Soluble Interleukin 2 Receptor and Interleukin 1α in Toxic Epidermal Necrolysis

A Comparative Analysis of Serum and Blister Fluid Samples

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Background: Toxic epidermal necrolysis (TEN) is a rare but severe adverse drug disease, characterized by extensive skin and mucosal detachment with participation of different immunoinflammatory pathways, in particular with early participation of activated CD8+ T lymphocytes.

Objective: To further study the potential role of T lymphocytes in the early phase of keratinocyte necrosis.

Design: Prospective study.

Setting: University hospitals.

Patients: Thirteen patients with clinical and histopathologic criteria of TEN and 6 patients with second-degree burns.

Main Outcome Measures: Measurement of soluble interleukin (IL) 2 receptor (sIL-2R) and IL-1α in serum samples and fluid of recent blisters.

Results: In the blister fluid of patients with TEN, we found significantly higher levels of sIL-2R than in patients with burns, whereas IL-1α levels were higher in the blister fluid of burned patients. No significant differences were found in serum samples of patients with TEN and burns, in either sIL-2R or IL-1α. In TEN we also found significantly higher levels of sIL-2R in the blister fluid compared with serum samples, pointing to a predominantly local production contrasting with the low concentration of sIL-2R in the blister fluid of burned patients.

Conclusions: Our findings of elevated sIL-2R levels in blister fluid of patients with TEN are probably related to a local down-regulation of an immunologically mediated cytotoxic reaction and further support the involvement of activated T lymphocytes in the early blisters of TEN.

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PATIENTS AND METHODS

We studied 13 patients with clinical and histopathologic findings of TEN, according to established criteria. We collected fluid from recent blisters (evolution <48 hours), early in the beginning of the disease and before the introduction of any immunosuppressive agent. In 7 of these patients, blood samples were also obtained at the same time as blister fluid. As a control, we took blood and blister fluid from 6 patients with second-degree burns. Samples were centrifuged and stored at -20°C until use. The IL-1α and sIL-2R were measured in duplicate samples by means of commercially available specific enzyme-linked immunosorbent assays (Immunotech, Marseille, France), according to the manufacturer's instructions. Results are presented as medians and ranges. For statistical analysis, we used the Mann-Whitney test, the Wilcoxon matched-pairs signed rank test, and the Spearman rank correlation. A level of P<.05 was considered statistically significant.

10 patients were female and 3 were male. Median skin detachment on the day the samples were collected was 27% of body surface area (range, 18%-58%), but it increased to a maximum of 40% (20%-95%). In all 13 patients, we collected blister fluid from recent blisters (evolution <48 hours) and early in the course of the disease. At that time, all patients had fever (median temperature, 39.0°C), without documented associated infection. Of the 13 patients, 5 (38.5%) died, most of them achieving the higher percentages of skin detachment. Associated drugs were found in 12 of the 13 patients, according to the imputability methods used for toxic drug reactions. For the 6 control patients with second-degree burns, median age was 42 years (range, 20-46 years); 5 were female and 1 was male. Median skin detachment on the day the samples were collected was 15% of body surface area (range, 10%-40%). At that time, the median temperature was 37.4°C (range, 37.0°C-38.2°C).

The sIL-2R and IL-1α levels in blister fluid and serum of the patients with TEN and burns are shown in Table 2. In TEN blisters, we found significantly higher levels of sIL-2R than in burns (P=.005), whereas IL-1α levels were higher in the blister fluid of burned patients (P=.02). No significant differences were found in serum samples of patients with TEN and burns, in either sIL-2R or IL-1α.

Table 3 compares the levels of sIL-2R and IL-1α in the blister fluid and serum for both disorders, taken at the same time. In TEN we found significantly higher levels of sIL-2R in the blister fluid (P=.03) and no correlation between levels of sIL-2R in serum and blister fluid (r = 0.46, P = .29, Spearman rank correlation). In contrast, burned patients had a significantly lower concentration of sIL-2R in the blister fluid, which correlated positively with serum concentrations (r = 0.83, P = .04). We did not find a correlation between blister and/or serum sIL-2R level and the severity (skin detachment) or outcome of the patients. No significant differences were found between serum and blister fluid levels of IL-1α in both patients with TEN and burned control patients.

COMMENT

Toxic epidermal necrolysis is rare, with an incidence of 0.4 to 1.2 cases per 1 million people, but frequently fatal, with an estimated rate of death around 30%. The patients included in our series (Table 1) were all classified according to a recognized classification. The most probable culprit drug was found according to an imputability method used for adverse drug reactions. Toxic epidermal necrolysis is usually associated with drug intake, but the pathophysiologic characteristics are not fully understood; however, immunologic mediation is unquestionable. Since the blistering eruption is a rapid event after bulla appearance. With this rule in mind, we demonstrated that CD8+ T lymphocytes with the phenotype of memory and cytotoxic T cells were the predominant cells in the first blisters and within the 48 hours of their appearance. Most of the blister fluid T lymphocytes express the skin-homing receptor cutaneous lymphocyte–associated antigen. In addition, drug-specific CD8+ T-cell clones have been described in skin adverse drug reactions, and T-cell–mediated cytotoxic effects against keratinocytes seem to be drug specific and mediated by perforin. The present study, with the finding of high levels of sIL-2R in patients with TEN, mainly in early blisters, further corroborates the participation of activated T lymphocytes or associated cytokines in the first steps of this disease.

Soluble IL-2 receptor represents the extracellular domain of the 55-kd α-chain of the IL-2 receptor. In vitro studies have shown the release of sIL-2R from activated T cells, correlating with the rate of expression of the membrane-bound receptor and the degree of activation. A similar release, but at a much lower rate, has also been described in B cells and monocytes, and therefore sIL-2R is usually considered a marker of T-cell activation. Elevated serum sIL-2R concentrations have been reported in burned patients, as part of the inflammatory response and immune dysfunction of the postburn period, and in disorders involving the activation of T cells, like autoimmune diseases or organ transplant rejection. In transplant rejection, serum sIL-2R concentrations correlate with the severity of the disease, and, interestingly, they are a sensitive indicator for acute graft-vs-host disease in allogeneic bone marrow transplantation, a clinical and biological disorder that, in severe cases, mimics TEN. This soluble protein (sIL-2R) can be measured in biological fluids by means of an enzyme-linked immunosorbent assay and thus provides an indirect indicator of activated T cells, which are known to be present in the early blisters of TEN, where cytotoxic CD8+ lymphocytes predominate, as recently shown. A small subset of natural killer cells may also express the 55-kd α-chain of the IL-2 receptor (the CD16+ CD56high subset), representing less than 10% of natural killer cells. However, this dis-
cretes natural killer subset lacks killer cell immunoglobulin-like receptor expression, 24 in contrast to blister fluid lymphocytes reported in TEN. 13 In the blister fluid of patients with TEN, we found significantly increased levels of sIL-2R compared with control patients with second-degree burns (P = .005; Table 2). Moreover, only in TEN cases, blister levels were significantly higher than levels in corresponding serum samples taken at the time of blister puncture (P = .03; Table 3). This finding, and the lack of correlation with serum levels (r = 0.46, P = .29, Spearman rank correlation), points to a release of sIL-2R early at the site of blister formation in TEN and supports the presence of activated T cells in lesional skin. 16 In contrast, sIL-2R levels in control blisters of burned patients were significantly lower than levels in corresponding serum samples and positively correlated with serum concentrations (r = 0.83, P = .04), consistent with a diffusion from the blood compartment. In 2 patients with TEN, we measured sIL-2R level in blisters with less than 48 hours of evolution, but appearing in the subsequent days, and we found a decrease in sIL-2R level associated with a decrease in the number of CD8+ lymphocytes (data not shown), further reinforcing the importance of knowing when the different studies were performed.

A significant reservoir of preformed sequestered IL-1α exists in epidermis, mainly in keratinocytes, and may be released on injury. External stimuli, such as burns, or internal triggers, such as local cytokine production, can induce the release of IL-1 for local or systemic delivery. 23 Fluid taken from blisters on thermally injured skin, early after burn injury, contains substantial amounts of IL-1, and the source is the injured keratinocytes. 26 In fact, human keratinocytes contain predominantly IL-1α, but more than 50% of the IL-1 activity within the epidermis is confined to the stratum corneum. 27 Our data confirm the rapid and intense aggression to keratinocytes in burned patients, with consequent significantly higher levels of IL-1α in the early blisters, suggesting that thermal injury is a major stimulus for the production of this inflammatory cytokine by keratinocytes. However, different lesional mechanisms may explain the significant difference we found in blister fluid IL-1α level of burned patients compared with those with TEN—an exogenous thermal injury that induces keratinocyte necrosis at the epidermal surface (in burned patients) vs an endogenous immunologic cytotoxic reaction that induces a Fas-ligand–mediated keratinocyte apoptosis 7 at the dermal-epidermal surface.

### Table 1. Relevant Clinical Characteristics of Patients With TEN*

<table>
<thead>
<tr>
<th>Patient No./</th>
<th>Sex/Age, y</th>
<th>Skin Detachment, % BSA</th>
<th>Temperature, °C</th>
<th>Death</th>
<th>Suspected Drug</th>
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<tr>
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<tr>
<td>3/F/52</td>
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<td>4/F/33</td>
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<tr>
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<td>38.2</td>
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<tr>
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<td></td>
<td>20</td>
<td>40.0</td>
<td>No</td>
<td>Fenofibrate</td>
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</table>

*TEN indicates toxic epidermal necrolysis; BSA, body surface area. Time 1 is the time of collection of blood and blister fluid samples; time 2, the time of maximum skin detachment.

### Table 2. Blister Fluid and Serum Levels of sIL-2R and IL-1α in Patients With TEN and Control Subjects With Burns*

<table>
<thead>
<tr>
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<th>Median (Range)</th>
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<tr>
<td></td>
<td>TEN Blister</td>
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<tr>
<td>sIL-2R, pmol/L</td>
<td>300.0 (13.0-632.0)</td>
</tr>
<tr>
<td>IL-1α, pg/mL</td>
<td>2.5 (2.5-180.3)</td>
</tr>
</tbody>
</table>

### Table 3. Comparison of Blister Fluid and Serum Levels of sIL-2R and IL-1α in TEN*

<table>
<thead>
<tr>
<th></th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TEN Blister</td>
</tr>
<tr>
<td>sIL-2R, pmol/L</td>
<td>308.3 (160.3-632)</td>
</tr>
<tr>
<td>IL-1α, pg/mL</td>
<td>4.4 (2.5-26.4)</td>
</tr>
</tbody>
</table>

* sIL-2R indicates soluble interleukin 2 receptor; IL-1α, interleukin 1α; and TEN, toxic epidermal necrolysis. In the TEN group, n = 13 for blister fluid and n = 7 for serum; the burn group, n = 6.
The significance of our findings of high levels of sIL-2R in the blister fluid of patients with TEN is difficult to establish, as the biological consequences of high concentrations of sIL-2R in several diseases are still poorly understood. In TEN, CD8+ cytotoxic T lymphocytes directly and/or by induced cytokines are probably the main effectors to induce blister formation, and an increased number of lymphocytes with the natural killer phenotype and cytotoxic function were recently described in the blister fluid of patients with TEN. As IL-2 is especially relevant for the generation of cytotoxic T cells, natural killer cells, and lymphokine-activated killer cells, it is possible that high levels of sIL-2R in the skin, even resulting from immune activation, may be involved in a local down-regulation of an immunologically mediated cytotoxic reaction. In this regard, Jobin et al.18 studying serum samples from burned patients, showed that purified sIL-2R inhibited natural killer cell activity by 50% and suppressed IL-2–induced interferon-γ production by peripheral blood mononuclear cells.

As the recently described expression of the killer cell immunoglobulin-like receptor in most lymphocytes present in the early blister fluid of patients with TEN,13 elevated sIL-2R levels in blister fluid could be another way to down-regulate a massive cytotoxic reaction against keratinocytes. In fact, recent reports indicate that sIL-2R suppresses both natural killer cell activity and IL-2–induced interferon-γ, a cytokine required for the efficient specific killing of keratinocytes by cytotoxic T cells30 and drug-specific T-cell clones.8

While the cellular sources of the cytokines we measured in the blister fluid of patients with TEN were not established in our study, their expression and/or secretion in situ may participate in a cytokine cascade that culminates in the extensive skin and mucosal injury seen in the disease. A better understanding of the contribution of these and other immunoregulatory cytokines to the pathogenesis of TEN may allow the design of more specific and more effective therapy.

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REFERENCES