CONCENTRATIONS OF CYTOKINES IL-6 AND IL-10 IN PLASMA OF BURN PATIENTS: THEIR RELATIONSHIP TO SEPSIS AND OUTCOME

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SUMMARY. Burn injury induces a suppression of the Th1 response, which is associated with an increased susceptibility to conditions of infection, morbidity, and mortality. It is well established that cytokines modulate the pathogenesis of burn injury. In this study, plasma levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) were determined in burn patients and correlated with the severity of sepsis. Sixty adult burn patients (total body surface area burned, 8-80%) were included in the study, of whom 34 developed sepsis and 14 died. The nonseptic group consisted of 26 patients. Thirty-one healthy blood donors served as controls. Patients were not treated with antibiotics until sepsis occurred. Plasma samples were collected immediately post-burn and after several days, and cytokine concentrations were determined by ELISA. Within three days, all the patients presented high levels of circulating IL-6, which were significantly higher in septic patients than in nonseptic patients (349 ± 278 vs 63 ± 56 pg/ml, $p < 0.001$). IL-10 levels were higher in septic patients than in nonseptic patients at all times in our study. The value of 60 pg/ml shows a sensitivity of 92% and a specificity of 93% in the differentiation of survivor from nonsurvivor septic patients. In this study the high value of circulating IL-10 on day 3 suggests that cytokine may discriminate between nonsurvivor septic and survivor septic patients.

Introduction

Burn injury results in increased susceptibility to infection, which can lead to increased morbidity and mortality.

Cytokines have recently begun to be regarded as important players in the post-burn pathophysiological process and the pathophysiology of sepsis and septic shock.[1-3] The elevation of pro- and anti-inflammatory cytokines alters immune function, which can lead to decreased resistance to infection in severely burned patients.[4,5]

Increased levels of cytokines such as interleukin-6 (IL-6), IL-8, IL-10, and tumour necrosis factor-α (TNF-α) have been reported in adult burn patients, but these cytokines were not consistently found to be predictors for the development of sepsis.[1-6]

In the present study we measured dynamic changes in IL-10 and IL-6 serum levels in burn patients with and without sepsis. The aim of our study was to determine whether early post-burn serum IL-6 and/or IL-10 could be used to identify patients at high risk of developing ultimately fatal sepsis.

Materials and methods

Patients

The patients studied were all admitted to the Plastic Surgery and Burns Operating Unit, ARNAS Civic Hospital, Palermo, Italy.

A series of serum samples were obtained from 60 burn patients (44 males, 16 females; total body surface area [TBSA] range, 15-80%; mean age, 50.2 ± 14.6 yr) and 31 healthy subjects (20 males, 11 females; mean age, 43.0 ± 7.5 yr). Patient samples were collected at 1, 3, 7, 10, 14, and 20 days; serum was separated from whole blood by centrifugation and stored in aliquots at -70° C.

Thirty-four patients developed sepsis, of whom 14 died after a mean period of 15 ± 13 days. There was no mortality among the nonseptic patients.

Patients were not treated with antibiotics until sepsis occurred.

Informed consent for sera analysis was obtained from the patients and the healthy subjects.

Cytokine determination

IL-10 and IL-6 serum levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (Euroclone, Wetherby, Yorkshire, UK), following the instructions given by the manufacturer.

The minimum detectable doses of IL-10 and IL-6 were respectively 12.5 and 7.8 pg/ml.

C-reactive protein determination

C-reactive protein (CRP) was determined by standard procedures using an automated haematology analyser (Sysmex XT 1800 L).

Statistical analysis

Data were expressed as mean ± SD.

Student's t-test was used to compare responses in different groups ($p < 0.05$ was chosen for rejection of the null hypothesis).

Results

Patient characteristics

Table I summarizes the clinical and pathological characteristics of the burn patients and the healthy subjects. The development of sepsis was correlated to the extent of the burn area, while age was a determinant factor distinguishing survivors from nonsurvivors among the septic burn patients.

Patients were classified as being septic on the basis of one of the following criteria (signs and symptoms):

- positive blood or tissue culture for bacteria and fungi
- hyperthermia (> 38 °C)
- altered mental status
- haemodynamic instability usually requiring vasopressors

In 34 of the 60 patients, signs of sepsis were observed after a mean period of 3 ± 1 days.

Table I - Clinical characteristics of burn patients and healthy subjects

Subjects	Number of subjects	Mean age (yr)	Percentage TBSA[1]
Survivor septic patients	20	44 ± 18*	26.3 ± 21
Nonsurvivor septic patients	14	61 ± 19*	35 ± 17**
Nonseptic patients	26	44 ± 22*	17.5 ± 7**
Healthy subjects	31	43 ± 7.5*	–

[1] TBSA = total body surface area.
No significant differences in mean age were found in the groups studied (* = p nonsignificant).
No statistical differences in TBSA percentage were found between survivor and nonsurvivor septic patients, while significant differences in TBSA percentage were found between nonsurvivor septic patients and nonseptic patients (** = $p < 0.01$).

Cytokine determination

Serial changes in circulating IL-6 and IL-10 in the 60 burn patients were determined by ELISA.

The results shown in *Figs. 1* and *2* demonstrate increased serum levels of these mediators shortly after burn injury.

At all times in our study IL-6 levels were higher in septic patients than in nonseptic patients ($p < 0.05$) and in healthy subjects ($p < 0.001$).

No significant differences in the maximum serum IL-6 levels were detected between patients who survived and those who did not survive, from the time of admission to 15 days post-burn.

The results presented in *Fig. 2* demonstrate an increase in serum IL-10 shortly after burn injury. In nonseptic and in survivor septic patients, the levels were lower than those of nonsurvivor septic patients (13 ± 7 and 14 ± 6 versus 47 ± 25 pg/ml; $p < 0.05$). In particular, a peak level of serum IL-10 was observed on 3 day post-burn only in septic nonsurvivor patients (180 pg/ml). In nonseptic and survivor septic patients, IL-10 levels remained at relatively low levels at all moments the study (*Fig. 3*).

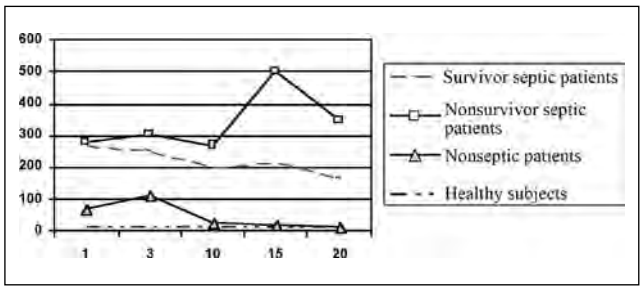

Fig. 1 - Plasma IL-6 levels (pg/ml) in the groups studied on days indicated. IL-6 levels were higher in septic than in nonseptic patients, but until day 15 day these values did not distinguish survivor from nonsurvivor septic patients.

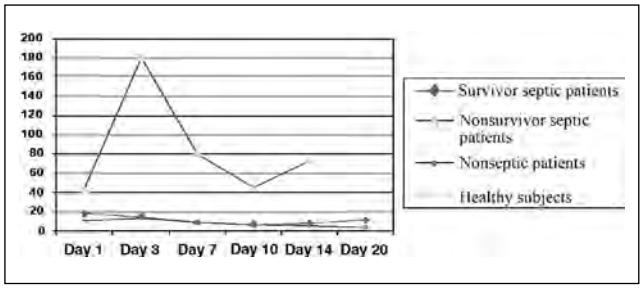

Fig. 2 - Plasma IL-10 levels (pg/ml) in the groups studied on days indicated. IL-10 levels were higher in septic than in nonseptic patients at all times of the study.

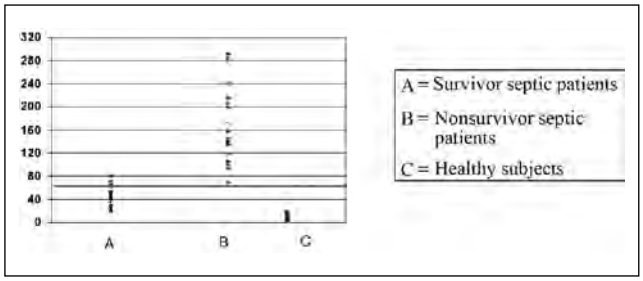

Fig. 3 - The IL-10 values (pg/ml) on day 3 discriminated between survivor and nonsurvivor septic patients. Taking 60 pg/ml as the cut-off value, it was possible to differentiate survivor from nonsurvivor septic patients with a sensitivity of 92% and a specificity of 93%.

As shown in *Fig. 3*, the IL-10 values on day 3 discriminated between survivor and nonsurvivor septic patients. Taking 60 pg/ml as the cut-off value, it was possible to differentiate survivor from nonsurvivor septic patients with a sensitivity of 92% and a specificity of 93%.

C-reactive protein determination

CRP was determined by standard procedures using an automated haematology analyser.

The results shown in *Fig. 4* indicate that CRP levels were higher in septic patients than in nonseptic patients ($p < 0.05$), but until day 15 CRP values did not discriminate between survivor and nonsurvivor septic patients.

As a representative example, the results presented in *Fig. 5* show the values of IL-6, IL-10, and CRP on day 3 in the various groups. IL-6, IL-10, and CRP concentrations were highest on day 3 in the septic patients, but only IL-10 levels discriminate between survivor and nonsurvivor septic patients.

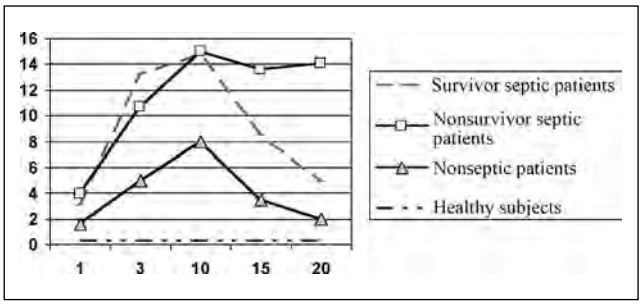

Fig. 4 - C-reactive protein levels (mg/dl) in the groups studied on days indicated. CRP levels were higher in septic patients than in nonseptic patients, but until day 15 CRP values did not discriminate between survivor and nonsurvivor septic patients.

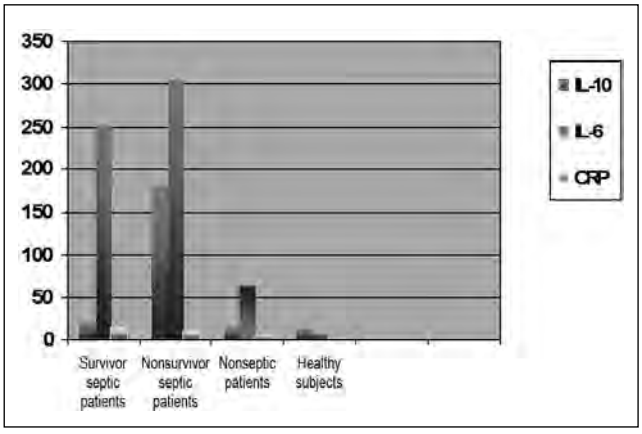

Fig. 5 - IL-6, IL-10, and CRP levels on day 3 in the groups studied. IL-6, IL-10 (pg/ml), and CRP (mg/dl) concentrations were highest on day 3 in septic patients but only IL-10 levels discriminated between survivor and nonsurvivor septic patients.

Discussion

A burn wound is perhaps the most intense stress experienced by the human body.

The post-burn systemic inflammatory response leads to the production of mediators, including cytokines. Results obtained previously showed that there was a post-burn increase in serum levels of inflammatory cytokines, including TNF-α, IL-8, and IL-6.[6,7] In addition to the pro-inflammatory mediators mentioned above, other cytokines have recently been observed to play a role in the regulation and inhibition of both inflammatory and immune responses.[8,9]

It was found that after major injury patients had high concentrations not only of pro-inflammatory mediators (IL-6) but also of anti-inflammatory mediators, such as IL-10.[7,10,11]

The aim of the present study was to determine serum cytokines such as IL-6 and IL-10 from the time of hospital admission to day 20 post-burn.

Our results show that these cytokines increased on admission in all groups compared to healthy subjects.

Subsequently, we observed high levels of IL-6 in septic patients rather than in those who were nonseptic and in healthy subjects, suggesting that this cytokine may play a role in the pathogenesis of sepsis following burn injury. However, no significant differences in the maximum serum IL-6 levels were detected between patients who survived and those who did not survive, from the time of admission to day 15 post-burn injury.

Serum IL-10 remained low in the survivors, while increases in serum IL-10 were detected in the nonsurvivors with proven sepsis.

In particular, among the patients who developed sepsis, the analysis of IL-10 on day 3 was a determinant discriminator between survivors and nonsurvivors.

This result suggests that, in addition to its anti-inflammatory effect, IL-10 also appears to be correlated with the development of septic complications.

It was found in burned mice that IL-10 appeared to induce decreased resistance to infection.[12] A similar finding was reported by O'Sullivan et al., who found that a major injury led to predominance of the T helper-2 lymphocyte phenotype with an increased production of IL-4 and IL-10 that is associated with decreased resistance to infection.[13-15]

Conclusion

The pro- and anti-inflammatory responses following infection are complex and involve many mediators with multiple interrelationships between them.

We suggest that IL-10 levels can be used as a diagnostic tool to identify patients who risk developing sepsis, which may lead to their death.

RÉSUMÉ. Les brûlures causent la suppression de la réponse Th1, qui est associée avec une susceptibilité augmentée aux conditions d'infection, de la morbidité et de la mortalité. Il est reconnu que les cytokines modulent la pathogenèse de la maladie de la brûlure. Les Auteurs de cette étude ont déterminé les niveaux plasmatiques de l'interleukine-6 (IL-6) et de l'interleukine-10 (IL-10) chez les patients brûlés en corrélation avec la gravité du sepsis. Soixante patients brûlés adultes atteints de brûlures dans 8-80% de la surface corporelle ont été inclus dans l'étude, dont 34 ont développé le sepsis et 14 sont décédés. Le groupe non septique comprenait 26 patients. Trente-et-un donateurs sains de sang ont été utilisés comme témoins. Les patients ont reçu un traitement antibiotique seulement après la manifestation du sepsis. Les exemplaires de plasma ont été recueillis immédiatement après la brûlure et encore après divers jours, et les concentrations de cytokine ont été déterminées en utilisant ELISA. Dans trois jours tous les patients présentaient des valeurs élevées d'IL-6 en circulation qui étaient significativement plus hautes chez les patients septiques que chez les patients non septiques (349 ± 278 vs 63 ± 56 pg/ml, $p < 0.001$). Les valeurs d'IL-10 étaient continuellement plus élevées chez les patients septiques que chez les patients non septiques pendant la durée entière de l'étude. La valeur de 60 pg/ml indique une sensibilité de 92% et une spécificité de 93% pour ce qui concerne la différenciation entre les patients septiques survivants et non survivants. Selon les Auteurs la valeur élevée d'IL-10 en circulation au troisième jour après la brûlure pourrait indiquer que cette cytokine sert à distinguer entre les patients septiques non survivants et les patients septiques survivants.

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This paper was received on 25 March 2008.

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