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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-2 α , CINC-2 β , 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.

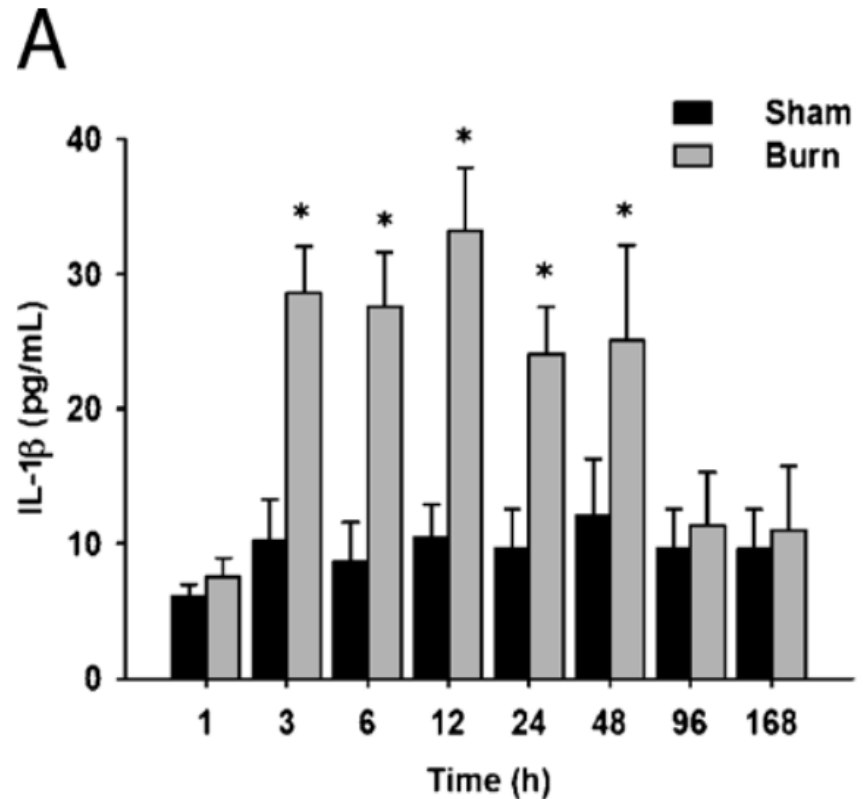
Statistical analysis

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

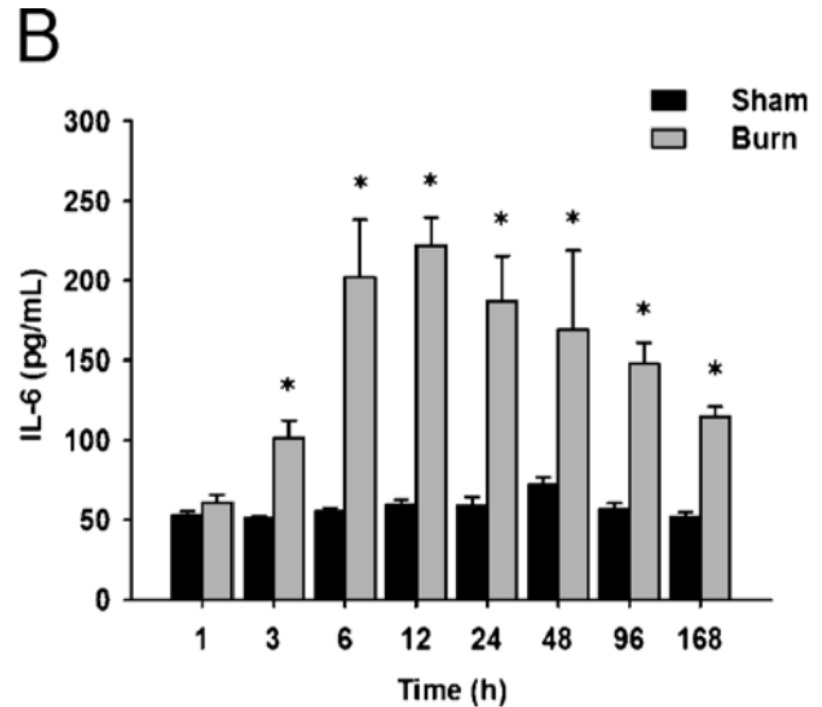
RESULTS

- 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.

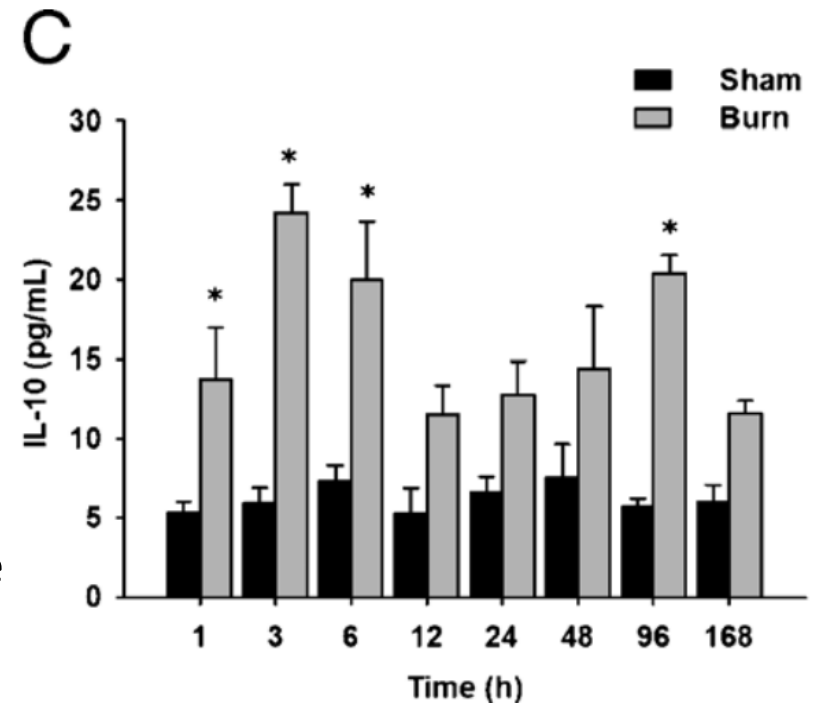
- IL-1 beta showed a significant increase 3 h postburn injury - These levels peaked at 12 h, remained significantly elevated for up to 48 h, and then rapidly decreased to basal levels at 96 and 168h



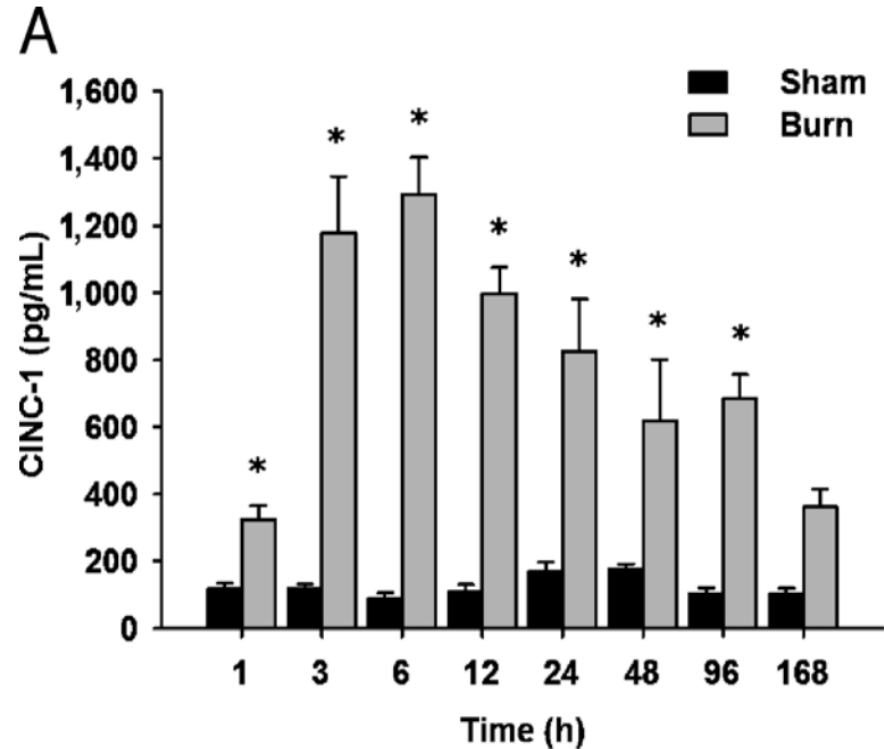
- IL-6 increased immediately after burn injury - peak at 6 h and then gradually decreased for the remaining of the study



- 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

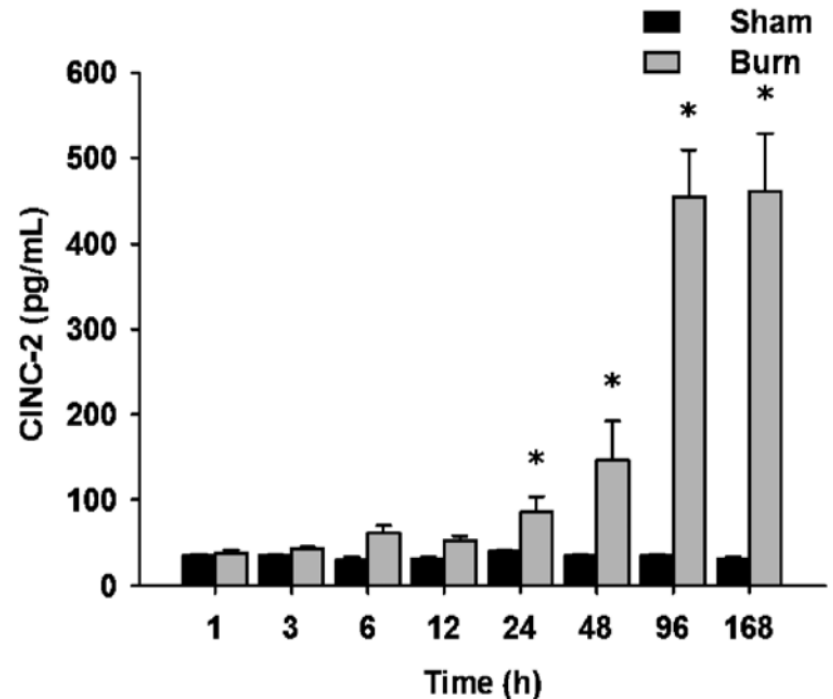


- CINC 1 concentrations increased immediately after burn injury with a peak at 12 h
- During the remaining time of the study, CINC-1 levels decreased gradually but remained significantly elevated until the end of the investigation



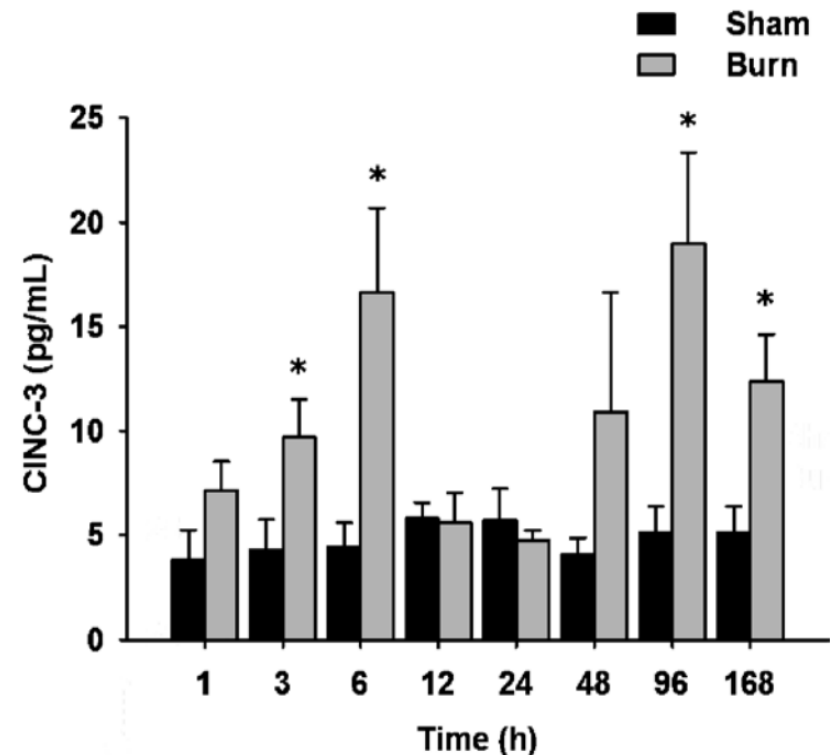
- Unlike CINC-1, the levels of CINC-2 did not dramatically increase immediately after burn injury.
- Instead, CINC-2 levels displayed relatively constant values for up to 24 h after burn.
- At 48 h, a dramatic increase in the levels of this cytokine was observed, with a further increase at 96 h that was maintained for the remainder of the study .

B

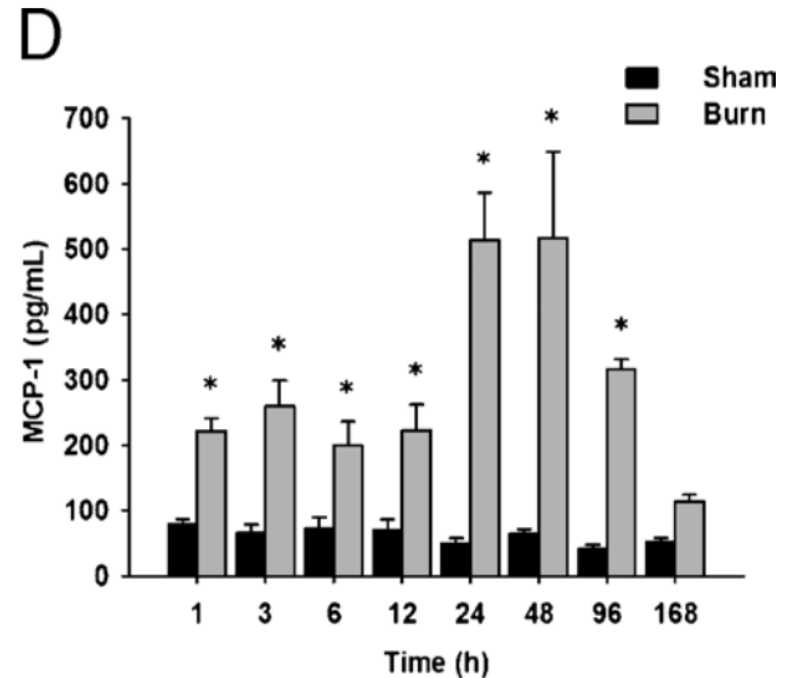


- The serum concentrations of CINC-3 followed a biphasic progression during the time line studied.
- Levels of this particular chemoattractant increased significantly at 3 h postburn injury, with a peak at 6 h. After a second dramatic decrease at 12 and 24 h, CINC-3 levels increased again with a late peak at 96 h and maintained significantly elevated for the remainder of the study.

C



- 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-1 β , 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-1 β , 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 \rightarrow 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.



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Thank you for your attention!