Soluble Fas in Pemphigus Vulgaris

Hamideh Moravvej MD, Maryam Yousefi MD*, Babak Farrokhi MSc, Nariman Mosaffa DVM PhD

Abstract

Background: The Fas/Fas ligand (FasL) system has been recognized as an important pathway for apoptosis induction in cells and tissues. It has recently been shown that skin lesions of pemphigus vulgaris are associated with Fas mediated apoptosis. The aim of this study was to evaluate the level of serum soluble Fas of ten newly diagnosed patients with pemphigus vulgaris.

Methods: Sera were collected from ten patients with pemphigus vulgaris. Commercial sandwich enzyme-linked immunosorbent assay (ELISA) for quantitative detection of soluble Fas was applied.

Results: Patients with mucosal skin involvement had higher median values in contrast to patients with cutaneous involvement.

Conclusion: Elevation of soluble Fas in our study may give insights for the pathogenesis of pemphigus vulgaris. Suppression of this underlying mechanism may be an important target for novel therapies and relapse prevention.

Keywords: apoptosis, Fas, Fas ligand, pemphigus vulgaris

Introduction

Pemphigus vulgaris (P.V.) is a life-threatening mucocutaneous auto-immune disease characterized by cell-cell detachment within the stratified epithelium due to IgG auto-antibodies against autoantigens expressed on the human suprabasal keratinocyte plasma membrane.

Binding of pemphigus antibodies to keratinocytes causes these cells to separate from each other, a process called acantholysis. Acantholysis leads to extensive intra-epidermal clefting, gross blisters, and erosions of the skin and oral mucosa in patients with P.V.

The Fas/Fas ligand (FasL) system has been recognized as an important pathway for apoptosis induction in cells and tissues. Fas is widely expressed in normal and neoplastic cells. FasL expression in normal tissues is limited to activated T-cells, natural killer cells and macrophage lineage. Ligation of Fas by either agonistic antibody or its natural ligand, FasL, induces a death signal to the targeted cells, thus triggering apoptosis.

In human skin, Fas has been observed in the basal and spinous layers of the normal epidermis and dermal adnexae, and in various disease states such as malignancies.

Healthy patient sera does not harbor detectable levels of soluble FasL (sFasL), whereas it is present in sera from patients with large granular lymphocytic leukemia and natural killer cell lymphoma.

It has recently been shown that P.V. skin lesions are associated with Fas mediated apoptosis and Gerando et al. have recently postulated the role of apoptosis (the result of a cascade initiated by apoptosis) in skin blistering of P.V.

Also a marked increase in serum soluble Fas (sFas) has been shown in toxic epidermal necrolysis (TEN) and drug induced hypersensitivity syndrome. In present report we measured sFas in ten new onset patients with P.V. in order to determine if this survival mechanism would be blocked in omitting auto-reactive B lymphocytes, thus trigger auto-immunity in organ-specific disorders such as P.V.

Patients and Methods

Patients

A diagnosis of P.V. was made from clinical, histological, and immunological studies after which patients were classified into three groups according to site of involvement: 1) mucosal, 2) mucocutaneous, and 3) cutaneous.

Blood samples (5 mL) were taken from ten newly diagnosed P.V. patients before starting systemic treatment. Sera were collected from clotted blood and kept frozen at -20°C until assayed by Enzyme-Linked Immunosorbent Assay (ELISA).

Determination of sFas

A commercial sandwich ELISA for quantitative detection of sFas [APO-1 (sFas/APO-1) ELISA Kit Bender Med System, BMS245 GmbH, Vienna, Austria] was used to determine serum sFas levels. The assay is based on an anti-sFas monoclonal coating antibody which is adsorbed onto micro wells. Optical Density (OD) was measured out at 450 nm with 620 nm as an optical reference wave length in an automated plate reader (Anthos 2020). Levels of sFas were determined by comparison with the standard curve, which was determined by generating two rows of sFas at standard dilutions (range: 15.6 – 1000 pg/mL).

Statistical analysis

The median values of the individual sFas levels were calculated for each group.

Results

In this study, there were a total of ten patients (6 female; 4 male) whose median age was 50.5 years. As seen in Table 1, patients were classified according to the following levels of involvement: mucosal, mucocutaneous, and cutaneous.

The overall median sFas level was 2095 pg/mL; however, amongst the three groups of patients median levels were as follows: mucosal (2775 pg/mL), mucocutaneous (1820 pg/mL), and...
cutaneous (1130 pg/mL). Patients with mucosal involvement had a higher median value (2775) in contrast to patients with cutaneous involvement who had the lowest median value (1130).

**Discussion**

Apoptosis is believed to play a role in the mechanism of keratinocyte death in P.V. The occurrence of apoptosis markers has been observed in early lesions of P.V. patients prior to acantholysis. Pemphigus vulgaris IgG and sera have been shown to induce biomolecular markers of apoptosis in keratinocyte monolayers and skin organ cultures, with caspase inhibitors abolishing P.V. IgG-induced acantholysis.

In autoimmune diseases, elevated sFas levels have been observed and it is suggested that sFas may play a role in pathogenesis by inhibiting Fas-mediated apoptosis of activated lymphocytes.

Wang et al. have shown that autoimmunie antibodies from pemphigus patients can induce the Fas-dependent pathway of apoptosis in human keratinocytes. Their study revealed increased susceptibility of senescent keratinocyte to P.V. IgG-mediated apoptotic death and culture lesions.

Puviani et al. studied Fas ligand levels in sera from patients with P.V. and pemphigus foliaceus. That study showed marked increase of Fas levels in the sera from pemphigus patients, whereas Fas levels were undetectable in those who received steroids. Addition of anti-FasL neutralizing antibody partially inhibited pemphigus sera-induced keratinocyte apoptosis. FasL additionally plays a crucial role in the pathogenesis of TEN, a disease similar to pemphigus that is characterized by apoptotic keratinocytes.

In summary Fas/FasL may be involved in the pathogenesis of various diseases including autoimmune diseases, melanoma, TEN, and P.V.

Elevation of sFas in present study indicates that serum sFas can be a marker for the resistance of antibody secreting auto-reactive lymphocytes against keratinocytes, thus demonstrating the inability of CD8+ regulatory T lymphocytes in omitting auto-reactive B cells. We can postulate that serum sFas, particularly in the initial phases of auto-immune disorders, may indicate the resistance of auto-reactive lymphocytes to death. We recommend further studies on larger groups of patients that consider sFas as a prognostic indicator for stopping treatment in addition to clinical improvement.

**Table 1.** Demographic features and soluble Fas values in patients with pemphigus vulgaris according to type of clinical manifestation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patient No.</th>
<th>Age (year)</th>
<th>Sex</th>
<th>Serum concentration of sFas (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal</td>
<td>1</td>
<td>51</td>
<td>Male</td>
<td>3040</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>46</td>
<td>Female</td>
<td>2510</td>
</tr>
<tr>
<td>Mucocutaneous</td>
<td>3</td>
<td>60</td>
<td>Female</td>
<td>4230</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>45</td>
<td>Female</td>
<td>1340</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>59</td>
<td>Male</td>
<td>1780</td>
</tr>
<tr>
<td></td>
<td>6</td>
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<td>Male</td>
<td>3480</td>
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<tr>
<td></td>
<td>7</td>
<td>44</td>
<td>Female</td>
<td>1820</td>
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<tr>
<td>Cutaneous</td>
<td>8</td>
<td>53</td>
<td>Male</td>
<td>2370</td>
</tr>
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<td></td>
<td>9</td>
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<td>1130</td>
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<tr>
<td></td>
<td>10</td>
<td>56</td>
<td>Female</td>
<td>920</td>
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**References**