Serum Interleukin-18 Concentrations in Burn Patients

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Abstract
Interleukin(IL)-18 is a recently cloned cytokine thought to participate actively in various inflammatory reactions. We investigated relationships between serum IL-18 concentrations and clinical parameters in burn patients, from whom serum samples were collected serially beginning within 24h after admission. Patients (17 male, 8 female; 22 survivors, 3 nonsurvivors) had a mean age of 49.2 years, a mean total burn surface area (TBSA) of 27.1% (range, 1 to 93%), and a mean burn index (BI) of 19.6 (range, 0.5 to 88). Serum IL-18 concentrations were determined by enzyme-linked immunosorbent assays (ELISA) to examine correlations with TBSA, BI, white blood cell (WBC) count, C-reactive protein (CRP), and oxygenation index (OI; Pao2/Fio2; P/F). On Day 1 serum IL-18 correlated negatively with TBSA (r = −0.53, P < 0.05), BI (r = −0.48, P < 0.05), and WBC (r = −0.49, P < 0.05), while on Day 7 IL-18 correlated positively with TBSA (r = 0.47, P < 0.05), BI (r = 0.48, P < 0.05), WBC (r = 0.47, P < 0.05), and CRP (r = 0.56, P < 0.01). Serum IL-18 had a negative correlation with OI (r = −0.30, P < 0.01), and a positive correlation with the Sequential Organ Failure Assessment (SOFA) score (r = 0.56, P < 0.01). The mean ± SEM for peak IL-18 concentrations in individual survivors was significantly lower than the peak value among nonsurvivors (334 ± 25 vs. 626 ± 215 pg/mL, P < 0.01).

In conclusion, IL-18 in serum showed significant relationships with TBSA, BI, severity of inflammation, respiratory function, multiple organ dysfunction, and outcome. IL-18 is likely to be involved in the pathophysiology of inflammatory reactions following burn injury.

Key words Interleukin (IL)-18, Burn, Inflammatory response, Multiple organ dysfunction syndrome

Introduction
Interleukin(IL)-18 was initially described in 1989 as IGIF (interferon-gamma inducing factor), and was cloned in 1995.1 IL-18 is considered a proinflammatory cytokine showing marked synergistic action with IL-12 in inducing interferon-gamma (IFN-γ) in T cells. IL-18 also appears to activate natural killer (NK) cells independently of IL-12.2,3

While IL-18 is important in the activation of immunity,4 excessive IL-18 production by activated macrophages may induce dysfunction in multiple organs including disruption of the immune system.5

Experimental studies have explored the pathophysiologic and immunologic reactions of IL-18 in various acute or chronic inflammatory diseases including endotoxic shock, hepatitis, cryptococcal infection and mycobacterial infection.2,6,7 However, in critically ill patients in the intensive care unit (ICU) who undergo life-support procedures to augment the function of various organs, the actions and time-concentration relationships of IL-18 are little known.

We investigated the relationship in burn patients between serum IL-18 concentrations and various clinical parameters, aiming to better understand the effects of IL-18.
Table 1 Characteristics of patients by burn extent

<table>
<thead>
<tr>
<th></th>
<th>TBSA&lt;30%</th>
<th>TBSA≥30%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>10/5</td>
<td>7/3</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>49±4</td>
<td>49±6</td>
<td>NS</td>
</tr>
<tr>
<td>TBSA (%)</td>
<td>11.7±2.0</td>
<td>50.2±6.5</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>BI</td>
<td>7.0±1.3</td>
<td>39.0±6.5</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Inhalation injury (present/absent)</td>
<td>8/7</td>
<td>4/6</td>
<td>NS</td>
</tr>
<tr>
<td>Fatal outcome (survival/nonsurvival)</td>
<td>15/0</td>
<td>7/3</td>
<td>NS</td>
</tr>
</tbody>
</table>

TBSA: total burn surface area, BI: burn index, NS: not significant. The data are expressed as the mean±SEM.

Patients and Methods

Patients

Between August 1999 and March 2002, twenty-five consecutive patients (mean age, 49.2 years; range: 22 to 85) admitted to the burn care unit of our hospital were enrolled in this study. The exclusive criteria were a patient age of under 8-years, and incomplete documentation. The mean for the total burn surface area (TBSA) was 27.1% (range, 1 to 93). The mean for the burn index [BI=1/2 of second-degree TBSA(%) + third-degree TBSA(%)] was 19.6 (range, 0.5 to 88). Inhalation injury was diagnosed by fiberoptic bronchoscopy in 12 cases. Of the 25 patients, 3 died of their burns. Patient characteristics in two groups defined by TBSA are presented in Table 1.

During the acute phase of treatment, all patients were resuscitated according to Parkland’s formula with lactated Ringer’s solution. Fresh frozen plasma and vasopressors were used additionally if needed. Systemic blood pressure, cardiac output, and pulmonary capillary wedge pressure were monitored. Artificial ventilatory support was performed when indicated. Silver sulfadiazine and vaseline ointment containing polymyxin B powder were applied as topical agents for the care of burn wounds.

After the phase of fluid replacement, total parenteral nutrition with or without enteral nutrition was initiated. Operations necessary for wound debridement and skin grafting were performed as early as possible.

Methods

Blood samples were collected on Day 1 (within 24h after admission), and on Days 3, 5, 7, 10, and 14. After centrifugation at 3000 rpm for 10 min, serum was stored at −80°C until assay. The serum concentrations of IL-18 were measured by an enzyme-linked immunosorbent assay (human IL-18 ELISA kit; Medical & Biological Lab., Nagoya, Japan).

White blood cell (WBC) count in blood, serum concentration of C-reactive protein (CRP), arterial carboxyhemoglobin (CO-Hb) concentration, arterial partial oxygen saturation (Pao2), and the Sequential Organ Failure Assessment (SOFA) score were determined on the same days as serum sampling for IL-18. The SOFA score, considered useful for the prediction of subsequent organ dysfunction, was calculated based on Pao2/FIo2, platelet count, serum bilirubin concentration, degree of hypotension, Glasgow Coma Scale, and serum creatinine concentration.

Statistical correlations were examined between serum IL-18 concentration and TBSA, BI, WBC, CRP, and SOFA score.

To investigate the relationship between IL-18 and respiratory dysfunction, the correlation with blood gas parameters (pH, Paco2, Pao2, and base excess), serum CO-Hb, and the values of the oxygenation index (OI; Pao2 divided by the fraction of O2 in inspired air, or FIo2) was investigated. Differences in serum IL-18 concentrations were also evaluated between patients with and without endobronchial inhalation injury as well as between survivors and nonsurvivors.

Patient numbers (n) for the data sets vary in the figures and tables because of early discharges from the burn care unit.

This study was approved by the local ethics committee of our university.

Statistical analysis

All values are expressed as means±SEM. Comparisons between data sets were performed with unpaired Student’s t-test or one-way analysis of variance (ANOVA) followed by F analysis. Post hoc correction was performed by applying Fisher’s protected least significant difference (PLSD) to the ANOVA findings. When parametric methods were not appropriate, the nonparametric method used was the Mann-Whitney U
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The serum IL-18 concentrations in 46 healthy volunteers were 126 ± 44.5 pg/mL. In the patients, serum IL-18 concentrations over time are presented in Fig. 1. On Day 1, initial IL-18 concentrations in patients with TBSA ≥30% were significantly lower than in patients with TBSA <30%. IL-18 in the group with TBSA ≥30% then gradually increased to maximal levels on day 14, which were significantly higher than at earlier time points in the same group, but were not statistically different from concentrations on Day 14 in the group with TBSA <30%. These two groups divided by burn size, had no statistical difference in their characteristics, age, sex, complications or outcome (Table 1).

A significant negative correlation between serum IL-18 and TBSA was observed on Day 1, and a significant positive correlation was seen on Day 7 (Fig. 2). However, a significant correlation was not observed on Day 14.

BI showed relationships with IL-18 that were similar to those of TBSA. On Day 1, WBC correlated negatively with IL-18, while both WBC and CRP correlated positively with IL-18 on Day 7. On Day 14, only CRP correlated positively with IL-18 (Table 2).

Results

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As shown in Fig. 3, IL-18 correlated negatively with OI, and also correlated positively with the SOFA score determined on the same day (Fig. 4).

To analyze the relationship between the intensity of inflammatory response and serum IL-18 concentration, the peak IL-18 value for each case was considered in terms of various parameters.

Peak IL-18 concentrations in burn patients (369 ± 182.1 pg/mL) were higher than in normal controls (P<0.0001). In addition, the peak IL-18 value correlated positively with CRP on the day of the IL-18 peak (Fig. 5). However, no statistical correlation was observed between the peak concentration of IL-18 and simultaneous WBC count.

As for respiratory status immediately after burn injury, serum IL-18 concentrations were not associated with blood gas parameters or CO-Hb on admission. Although the difference fell short of statistical significance, patients with
endoscopically demonstrable inhalation injury tended to have higher serum concentrations of IL-18 (265 ± 35 pg/mL) than those without (199 ± 18 pg/mL).

Peak concentrations of IL-18 in nonsurvivors, were higher than those in survivors (Fig. 6).

Discussion

IL-18 has been reported to act importantly to maintain and reinforce T helper cell-type 1 (Th1) responses, acting together with IL-12. IL-18 also stimulates NK cell function and can augment antibody production by B cells. If upregulation of this pathway becomes excessive, macrophages activated by IFN-γ will produce excesses of tumor necrosis factor alpha (TNF-α), nitric oxide, and superoxide, causing cell damage and tissue injury. However, IL-18 also stimulates macrophages to produce prostaglandin E2, which results in the upregulation of Th2 responses. The latter include the release of IL-4, IL-10, and IL-13, and tend to inhibit excessively activated Th1 immune responses.5,9

Another important action of IL-18 is to act directly on murine NK cells10,11 and murine Th1 cells10,12 to induce the expression of Fas ligand, augmenting their cytotoxicity.

In addition, caspase-1 [IL-1 beta(β)-converting enzyme, or ICE] acts in producing mature bioactive IL-1β from an inactive precursor form.2 IL-1 is a potent proinflammatory cytokine that regulates the acute-phase gene expression of CRP.13,14 Since IL-18 also requires proteolytic processing by ICE to acquire activity, IL-1 and IL-18 are regarded as members of the same family.

Recent studies suggest that IL-18 may be activated prior to other cytokines, and has proinflammatory actions involving the direct stimulation of gene expression and the synthesis of TNF-α and the activation of NK cells with the subsequent production of IL-1β and IL-8.15 These reports also indicate that IL-18 is involved in the regulation of cytokine production during the early phase of bacterial infections.9

The pathophysiology of the various mediators following severe burns or trauma is extremely complex. Especially in patients with burn injury, cytokines have a variety of roles in modulating immunologic and inflammatory responses by the up- or downregulation of immune responses3,16-23

Changes in the concentrations of plasma cytokines over time [e.g. IL-1β, IL-1 receptor antagonist (ra), IL-6, TNF-α, and IFN-γ], as well as their relationships to various clinical parameters after burn injury, have been described in previous studies.24-29 Serum concentrations of IL-6 increase during the early phase after burn injury, and then decline over time.24,26,27 IL-6 correlates positively with burn size,24,27 and also shows a relationship with complicating infections.29 Higher serum concentrations are seen in nonsurvivors than in survivors.25,26

In our study, IL-18 showed similar patterns to those of IL-6 in terms of burn size and mortality. Changes in IL-18 were time-dependent, with IL-18 being significantly more abundant on Day 14 in patients with extensive burns.

These results suggest that thermal damage evidence immediately after burn injury, may have little direct effect in inducing IL-18. On the other hand, wound infections that begin to occur on Day 4–5 to 2 weeks are likely to stimulate IL-18, since serum concentrations of IL-18 correlated positively with TBSA, BI, WBC count, and CRP on Day 7, but not Day 1. Few correlations persisted up to Day 14, although a continuing correlation between peak IL-18 levels and CRP presumably reflected the magnitude of the inflammatory response.30

This reason is supported by our result that the incidence of the positive rate of pathogens (e.g. Staphylococcus aureus, Pseudomonas aeruginosa) isolated from burn wounds, were increased daily after Day 4–5. It is also supported by the report that IL-18 levels are increased in patients with sepsis, and Staphylococcus aureus markedly increased the release of IL-18 while endotoxin was ineffective.31

On Day 1, the patients with a larger size of burn wound had significantly lower IL-18 concentrations (Fig. 1 and Fig. 2). This result suggests that thermal impact plays an immunosuppressive role in IL-18 induction, although this speculation is not be supported by any other recent study.

The relationship between IL-18 and the inflammatory response on Day 7 may involve the need for activation by ICE protease that is common to both IL-18 and proinflammatory cytokine IL-1β. As mentioned earlier, IL-12 and IL-18 induce T cells to produce IFN-γ. This synergistic action is a Th1 response, so that IL-18 is a co-stimulatory factor in the activation of Th1 but not
Th2 cells.3,10,32

Once inflammatory reactions are activated in burn patients, Th1-cellular immunity might be augmented by the action of IL-12 and IL-18. Other cytokine responses were not investigated in this study, so this hypothesis will be the subject of a future study.

In a previous report,6 combined treatment with IL-18 and IL-12 prolonged survival in mice with experimental Cryptococcus neoformans infection. These protective effects were associated with elevated IFN-γ,33 and reduced IL-4 in bronchoalveolar fluid.6,34 On the other hand, IL-18 also acts alone to increase Th2 cytokine production, serum IgE levels, and eosinophil recruitment, which may result in allergic sensitization and contribute to the pathogenesis of allergic asthma.35,36

Since we have found that IL-18 shows a relationship with OI as a respiratory parameter, measurements of this cytokine might play a role in respiratory dysfunction via systemic inflammatory response. Inhalation injury could have some influence on IL-18, although this association did not attain statistical difference.

SOFA score and mortality were both related to IL-18 in our burn patients. These results agree with those of previous reports,31,32 and the higher the IL-18 values were, the higher the Acute Physiology and Chronic Health Evaluation (APACHE) II score was in the septic burn group.29

In conclusion, increased IL-18 in burn patients appears to be a response to wound infection and the magnitude of systemic inflammation, and is also related with mortality.

Further study is needed to delineate the role of IL-18 in the pathophysiology of burn patients in the future.

References

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