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PLASMA CYTOKINES FOLLOWING THERMAL INJURY AND THEIR RELATIONSHIP WITH PATIENT MORTALITY, BURN SIZE, AND TIME POSTBURN

Adriana C. Drost, MS, David G. Burleson, PhD, William G. Cioffi, Jr., MD, Bryan S. Jordan, RN, MS, Arthur D. Mason, Jr., MD, and Basil A. Pruitt, Jr., MD

We measured plasma levels of interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) following thermal injury. Cytokine levels in the plasma of 27 burned patients were serially screened by ELISA and compared with cytokine levels in 16 healthy laboratory employees. The relationships between cytokine concentrations and patient mortality, burn size, and time postburn were examined. Plasma samples with detectable amounts of IL-1β and IL-6 were significantly more frequent in burned patients than in controls, whereas TNFα was undetectable in most plasma samples. All nonsurviving burned patients had detectable IL-6 levels; these were significantly higher than those of surviving patients. The IL-1β and IL-6 concentrations were highest during the first week after injury and declined over time. The IL-1β concentrations were positively correlated with burn size. These findings suggest that IL-1β and IL-6 may influence metabolic and immunologic responses in the first few weeks following thermal injury. Tumor necrosis factor α was transiently elevated in a small subpopulation of burned patients with no obvious relationship to burn size or time postburn.

DESPITE recent improvements in burn care, infection contributes significantly to mortality after thermal injury. All parts of the immune system appear to be altered by burn injury. Cytokines modulate a number of immunological functions following thermal injury and may influence the resistance of burned patients to infection.

Interleukin-1 (IL-1) is an endogenous pyrogen that induces a variety of acute-phase reactions.[1] Interleukin-1 also induces IL-2-dependent T-cell proliferation, leading to specific antigen-dependent immune responses.[2] Furthermore, IL-1 is involved in a cascade of regulatory events, stimulating or suppressing the production of other cytokines such as IL-6.[3,4,5]

Interleukin-6 was originally identified as a B-cell differentiation factor[6] inducing antibody production. Its known biological effects include T-cell activation,[7] induction of hepatic acute phase protein synthesis,[8] stimulation of hematopoiesis,[9] and inhibition of tumor cell growth.[10]

Tumor necrosis factor (TNF), or cachectin, is classically known for its ability to cause necrosis of tumors.[11] This cytokine also stimulates fibroblast growth,[12] modulates granulopoiesis, and affects bone resorption, hemostasis, and lipid metabolism.[13] Furthermore, TNF can induce IL-6 and IL-1.[14,15,16] Most importantly, TNF is thought to be a primary mediator of the host response to inflammation.[17]

All three cytokines have been studied in a variety of disease states. Scleroderma, inflammatory bowel disease, bacterial infections, mechanical trauma, sepsis, septic shock, and rheumatoid arthritis are examples of conditions in which concentrations of IL-1, IL-6 and TNF are altered in various body fluids.[18-24] The production of all three cytokines is reported to be increased in most of these conditions. We have previously reported that serum IL-1 activity is increased in burned rats compared with controls.[25] The purpose of the current study was to monitor plasma cytokine concentrations in patients with thermal injury and to determine the relationships between these concentrations and clinical status.

METHODS

Twenty-seven patients with burns ranging from 17.5% to 89% of total body surface area and an average age of 35.8 years (21 to 70 years) were studied. They were normotensive and hemodynamically stable after uneventful resuscitation. Blood was drawn three times a week between 5:00 and 6:00 AM into blood collection tubes containing EDTA. The specimens were centrifuged at 750*g* for 20 minutes, the plasma removed, and 1

From the U.S. Army Institute of Surgical Research, Fort Sam Houston, Texas.

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Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Reg 70-25 on Use of Volunteers in Research.

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mL aliquots stored at −70°C until assay. Remaining platelets were removed by further centrifugation at 11,000*g* for 1 minute immediately before assaying. Plasma IL-1β concentrations were measured in 423 samples from 27 patients. Of these, 253 samples from 21 patients were included in data analysis, since they were measured in an ELISA with a greater sensitivity. The TNFα concentration was measured in 409 samples from 27 patients; IL-6 was measured in 419 samples from 27 patients. Seventeen plasma samples from 16 healthy laboratory employees were used as controls.

The cytokines were detected by enzyme-linked immunosorbent assay (ELISA). The IL-1β and TNFα ELISA kits were purchased from Cistron Biotechnology, Pine Brook, NJ and the IL-6 ELISA kits from Genzyme Corporation, Cambridge, Mass. All three procedures were sandwich ELISAs in which the molecule of interest was first bound by an immobilized primary monoclonal antibody, then washed free of plasma components and subsequently bound by a rabbit polyclonal antibody. The reactions were amplified using goat anti-rabbit IgG conjugated to horseradish peroxidase, and visualized by production of color after addition of peroxidase substrate. The color intensity was proportional to the amount of bound conjugate and therefore to the amount of cytokine present. After color development, absorbance was measured using a MR600 microplate reader (Dynatech Laboratories Inc., Alexandria, Vir) at 490 nm or 450 nm.

Cytokine concentrations were calculated by comparing sample absorbance with the absorbance of pooled plasma from controls enriched with increasing amounts of recombinant human cytokine. The pooled control plasma did not contain detectable concentrations of endogenous cytokines. Control plasma was used for the standard curve instead of bovine serum albumin to account for possible interference of plasma factors in the ELISA assay. Sample measurements were accepted as different from zero (minimum detectable level) when their mean absorbance was 2 standard deviations or more above that of the nonspecific binding (NSB) of controls. The NSB for all ELISAs was determined in pooled control plasma without cytokine added. The average minimum detectable level for each ELISA was used to determine which samples were included in the analysis of plasma cytokine concentrations (IL-1$\beta \geq 2.5$ pg/mL; IL-6 ≥ 0.03 ng/mL; TNF$\alpha \geq 3.42$ pg/mL).

Statistical significance was determined by the Mann-Whitney U-test or Spearman's Rank Correlation as appropriate (BMDP Statistical Software, Los Angeles, Calif).

RESULTS

Plasma Cytokines Following Thermal Injury

Free circulating IL-1β, IL-6, and TNFα concentrations were measured three times a week for 6 weeks in plasma samples from 27 patients with severe thermal injury. Cytokine concentrations were compared with those of healthy laboratory personnel. Table 1 shows that 58.1% of all patient samples (147 of 253) contained detectable amounts of IL-1β. Mean IL-1β concentrations of detectable samples were 2.04 ± 0.43 pg/mL and 3.83 ± 0.29 pg/mL for control and patient samples, respectively. Interleukin-6 could be measured in 69.2% (290 of 419) of all patient samples, with mean concentrations of 0.26 ± 0.11 ng/mL for detectable controls and 0.46 ± 0.04 ng/mL for detectable patient samples. The percentages of samples with detectable amounts of IL-1β and IL-6 were significantly higher in the burned population than in controls ($p < 0.05$). Only 21.9% of samples from burned patients (92 of 421) contained detectable amounts of TNFα, with concentrations of 5.65 pg/mL for one detectable control sample and 7.43 ± 0.76 pg/mL for 92 detectable patient samples.

Plasma Cytokines and Mortality

To examine the relationship between cytokines and the outcome of thermal injury, plasma levels of TNFα and IL-6 from 24 surviving and three nonsurviving burned patients were compared. The number of IL-1β samples from nonsurviving patients was too small to be included in the data analysis.

Table 2 shows that of 403 samples from the surviving population, 274 contained detectable amounts of IL-6 (68%); all 16 samples in the nonsurviving group contained detectable amounts of IL-6 (100%). The mean plasma IL-6 concentration in nonsurviving patients was 1.62 ± 0.45 ng/mL, which was significantly higher than the 0.39 ± 0.03 ng/mL mean concentration found in detectable samples from surviving patients ($p < 0.001$). Only 22.2% (90 of 405) of samples from surviving patients and 12.5% (2 of 16) of samples from nonsurviving patients contained detectable amounts of TNFα. Mean TNFα concentrations were 7.23 ± 0.75 pg/mL from 90 detectable samples of surviving patients and 16.70 ± 10.17 pg/mL from two detectable samples of nonsurviving patients. Subsequent data analyses excluded samples from nonsurviving patients to remove possible effects associated with only that subpopulation.

Cytokines and Time Postburn

To explore the temporal relationship between cytokines and burn injury, patient samples were grouped by postburn week. Figure 1 displays the percentages of patient samples containing detectable amounts of cytokines. More than 90% of the patient samples contained

Table 1
Percentages of detectable plasma cytokines in burned patients

	IL-1β	IL-6	TNFα
Controls	23.5	29.4	5.9
Burn	58.1	69.2	21.9

Plasma cytokines were measured by ELISA in healthy laboratory personnel (Controls) and burned patients (Burn) for up to 6 weeks. The percentage of samples in which cytokines were detectable is displayed.

Table 2
Percentages of detectable plasma cytokines in surviving and nonsurviving burned patients

	IL-6	TNFα
Survivors	68 (274/403)	22.2 (90/405)
Nonsurvivors	100 (16/16)	12.5 (2/16)

Plasma cytokines measured by ELISA in surviving and nonsurviving burned patients. The percentage of samples in which cytokines were detectable is displayed. The actual numbers are given in parentheses.

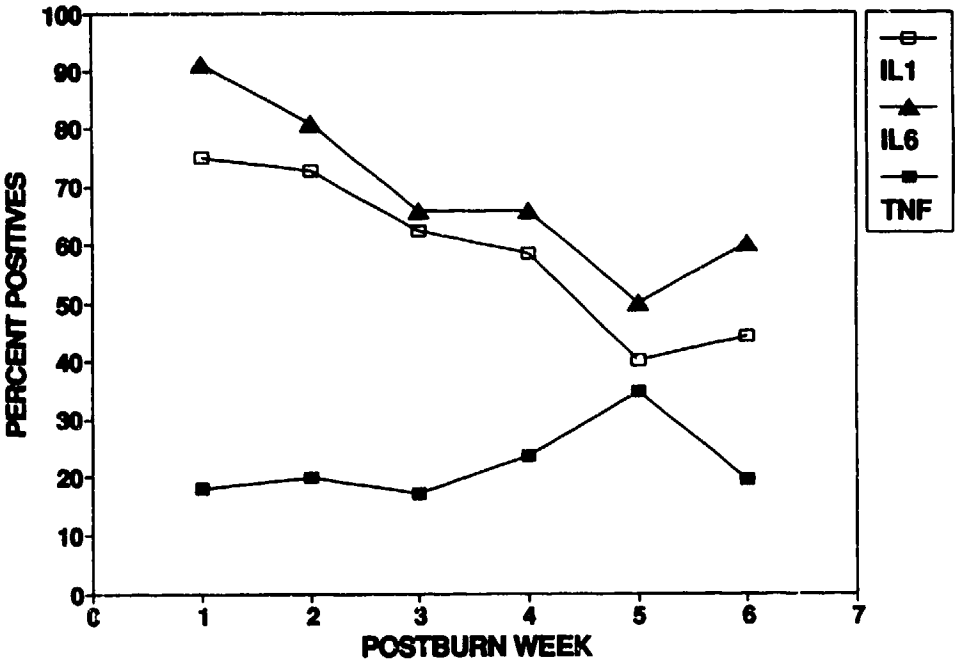

Figure 1. The proportion of plasma specimens positive for cytokines in surviving burned patients over time. Cytokine values were grouped by postburn week. Data points represent the percentage of samples with cytokine levels above the minimum detectable level in each ELISA.

detectable amounts of IL-6 during the first week postburn. This percentage decreased to 50% by postburn week five. The distribution of positive IL-1β samples was similar to that of positive IL-6 samples. In the first week postburn, 75% contained detectable levels of IL-1β, declining to 20.6% during the following 5 weeks. In contrast, TNFα was elevated in only a small percentage (17.1%–34.8%) of patient samples throughout the study period, with no apparent trend. The mean plasma cytokine concentrations of positive samples from surviving patients grouped by postburn week are displayed in Figure 2. All cytokine concentrations were highest during the first week postburn and decreased at various rates over the next 5 weeks. Statistically, both IL-1β and IL-6 were negatively correlated with time postburn (r^2 = 0.1060, $p < 0.0001$ and $r^2 = 0.0294$, $p < 0.001$, respectively).

Plasma Cytokines and Burn Size

The relationship between cytokine levels and percentage of total body surface area burn was examined. Figure 3 shows that the percentage of patients with positive IL-1β levels increased with increasing burn size ($r^2 = 0.0414$, $p < 0.05$). There was no apparent relationship between burn size and positive TNFα or IL-6 patient samples.

DISCUSSION

Plasma IL-1β and IL-6 concentrations were increased following thermal injury in this group of study patients. Since each cytokine had a different relationship with time postburn and burn size, it seems reasonable to postulate that they may play distinct roles in modulating pathophysiologic events following severe thermal injury. The TNFα levels were increased in only a subpopulation of patients and did not correlate with burn size, suggesting that something other than severity of injury may have triggered the increases.

Interleukin-1β levels were detectable in 75% of patient samples during the first week postburn and in progressively fewer patients thereafter. This decrease in the frequency of IL-1β-positive patients and the decrease in mean IL-1β concentration roughly coincides with the decrease in hypermetabolism, return to normal hormone levels, and positive nitrogen balance that occur in most patients after the third postburn week. Another suggestion of a relationship between plasma IL-1β levels and burn injury is the positive correlation observed between IL-1β and burn size.

The relationship between IL-6 and mortality was striking. Mean IL-6 concentration was almost tenfold higher in nonsurviving than in surviving patients, with 100% of the sample population exhibiting detectable levels. These data are in agreement with a report from Hack et al.,[23] who measured plasma IL-6 levels in patients with sepsis. They reported that IL-6 levels were significantly higher in patients who died than in patients who recovered from their septic episode. Whether the IL-6 response was caused by sepsis or by other events, such as multiple organ failure, was unclear.

Interleukin-6 was measurable in almost all patients soon after injury. The frequency of positive samples and mean concentrations decreased over time. This relationship of IL-6 with time postburn suggests that IL-6 may be associated with early posttraumatic events. Since there was no strong relationship to burn size, early events

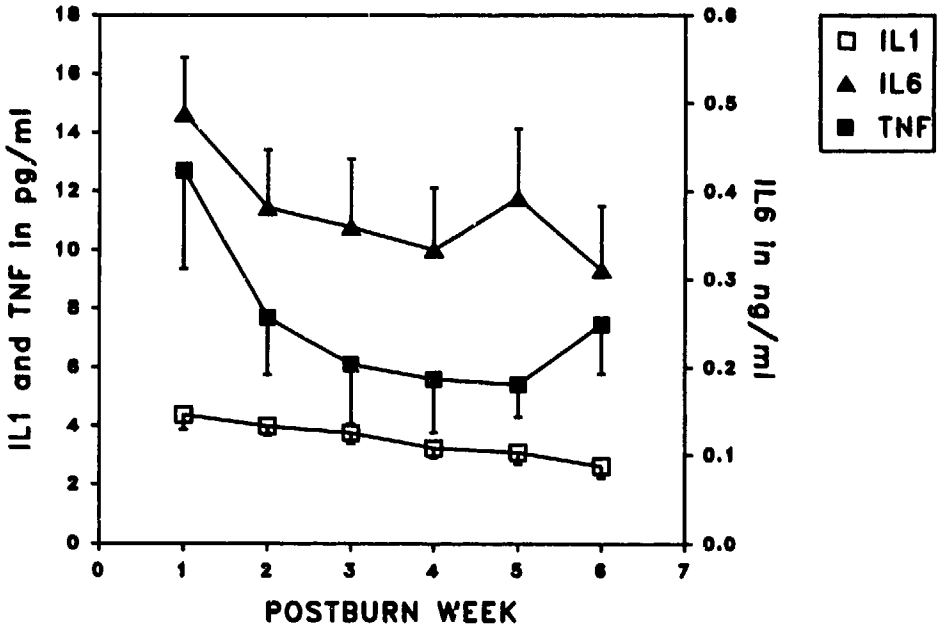

Figure 2. Plasma cytokine concentrations in surviving burned patients over time. Cytokines were monitored three times a week for 6 weeks postburn, positive cytokine values were grouped by week, and the means ± SEM plotted against postburn week.

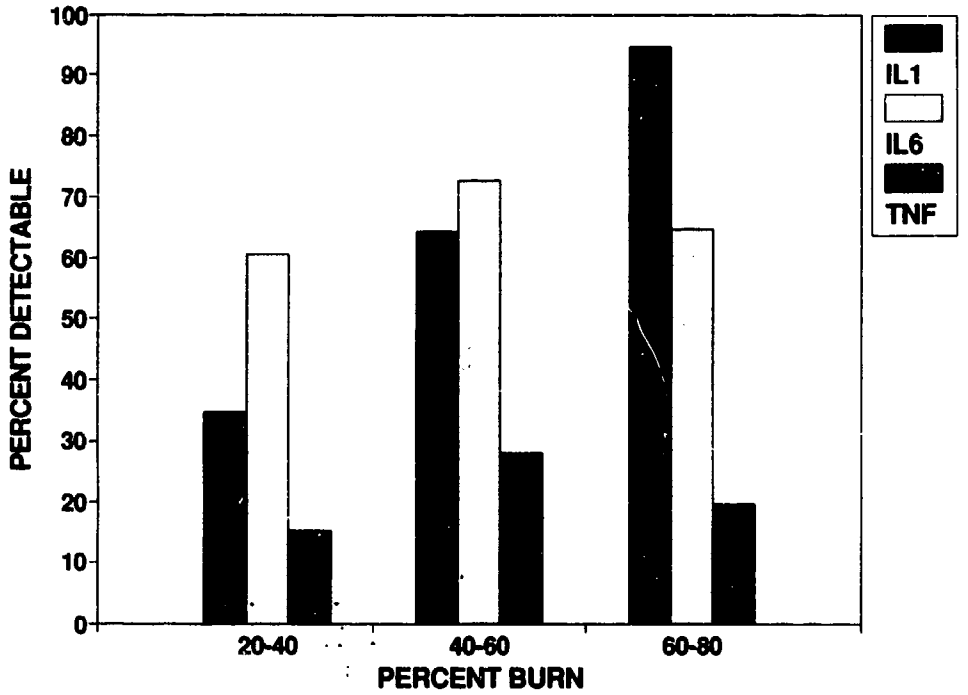

Figure 3. Plasma cytokines in surviving burned patients and area of total body surface burn. Cytokine values were grouped by burn size as displayed. Bars represent the percentage of samples with detectable levels of cytokine.

independent of the extent of thermal injury may trigger changes in plasma IL-6 levels.

The relationship between IL-6 and time postburn is consistent with data reported by Ertel et al.[22] These authors measured IL-6 production by T lymphocytes from patients with major mechanical trauma stimulated in vitro with PHA. In that system, IL-6 production was increased over controls throughout the 21-day study period. The amount of IL-6 produced decreased over time, but never returned to control levels during the study period.

Even though plasma TNFα levels in burned patients were not elevated in the group as a whole, 21.9% of the patient population had transiently detectable TNFα concentrations. In similar findings, Marano et al.[27] reported increased serum rather than plasma TNF levels in 26% of patients with thermal injury. They reported no correlation between TNF levels and burn size, but did find a correlation between the frequency of TNFα positives

and mortality. They reported a maximum of 1200 pg/mL of TNFα in burned patients, whereas our highest TNFα concentration was 27 pg/mL. The difference between the levels of TNFα we measured and those found by Marano et al. may be a result of the fact that their measurements were made in serum rather than plasma, since in vitro TNF generation in serum has been reported by Freeman et al.[28]

It is possible that the cytokines contribute to some of the pathophysiologic changes seen after burn injury. The fact that the cytokines vary independently with respect to burn size and time postburn suggests that their release into the circulation may be triggered by different mechanisms. The position of these agents in the pathophysiology of burn injury remains to be determined.

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