. We have previously demonstrated a reproducible hepatic injury using this technique, and it consistently results in an acute, progressive systemic response and pulmonary inflammatory injury within 24 hours.[10,11,22] Following complete thawing and reperfusion of the injured liver, warmed PBS (2 ml) was instilled in the abdomen. The abdomen was then closed using a running 3-0 silk suture and metallic clips. Mice were killed with an overdose of anesthetic and rapid bilateral pneumothoraces at 4 or 24 hours postoperatively. The inferior vena cava was cannulated for phlebotomy. The liver (nonablated remnant) and lungs were collected sterilely, briefly rinsed in PBS, snap-frozen in liquid nitrogen, and stored in RNA/DNA-free containers at −80° C until further processing.

Complement Quantification

Serum intact C3 levels were quantified in experimental animals at the time of death using the radial immunodiffusion method of Mancini over 72 hours (National Jewish Hospital Labs, Denver, CO).