# Persistent Elevation of TNF-$\alpha$ in Burn Patients May Contribute to Compromised Wound Healing

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**ABSTRACT.** Tumor Necrosis Factor-$\alpha$ (TNF-$\alpha$)/cachectin has been implicated as a mediator of many of the adverse host responses to injury and infection. We evaluated elevated levels of this mediator that persists in burn patients, leading to compromised wound healing.

Serum samples taken from 36 pediatric burn patients were collected over a period of six years and stored at −70°C until analyzed by an ELISA for TNF-$\alpha$. All patients who were less than 3 months post-burn with greater than 50% total body surface area (TBSA) burns had elevated TNF-$\alpha$ levels ($95\pm10$ pg/ml). Patients more than 6 months post-burn with greater than 30% TBSA burns also had elevated TNF-$\alpha$ levels (with 30-50% TBSA burns, the levels were $60\pm11$ pg/ml, and with $>50\%$ TBSA burns, the levels were $83\pm8$ pg/ml).

In the present study, we have demonstrated that serum TNF-$\alpha$ is detectable with greater frequency and in higher concentrations in patients with major burns, up to five years after the injury. This may be a factor contributing to the increased rate of infections and delayed wound healing seen in these patients. Studies also suggest a yin-yang relationship between TNF-$\alpha$ and scarring. These investigations support the implication that TNF-$\alpha$ in low concentrations helps wound healing, whereas higher concentrations may be detrimental.

**Key words: TNF-$\alpha$— burn patients — wound healing — scarring**

Tumor Necrosis Factor-$\alpha$ (TNF-$\alpha$)/cachectin is a monocyte/macrophage-derived polypeptide cytokine implicated as a primary mediator of many of the catastrophic host responses to infection of endotoxin.[1,2] It has also been suggested that TNF-$\alpha$ is involved in mediating septic shock as it is elevated in some patients with sepsis.[3,4] Apart from endotoxic shock, TNF-$\alpha$ has been reported to be a mediator of other processes such as cancer cachexia and hematopoiesis.[5] Recent experimental observations suggest that TNF-$\alpha$ may modulate wound healing.[5,6] TNF-$\alpha$ stimulates human diploid fibroblasts in culture, stimulates chemotaxis of bovine endothelial cells and modulates angiogenesis in the rat cornea and chick chorioallantoic membrane.[6] The purpose of this study was to evaluate the serum of burn survivors at various post-burn intervals, to ascertain elevated levels of TNF-$\alpha$ in these patients and

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relate its persistence to the severity of initial burn injury. This information may bear on the long-term healing response of the wound.

## PATIENTS AND METHODS

A total of 36 pediatric burn patients who were admitted to the Shriners Burns Institute in Galveston, Texas between 1987 and 1992 were assessed. They ranged in age from 4 to 19 years old, with the average age being 10.6 years old. There were 25 males and 11 females. Serum samples were collected over a period of six years and stored at −70°C. The size of burns ranged from 15% to greater than 90% total body surface area (TBSA) burns.

Serum TNF-$\alpha$ levels were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) Provided by Genzyme Corporation, Cambridge, MA, USA. Aliquots of freshly diluted standard concentrations of human recombinant TNF-$\alpha$ (rTNF-$\alpha$) and serum samples were incubated in duplicate on a 96-well ELISA plate to which monoclonal antibody had been bound. ELISA wells were then sequentially exposed to a second antibody, polyclonal rabbit anti-TNF-$\alpha$, a third antibody, polyclonal goat anti-rabbit lgG, and then to streptavidin-peroxidase for amplification and, finally, to substrate reagent to develop color ranges (Fig 1). Equivalent concentrations of rTNF-$\alpha$ were determined for experimental samples by interpolation of the rTNF-$\alpha$ standard curve run on each assay plate. Absorbance was determined on a microtiter plate ELISA reader (spectrophotometer) at 492 nm.

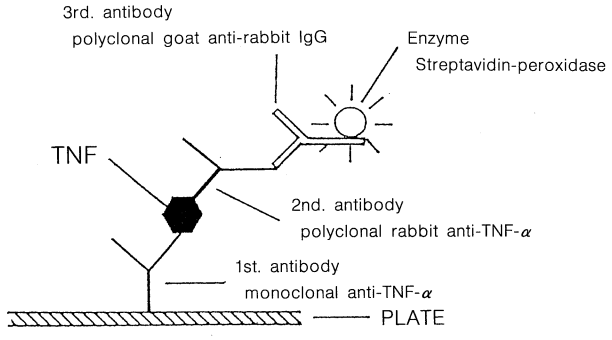


Fig 1. ELISA (Enzyme-Linked Immunosorbent Assay) of TNF-$\alpha$

The data were analyzed by dividing into the following categories (Table 1):

(1) Less than 3 months post-burn
All burns > 50% TBSA burns
8 patients in this group, serum collected between 7 days and 3 months post-burn.

(2) Greater than 6 months post-burn
All burns < 30% TBSA burns
6 patients in this group, serum collected between 13 and 65 months post-burn.

(3) Greater than 6 months post-burn

30-50% TBSA burns
11 patients in this group, serum collected between 15 and 52 months post-burn.

(4) Greater than 6 months post-burn
All burns>50% TBSA burns
11 patients in this group, serum collected between 8 and 66 months post-burn.

TABLE 1. The samples were divided into four groups

| | (1) <3 Months Post Burn | >6 Months Post Burn | | |
|---|---|---|---|---|
| | All >50% TBSA Burns | (2) <30% TBSA | (3) 30-50% TBSA | (4) >50% TBSA |
| Number | 8 | 6 | 11 | 11 |
| Months Post Burn | 0.25-3 | 13-65 | 15-52 | 8-66 |

**Statistical analysis**

Detectable serum concentrations were reported as the mean plus or minus the standard error. Comparison between groups was made using Student's t-test for paired or independent observations when appropriate. The value of $p<0.05$ is considered statistically significant.

## RESULTS

As seen in Table 2 and Fig 2, all patients less than 3 months post-burn had elevated serum TNF-$\alpha$ levels, as would be excepted according to previous studies.[3,4] Patients with less than 30% TBSA burns who were past the acute

TABLE 2. The data were analyzed by dividing the samples into four groups.

| | (1) <3 Months Post Burn* | >6 Months Post Burn | | |
|---|---|---|---|---|
| | All >50% TBSA Burns | (2) <30% TBSA | (3) 30-50% TBSA | (4) >50% TBSA |
| TNF-$\alpha$ (pg/ml) | 95±10 | 29±7 | 60±11 | 83±8 |
| Number | 8 | 6 | 11 | 11 |
| Months Post Burn | 0.25-3 | 13-65 | 15-52 | 8-66 |

±SEM
Acute* vs <30% $p<0.002$, Acute* vs 30-50% $p<0.05$
<30% vs 30-50% $p<0.05$, <30% vs >50% $p<0.002$
30-50% vs >50% $p<0.05$

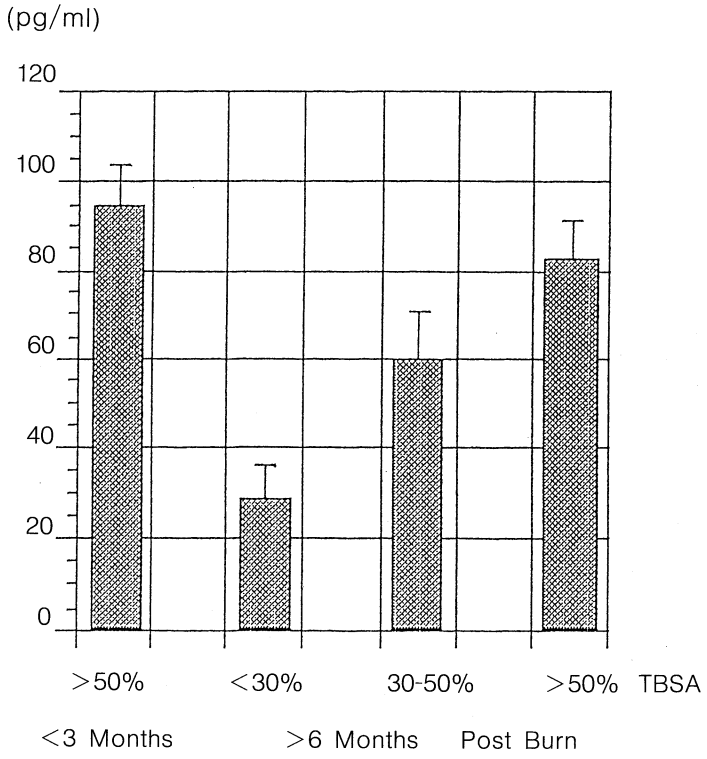


Fig 2. Comparison of serum TNF-$\alpha$ levels

stage in their recovery period had decreased levels of TNF-$\alpha$, but it was still detectable up to 65 months post-burn.

The mean detectable serum TNF-$\alpha$ concentrations in the groups of patients with major burns [>50% TBSA burns; group (1) $95\pm10$ pg/ml, group (4) $83\pm8$ pg/ml] were significantly higher than those in group (2) [$29\pm7$ pg/ml] and group (3) [$60\pm11$ pg/ml].

group(1) vs group(2), $p<0.002$
group(1) vs group(3), $p<0.05$
group(2) vs group(4), $p<0.002$
group(3) vs group(4), $p<0.05$
group(2) vs group(2), $p<0.05$

These results indicate that TNF-$\alpha$ is significantly elevated in patients with major burns, up to five years after injury.

## DISCUSSION

TNF-$\alpha$/cachectin, a biologically active cytokine synthesized by a variety of tissue types, including blood monocytes and tissue macrophages, in response to

a variety of exogenous stimuli, was initially described by Carswell *et al*, for its ability to induce the necrosis of implantable soft tissue sarcomas.[7] Since that time TNF-$\alpha$ has been implicated as a mediator of such diverse processes as cancer cachexia, endotoxin shock, and hematopoiesis. The endogenous production of TNF-$\alpha$ has been suggested as a pivotal mediator of several tissue-specific responses to injury and infection.[8] It has also been shown to modulate certain aspects of wound healing such as its local effects on wound disruption strength in mice.[6]

Until recently, assays for TNF-$\alpha$/cachectin activity were hampered by an inability to detect appreciable serum levels of this cytokine in injured or infected patients. Many reports have documented the presence of serum TNF-$\alpha$ in association with various human disease states, including parasitic and bacterial infections, as well as tumors[9-12] (Table 3).

TABLE 3. TNF-$\alpha$ Production in Disease

| Increased production in serum |
|---|
| Cancer |
| Chronic lymphatic leukemia |
| Sarcoidos |
| Rheumatoid arthritis |
| Meningococcal septicaemia |
| Parasitic infection : Malaria |
| AIDS |

TNF-$\alpha$ has been associated with fatal outcome in children with gram negative sepsis and purpura[13] and in patients with meningococcal disease.[10]

In this study, we demonstrated that serum TNF-$\alpha$ is detectable in higher concentrations in patients with major burns, up to five years after the injury. This may be a factor contributing to the increased rate of infections and delayed wound healing seen in these patients, although, another study[14] on the serum TNF-$\alpha$ response to bolus injections of endotoxin showed that peak concentrations of serum TNF-$\alpha$ were achieved within 90 minutes after the challenge and well before the clinical manifestations of endotoxemia (fever) were observed in healthy human volunteers. No detectable TNF-$\alpha$ levels were observed six hours after injection of endotoxin, suggesting that this cytokine appears transiently in the circulation. This may be because TNF-$\alpha$ is secreted in a phasic manner in response to a single injurious atimulus.

TNF-$\alpha$ levels in body tissues may be more important than actual circulating levels. Previous investigators recently demonstrated a novel transmembrane form of TNF, suggesting that cell-borne cytokines may be the primary mediators of directed inflammatory responses.[15] The finding suggests that serum levels may not accurately reflect the role of this cytokine in tissues during injury and infection.[4]

Marano *et al*[4] showed that serum TNF-$\alpha$ was detectable with greater frequency and in higher concentrations in patients with sepsis and in those who ultimately succumb to both septic and non-septic complications of burn injury. Serum TNF-$\alpha$ appears transiently and repetitively in the circulation during

injury. TNF-$\alpha$ was also detected in the serum of patients without sepsis and those who survived, although the incidence of such detection was less frequent and usually at lower concentrations. The early appearance of this cytokine, especially under conditions that are clearly survivable, may not necessarily be indicative of lethal toxicity.

The presence of serum TNF-$\alpha$ was not universally observed in any animal models.[16] There are several explanations for this observation. Cachectin is produced by tissue macrophages, including Kupffer cells, alveolar macrophages, renal mesangial cells, glial cells, endothelial cells, and other tissues.[17] TNF-$\alpha$ induces a graded local response, and in some tissues the biologic response is below the detection limit of current assay.[18] TNF-$\alpha$ also appears to be synthesized as a transmembrane protein with a higher molecular weight that confers biological activity by direct cell-to-cell contact but not in the circulation.[15]

Impaired wound healing is a significant clinical problem with a large number of patients affected annually. The increased incidence of wound complications noted after surgical procedures in a variety of clinical situations, including malnutrition, diabetes mellitus, chemotherapy and radiation therapy result in increased morbidity and mortality rates associated with surgical procedures.

Macrophages play a critical role in wound healing. Animals depleted of macrophages exhibit impaired wound healing, as do animals administered anti-macrophage antibodies locally to the wound.[3] TNF-$\alpha$ is a ubiquitous inflammatory mediator released by activated macrophages, which in turn induces macrophage to release a variety of factors including macrophage colony-stimulating factor (M-CSF) and Interleukin-1 (IL-1), and potentiates macrophage to respond to other lymphokines.[1,3] TNF-$\alpha$ acts on other cell types in the wound such as endothelial cells and fibroblasts.

The presence or absence of TNF-$\alpha$ in the wound or serum and the diverse effects of various levels of this factor are now beginning to be reported. The timing of the presence of TNF-$\alpha$ in the wound is also likely to be important.

Presently, we are prospectively looking at TNF-$\alpha$ levels and scar formation, as there seems to be a yin-yang relationship between TNF-$\alpha$ and scarring. At this time, these investigations support the view that TNF-$\alpha$ in low concentrations helps wound healing, whereas higher concentrations, may be detrimental.[6] Therefore, TNF-$\alpha$/cachectin in the appropriate concentrations, timing and vehicle may favorably modulate wound healing, whereas, in other circumstances, it may be deleterious.

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