CHARACTERIZATION OF THE INFLAMMATORY RESPONSE DURING ACUTE AND POST-ACUTE PHASES AFTER SEVERE BURN

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Content

• Introduction
• Material and Methods
• Results
• Discussion
Introduction

- The trauma of severe burn injury induces a distinct systemic inflammatory response.

- Cytokines are the primary mediators of this inflammatory reaction to injury.

- They constitute a group of proteins with autocrine and endocrine activities that provide communication among different types of cells.

- Cytokines regulate homeostasis and cellular repair through effects on cell growth and differentiation via receptor activation.

- Various cytokines such as IL-1, IL-6, and TNF have been used as markers of the severity of burn injury.

- In a pediatric cohort 16 of 17 measured cytokines were significantly elevated throughout the first week after burn when compared with healthy children.
- Increased proinflammatory cytokine synthesis posttrauma leads to hypermetabolism and catabolism.

- As a consequence, the structure and function of essential tissues are compromised and contribute to multiorgan failure and mortality.

- Changes in cytokine levels occur before alterations in metabolism.

- It may be possible to modulate the hypermetabolic response postburn by exogenously modulating cytokine expression levels.

- Rodents are particularly attractive because of the availability of genetically homogeneous individuals, low cost, and ease of handling.
• Rodents lack a homolog of human IL-8, but they express a recently characterized cytokine with similar properties and functions termed cytokine-induced neutrophil chemoattractant (CINC).

→ four isoforms (CINC-1, CINC-2α, CINC-2β, and CINC-3)
→ identified as potent chemotactic factors belonging to the IL-8 family

• Here, they studied the kinetics of alterations in the serum cytokine profiles of a rat burn model immediately postburn and throughout a period of 7 days.
MATERIALS AND METHODS

- Approved by the Institutional Animal Care and Use Committee of the University of Texas Medical Branch at Galveston

Burn injury

- Sprague-Dawley rats weighing 325 to 350 g were used
- Were housed in an institutional animal care facility and received a high-protein diet and water ad libitum throughout the study
- Animals were anesthetized with general anesthesia and received analgesia.
- The dorsum of the trunk and the abdomen were shaved and a 60% total body surface area burn was administered by placing the animals in a mold, which was placed in 96° to 98° water, scalding the back for 10 s and the abdomen for 2 s.
This method delivers a full-thickness cutaneous burn as confirmed by histologic Section.

Lactated Ringer solution was administered immediately after the burn for resuscitation.

After burn and resuscitation, animals were observed, received oxygen, and were then placed into cages.

**Cytokine determinations**

Animals were euthanized by decapitation without anesthesia 1, 3, 6, 12, 24, 48, 96, and 168 h after burn injury.

Blood was collected immediately after decapitation and stored on ice until serum preparation.

The levels of IL-1, IL-6, IL-10, TNF-α, VEGF, CINC-1, CINC-2, CINC-3, and MCP 1 were determined by double-sandwich, enzyme-linked immunosorbent assays.
**Statistical analysis**

- The data were analyzed using t test or Mann-Whitney rank sum test.

**RESULTS**

- Burn led to a significant reduction in weight during the duration of the study: weight reduction of approximately 10% of the original weight.

- During the 168-h study period, significant increases were found in the serum levels of certain cytokines.

- Thermal injury resulted in augmented serum CINC-1 and CINC-2 concentrations up to 24- and 16-fold - as well as IL-6 and MCP-1 levels up to 5- and 10-fold.

- IL-1 beta, CINC-3, and IL-10 were also found to be significantly increased after burn.

- Serum levels of TNF-α and VEGF in burned rats were not significantly different in nonburned rats.
- IL-1 beta showed a significant increase 3 h postburn injury. These levels peaked at 12 h, remained significantly elevated for up to 48 h, and then rapidly decreased to basal levels at 96 and 168 h.
- IL-6 increased immediately after burn injury - peak at 6 h and then gradually decreased for the remaining of the study
- IL-10 showed a biphasic progression with a dramatic increase upon burn injury - peaked at 3 h after burn injury and gradually decreased subsequently.

- IL-10 levels showed an additional increase at 96 h postinjury before decreasing to normal levels at the end of the period studied.
- CINC 1 concentrations increased immediately after burn injury with a peak at 12 h

- During the remaining time of the study, CINC-1 levels decreased gradually but remained significantly elevated until the end of the investigation
Unlike CINC-1, the levels of CINC-2 did not dramatically increase immediately after burn injury.

Instead, CINC-2 levels displayed relatively constant values for up to 24 h after burn.

At 48 h, a dramatic increase in the levels of this cytokine was observed, with a further increase at 96 h that was maintained for the remainder of the study.
- The serum concentrations of CINC-3 followed a biphasic progression during the time line studied.

- Levels of this particular chemoattractant increased significantly at 3 h postburn injury, with a peak at 6 h. After a second dramatic decrease at 12 and 24 h, CINC-3 levels increased again with a late peak at 96 h and maintained significantly elevated for the remainder of the study.
- The levels of MCP-1 showed a significant increase immediately after injury. These levels were maintained for up to 12 h.

- A peak at 48 h was observed, with subsequent decrease in the levels of this cytokine at 96 and 168 h.
DISCUSSION

• Severe burn trauma induces a distinct systemic inflammatory reaction in patients

• Release of proinflammatory mediators - protein wasting and organ dysfunction

• Contributes to increased incidence of infection and sepsis, factors that augment the risk of multiple organ failure and death

• Alterations in the levels of cytokines postburn occur before the observed metabolic abnormalities

• It may be possible to design therapeutic interventions that attenuate the hypermetabolic response by decreasing the expression of cytokines associated with it.
The aim of this study was to assess the cytokine profile of a rat burn model that can be used for the characterization in alterations of cytokine expression patterns after severe burn injury during the acute and postacute inflammatory phases. The results showed that proinflammatory and anti-inflammatory cytokines, including IL-1β, IL-6, IL-10, CINC-1, CINC-2, and CINC-3, as well as MCP-1, are significantly elevated in rodents up to 7 days after severe burn injury. This general behavior of the cytokines in our model was similar to the cytokine response in burned patients. Most cytokines recently measured in a pediatric cohort were elevated during the first week after burn and decreased significantly over time to approach concentrations of normal, unburned children - Similar, in this study. The levels of most cytokines measured were augmented during the first days postburn.
• In humans, early markers of inflammation include IL-1β, IL-6, IL-8, IL-10, and TNF-α. IL-1 and IL-8 typically display the highest levels at the time of patient admission to the hospital.

• IL-1 seems to be a key component of the inflammatory mediator cascade, regulating the host response to infection, injury, and inflammation.

• IL-8 is a key cytokine mediator of the acute-phase response to injury and infection.

• However, IL-8 does not exist as such in rodents → rats express CINC-1, CINC-2, and CINC-3 proteins (functional isoforms of IL-8).

• In this study, the expression profiles of CINC cytokines were not similar among themselves. CINC 1 was observed to increase immediately after burn in a manner similar to that of IL-8 in humans.
CINC 2 showed a considerable lag before displaying a significant increase. Finally, there were two peaks associated with CINC-3 expression: one immediately postburn and a second one near the end of our study.

Similarly to IL-8, IL-6 plays a pivotal role in mediating the acute-phase response - associated with complications and mortality.

The role of IL-6 during inflammation remains controversial → has both proinflammatory and anti-inflammatory properties.

IL-6 increased immediately after burn and remained elevated throughout the length of the study. (similar result was observed for this cytokine in a recent study of burned pediatric patients → rises within 2 to 4 h and peaks at 6 to 12 h)

The levels of IL-10 in the rat were also elevated immediately postburn and showed fluctuation throughout the study.
• The ratio of IL-6 to IL-10 has been reported to predict mortality in critically ill patients with systemic inflammatory syndrome.

• IL-10 is a critical mediator of immunosuppression after traumatic injury → it seems to induce decreased resistance to infection

• Monocyte chemotactic protein 1 is a member of the β-chemokines and plays a crucial role in the trafficking and recruitment of effector leukocytes to primary sites of immune responses and inflammation.

• In the rat, we found that the expression of MCP-1 was elevated immediately postburn, with a significant increase at 24 and 48 h after burn trauma.

• A similar result was observed for this cytokine in our recent study of burned pediatric patients: after an early peak postburn, plasma concentrations of MCP-1 started decreasing 6 days postburn injury.
• Vascular endothelial growth factor is one of the most potent of vascular regulation in angiogenesis and vascular permeability and has been shown to be elevated in severely burned patients.

• TNF is a cytokine produced mainly by macrophages and monocytes and primarily increased in burn patients with sepsis.

• In burn rats, VEGF and TNF serum levels were not significantly different to nonburned rats.

• The burn treatment led to a hypermetabolic state - as is observed in human patients.
The hypermetabolic response after a major burn is characterized by a hyperdynamic response with increments in a wide variety of metabolic outcomes, including body temperature, oxygen and glucose consumption, carbon dioxide production, and muscle proteolysis.

This response begins on the 5th day postinjury and continues up to 24 months postburn, leading to a significant loss of lean body mass, muscle weakness, and poor wound healing.
SUMMARY

• They analyzed a panel of serum cytokines in rats over time, known to increase in humans postburn that may serve as reference for future development of therapeutic interventions.

• Various cytokines were observed in our rat burn model to follow a similar kinetic profile to that of humans.
Thank you for your attention!