Tohoku J. Exp. Med., 2002, **198**, 23-29

Recombinant Human Interleukin-1α Increases Serum Albumin, Gc-Globulin, and α_1-Antitrypsin Levels in Burned Mice

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Department of Emergency and Critical Care Medicine, Graduate School of Medicine, Tohoku University, Sendai 980-8574, [1]*Department of Emergency and Critical Care Medicine, Nippon Medical School, Tokyo 113-8603,* [2]*Department of Surgery, University of Colorado, Health Sciences Center, Denver, CO, USA*

KOIKE, K., SHINOZAWA, Y., YAMAZAKI, M., ENDO, T., NOMURA, R., AIBOSHI, J., SAMARGHANDIAN, S., EMMETT, M. and PETERSON, V.M. *Recombinant Human Interleukin-1α Increases Serum Albumin, Gc-Globulin, and α_1-Antitrypsin Levels in Burned Mice.* Tohoku J. Exp. Med., 2002, **198** (1), 23-29 —— The response to thermal injury is a complex physiologic process requiring communication between sites of injury and distant target organs. The liver, one of these target organs, synthesizes a family of secretory proteins, the acute phase proteins, that carries out specific immunoprotective functions. In this study we investigated the effects of daily recombinant human interleukin-1α (rhIL-1α) administration on the serum levels of negatively regulated, i.e., albumin and Gc-globulin and positively regulated, i.e., α_1-antitrypsin, acute phase proteins in a murine model of thermal injury. Adult CF-1 female mice underwent a 6.5-seconds, 20% total burn surface area, full thickness steam injury, and received either intraperitoneal rhIL-1α (20 $\mu g \cdot kg^{-1} \cdot day^{-1}$) or diluent for 10 days. Seven and 14 days after injury, mice were sacrificed, and serum albumin, Gc-globulin and α_1-antitrypsin levels were measured by crossed immunoelectrophoresis technique. Thermal injury significantly lowered serum albumin levels, tended to decrease Gc-globulin levels, and increased serum α_1-antitrypsin levels. Daily rhIL-1α administration after burn injury prevented hypoalbuminemia, and increased serum levels of Gc-globulin and α_1-antitrypsin. IL-1 therapy might be helpful to maintain the homeostasis and immunity of the host after thermal injury. ———— thermal injury; acute phase proteins

Received May 30, 2002; revision accepted for publication September 18, 2002.

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Thermal injury is known to promote the synthesis of acute phase proteins in the liver. The biological purpose of the changes in plasma acute phase protein concentrations has been postulated to erect a protecting zone of demarcation around the area of burn injury (Schreiber and Howlett 1983). Proteinases, e.g., elastase and cathepsins, are released from lysozomes of destroyed cells and activated leukocytes, and participate in the destruction of normal tissues. The action of proteinases is inhibited by proteinase inhibitors such as α_1-antitrypsin. Most of the serum proteinase inhibitors are positive acute phase proteins, and the accelerated production of these proteins would pose increased demands on supply of aminoacyl transfer RNA and ATP. Such increases are compensated by a concomitant decrease in the synthesis of so-called negative acute phase proteins, such as albumin and Gc-globulin (Rothschild et al. 1988; Hiyama et al. 1991). The reduced serum levels of albumin and Gc-globulin, however, appear to inversely correlate with the mortality in burn and critically ill patients (Lee et al. 1989).

The biosynthesis of acute phase proteins in the liver is regulated by several factors including interleukin-1 (IL-1). IL-1 stimulates hepatocytes to synthesize a spectrum of positive acute phase proteins as well as complement components and clotting factors (Dinarello 1989). The property of IL-1 to induce acute phase proteins was also confirmed in vivo (Ramadori et al. 1985). Recent clinical studies demonstrated that burn patients who survived had significantly higher serum IL-1 concentrations than those who died (Cannon et al. 1992; Vindenes et al. 1998). This finding may indicate that IL-1 is an essential mediator of host defense after thermal injury. We, therefore, investigated the effects of recombinant human IL-1α (rhIL-1α) therapy on the serum concentrations of albumin, Gc-globulin, and α_1-antitrypsin after thermal injury in mice.

Materials and Methods

Thermal injury model

Female CF-1 mice were used for this study. Mice were fed standard lab chow and acidified water ad libitum. RhIL-1α was a generous gift from Hoffmann-La Roche Inc., Nutley, NJ, USA. Human serum albumin solution (Baxter Healthcare Corp., Glendale, CA, USA) was diluted to 0.05% in saline and used as vehicle for solutions for intraperitoneal (i.p.) injection. On the day of burn injury, mice were deeply anesthetized with methoxyflurane gas (Pitman-Moore, Inc., Washington Crossing, NJ, USA), and the shaved and depilitated dorsum of each mouse was exposed to steam for 6.5 seconds, resulting in a 20% total burn surface area (TBSA), total full-thickness burn (Koike et al. 1996). Immediately after thermal injury, mice were injected 1.0 mL of saline intraperitoneally as fluid resuscitation, caged individually, and given chow and water containing acetaminophen, 1 mg/mL. In the experimental group, daily morning i.p. injection of rhIL-1α (20 $\mu g \cdot kg^{-1} \cdot day^{-1}$) was begun 18 hours following burn injury and continued through postburn day 10. The dose of rhIL-1α was determined by the previous studies (Pojda and Tsuboi 1990; Winton et al. 1994). Equal amounts of vehicle were administered to burned-control animals. Seven or 14 days after thermal injury, mice were killed for the sampling of peripheral blood. In order to avoid studying mice with acutely elevated plasma levels of rhIL-1, injections were not performed in the morning of sacrifice. Venous blood was obtained from the inferior vena cava, allowed to clot at room temperature, and spun at $1000 \times g$, and the sera were frozen at $-70°C$ until assayed. Animal care and research protocols were approved in advance by the Institutional Animal Care and Use Committee of University of Colorado, Health Sciences Center.

Serum albumin, Gc-globulin, α_1-antitrypsin measurement

Serum levels of albumin, Gc-globulin, α_1-antitrypsin were measured by crossed immunoelectrophoresis technique (Emmett and Crowle 1981). In brief, pooled normal mouse serum, emulsified in incomplete Freund's adjuvant, served as immunogen to produce a polyspecific antiserum in female New Zealand white rabbits. Antiserum was stored at −20°C and, before use, was dialyzed overnight against TRIS barbital buffer, pH 8.6. Serum samples, 1 μL, were electrophoresed for 45 minutes at 18 V/cm and 4°C in the first direction with 1.4% agarose and 1% dextran T-10 in TRIS barbital buffer, pH 8.6, on double-width microscope slides as previously described (Emmett et al. 1984). Separated serum proteins were then electrophoresed for 2.5 hours at 18 V/cm and 4°C at right angles to their original travel into agarose previously charged with rabbit polyspecific antisera. The resultant presipitin loops were stained and examined, and serum protein levels were quantitated by determination of the area under the loop. Results for each test sample were compared with the mean value for normal mice, and data were expressed as a percent of normal control.

Statistics

Values were expressed as mean±S.E.M. Each data point includes 8 animals. Data were analyzed by two-way ANOVA followed by Fisher-PLSD post-hoc tests. Statistical significance was accepted at the 0.05 level.

RESULTS

Systemic response

Mice tolerated well the full-thickness 20% TBSA burn injury and the 14 days survival rate was ≧95%. Signs of unusual wasting, lethargy, or sepsis/endotoxemia (e.g., piloerection, conjunctivitis, diarrhea) were absent.

Serum albumin

Burn mice experienced statistically significant low albuminemia ($p<0.05$) at postburn days 7 and 14 (64.4±6.8%, 72.3±7.3%), compared to normal non-burned controls (Fig. 1). Administration of rhIL-1α significantly maintained burn-induced low albuminemia at postburn day 7 and 14 (day 7, 96.4±6.5%; day 14, 109.2±8.2%).

Serum Gc-globulin

Thermal injury induced a slight decrease in serum Gc-globulin levels at postburn day 7 (82.8±6.6%), and returned to normal by day 14 (109.4±12.1%) (Fig. 2). Administration of rhIL-1α after thermal injury significantly increased serum Gc-globulin levels on days 14 (168.1±17.3%).

Serum α_1-antitrypsin

Burned mice experienced a significant increase in serum α_1-antitrypsin levels by post-

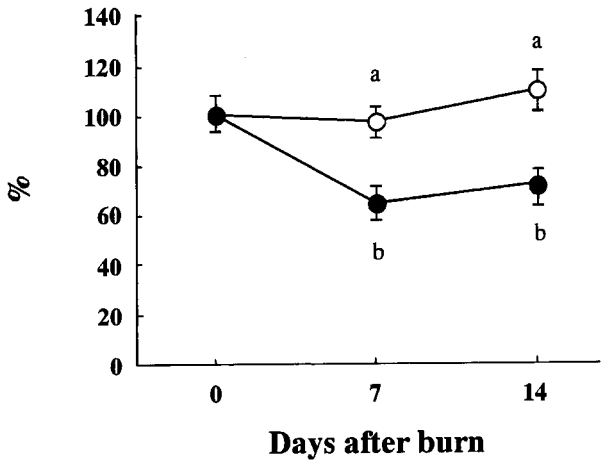

Fig. 1. The kinetics of serum albumin levels after thermal injury in groups of mice receiving daily injections of saline or rhIL-1α. Data are expressed as a percent of normal control. Values represent mean±S.E.M obtained from 8 mice.
[a] $p<0.05$, compared to the saline control mice at each postburn day.
[b] $p<0.05$, compared to day 0 in each group.
●, saline; ○, rhIL-1α.

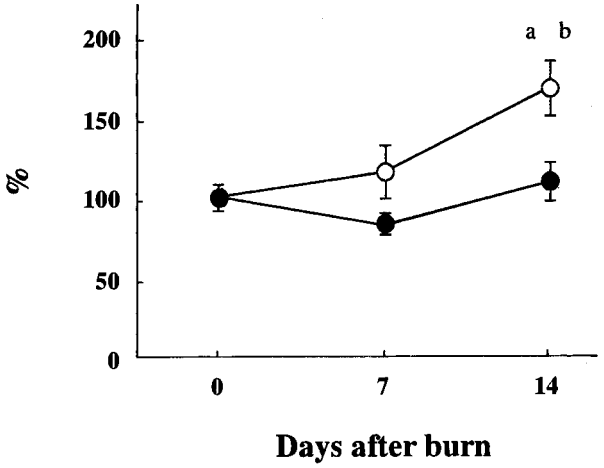

Fig. 2. The kinetics of serum Gc-globulin levels after thermal injury in groups of mice receiving daily injections of saline or rhIL-1α. Data are expressed as a percent of normal control. Values represent mean$\pm$S.E.M obtained from 8 mice.
[a]$p<0.05$, compared to the saline control mice at each postburn day.
[b]$p<0.05$, compared to day 0 in each group.
●, saline; ○, rhIL-1α.

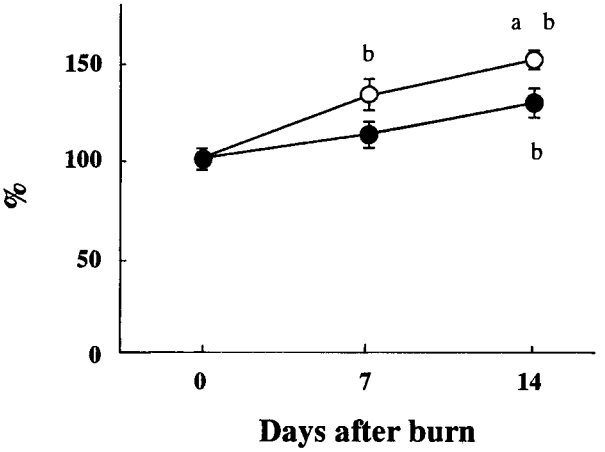

Fig. 3. The kinetics of serum α_1-antitrypsin levels after thermal injury in groups of mice receiving daily injections of saline or rhIL-1α. Data are expressed as a percent of normal control. Values represent mean$\pm$S.E.M obtained from 8 mice.
[a]$p<0.05$, compared to the saline control mice at each postburn day.
[b]$p<0.05$, compared to day 0 in each group.
●, saline; ○, rhIL-1α.

burn day 14 (day 7, 113.5$\pm$6.4%; day 14, 129.2$\pm$7.3%) (Fig. 3). Treatment with rhIL-1α further elevated these levels to 133.3$\pm$8.1% on day 7 and significantly to 151.2$\pm$4.9% on day 14.

Discussion

Thermal injury significantly lowered serum levels of albumin, tended to decrease Gc-globulin levels, and increased serum α_1-antitrypsin concentrations. Surprisingly, rhIL-1α administration following burn injury not only elevated serum α_1-antitrypsin levels, but also increased serum levels of negative acute phase proteins, albumin and Gc-globulin. Although in the current study we did not include a control group consisting of sham-burned animals that received injections of rhIL-1α, it has been documented that IL-1 injection in normal animals decreases serum concentrations of negative acute phase proteins and increases those of positive acute phase proteins (Ramadori et al. 1985; Dayer et al. 1989; Godson et al. 1995).

Albumin serves various physiological functions (Rothschild et al. 1988). It is a transport protein that tightly binds a wide range of physiologic substances and drugs such as bilirubin, hormones, and ions. Albumin also contributes to the maintenance of osmotic pressure and the pH of the body fluids. In the clinical setting, it is well known that the levels of serum albumin fall rapidly after severe burn injury by as much as 50%, and that low albumin levels often persist for many weeks (Moody et al. 1985). Jarrar et al. (1997) demonstrated using rats with 40% TBSA burn injury that albumin mRNA levels decreased on postburn day 7. We did not measure hepatic mRNA levels in this study, so that it is not clear why rhIL-1α therapy recovered serum albumin levels. So far, no previous report appears to explain this phenomenon. Recent clinical studies indicate that IL-1 could be an important mediator of host defence after thermal injury

(Cannon et al. 1992; Vindenes et al. 1998). RhIL-1α administration may have improved burn-induced hypoalbuminemia, somehow, by attenuating catabolism or extravascular protein leakage from the circulation.

Daily rhIL-1α administration following thermal injury also increased serum levels of Gc-globulin. While Gc-globulin is known as the main protein involved in plasma transportation of vitamin D, Gc-globulin also constitutes a central part of the extracellular actin scavenger system-a system responsible for the binding and clearance of extracellular actin (Lee et al. 1989, 1992). In conditions with massive cell death, the extracellular actin scavenger system may be exhausted, leading to the presence of F-actin, a polymerized form of actin, in the circulation. In animal experiments, F-actin was demonstrated to cause microthrombi and endothelial injury with organ failure (Haddad et al. 1990). Several clinical situations, e.g., septic shock and hepatic necrosis, were characterized by reduced serum Gc-globulin concentrations (Lee et al. 1989). An association between reduced concentrations of serum Gc-globulin, multiple organ dysfunction, and survival has also been demonstrated in patients with fulminant hepatic failure and trauma (Schiodt et al. 1997; Dahl et al. 1998). Only a few studies have been conducted to delineate the effects of cytokines on Gc-globulin synthesis. Using Hep 3B hepatocytes, Guha et al. (1995) measured changes in Gc-globulin mRNA synthesis and the level of secreted protein after treatment with cytokines or dexamethasone. While Gc-globulin belongs to the albumin gene family, treatment with IL-6 or dexamethasone increased those levels of Gc-globulin and decreased those levels of albumin. IL-1 did not cause any detectable changes in the levels of Gc-globulin mRNA and protein. These findings may suggest that the effect of cytokines on Gc-globulin synthesis is different from other negative acute phase proteins and that RhIL-1α administration in our current study have elevated serum concentration of Gc-globulin through the subsequent induction of IL-6 and anti-inflammatory hormones.

RhIL-1α therapy also increased serum α_1-antitrypsin levels after thermal injury. Alpha$_1$-antitrypsin belongs to a family of plasma serine protease inhibitors that constitute a subgroup of acute phase proteins including α_1-antichymotrypsin, angiotensinogen, C1-inhibitor, antithrombin, and antiplasmin (Carrell et al. 1982; Arnaud and Chapuis-Cellier 1988). These proteins are synthesized in response to inflammatory, infective, or traumatic stimuli, and they all serve protective functions. Alpha$_1$-antitrypsin has an inhibitory activity against neutrophil elastase, chymotrypsin, cathepsin G, trypsin, collagenase, plasmin, and thrombin (Carrell et al. 1982). Experimentally produced deficits in α_1-antitrypsin result in the death of experimental animals (Balldin et al. 1978). Using both Buffalo rats, which are highly resistant to burn injury, and Fisher 344 rats, which show very low resistance after burn injury, Xia et al. (1992) noticed that Buffalo rats showed higher levels of α_1-antitrypsin mRNA before and after burn injury. This finding may support the concept that α_1-antitrypsin contributes to the host's tolerance to thermal injury. Since IL-1β alone showed minimum effect on the induction of α_1-antitrypsin mRNA and the combination of IL-1β with IL-6 revealed synergistic effect in Hep 3B cell line (Jiang et al. 1995), rhIL-1α injection in our study may also have elevated serum concentration of α_1-antitrypsin by stimulation of IL-6. The other source of α_1-antitrypsin production appears to include peripheral blood mononuclear cells (PBMC) and intestinal epithelial cells (Knoell et al. 1998; Molmenti et al. 1993). When PBMC from normal healthy volunteers were treated with IL-1β, 2- to 3-fold increase of α_1-antitrypsin was observed in conditioned supernatants. Similar findings were seen in a human intestinal epithelial cell line Caco2.

CONCLUSION

Daily rhIL-1α administration elevated serum levels of albumin, Gc-globulin, and α_1-antitrypsin after thermal injury. It has not been ascertained whether the production of these acute phase proteins increased or the excretion of them decreased. Further study is needed to elucidate the underlying mechanism. However, this study may bring up a new hypothesis that IL-1 therapy yields beneficial effects on the clinical course of burn injury.

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