Soluble Interleukin-2 Receptor Levels in Patients with Dermatitis Herpetiformis

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To determine the role of T-cell activation in dermatitis herpetiformis (DH), soluble IL-2R levels were measured by enzyme-linked immunosorbent assay (ELISA) in the sera of 30 patients with DH. Levels of this shed receptor are considered to be a measure of in vivo T-lymphocyte activation, and are elevated in the sera of many patients with inflammatory and immune-mediated diseases. Fifteen of the thirty (50%) patients with DH had elevated levels of soluble IL-2R compared to one of 31 (3%) healthy HLA-B8 or HLA-DR3 control subjects (p < 0.00001) and one of 10 (10%) healthy non–HLA-B8/-DR3 subjects (p < 0.0018). In addition, the mean soluble IL-2R level in the patients with DH (744 ± 381 U/ml) was also significantly higher than that seen in 31 healthy HLA B8 or HLA DR3 individuals (388 ± 160 U/ml, p = 0.0001) and 10 healthy non–HLA-B8/DR3 individuals (397 ± 201 U/ml, p = 0.002). Only two of the 30 patients with DH had active skin lesions at the time of serum sampling, one of whom had elevated levels of IL-2R. Measurement of soluble IL-2R levels in sequential serum samples, available in four patients with DH at times of active and inactive skin disease, demonstrated a temporal association between soluble IL-2R level elevations and active skin disease in two patients and no association in two patients. In one patient a marked elevation in soluble IL-2R levels occurred with the onset of gastrointestinal symptoms, which decreased by 14% with institution of a gluten-free diet. In order to determine if soluble IL-2R levels are related to the mucosal immune response, the IL-2R levels were compared to the level of IgA antibodies directed against the dietary antigen β-lactoglobulin. Ten of eleven (91%) patients with circulating IgA anti-β-lactoglobulin antibodies were also found to have elevated levels of IL-2R. In contrast, in the patients with no detectable IgA anti-β-lactoglobulin antibodies, only four of 16 (25%) had elevated levels of IL-2R (p = 0.001). Because IL-2R levels are not related to activity of the skin disease in patients with DH but are associated with the presence of IgA antibodies against the dietary antigen β-lactoglobulin, these results suggest that some of the T-cell activation commonly present in DH reflects an ongoing immune response in the gastrointestinal tract. J Invest Dermatol 97:568–572, 1991

Dermatitis herpetiformis (DH) is a chronic papulovesicular skin disease characterized by granular deposition of IgA at the dero-mepidermal junction, the presence of a gluten-sensitive enteropathy (GSE), and a strong association with the extended HLA haplotype -B8, -DR3, -DQw2 [1–4]. Although the GSE found in patients with DH is most often asymptomatic, a pathophysiologic link between the skin and the gut disease has been suggested by a variety of findings [5]. IgA, the predominant immunoglobulin in secretions, is uniformly present in the skin of patients with DH [6,7]. In addition, approximately 50% of patients with DH have an IgA antibody response to dietary proteins and IgA-containing circulating immune complexes in their serum, suggesting an ongoing mucosal immune response in the gut [8–10]. The importance of the mucosal immune response in the pathophysiology of DH is further substantiated by the observation that the skin lesions of DH as well as the small bowel histologic changes resolve on a gluten-free diet (GFD) [5,11].

Small bowel biopsies of patients with DH reveal the presence of a lymphocytic infiltrate in the lamina propria, consisting of T and B lymphocytes and plasma cells [5,12–14]. T lymphocytes are integral components of immune responses, modulating B-cell responses as well as exerting a variety of effector functions upon exposure to an appropriate antigen. Activated T cells secrete several lymphokines, including interleukin-2 (IL-2), a 15,000-dalton protein that acts to initiate the proliferation and clonal expansion of activated T cells [15]. Lymphocyte responses to IL-2 are mediated by specific multi-chain cell-surface receptors, which are induced after antigen or mitogen-induced T-cell stimulation, and, in an autocrine manner, by IL-2 itself [16,17]. A shortened form of the Tac, or p55, chain of the IL-2 receptor (IL-2R) is shed from the surface of activated mononuclear cells and is detectable in serum or supernatants from in vitro cell cultures [18]. High concentrations of soluble IL-2R have been found in the sera of patients with a variety of neoplastic, infectious, and inflammatory conditions, including rheumatoid arthritis, systemic lupus erythematosus (SLE), and skin

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diseases such as atopic dermatitis and psoriasis that have a significant lymphocytic dermal infiltrate [19–23]. Recently, elevated soluble IL-2R levels have also been demonstrated in patients with untreated celiac disease [24]. Because activated T lymphocytes are likely the predominant source of soluble IL-2R, the elevated levels of soluble IL-2R in these conditions have been considered to represent T-cell activation and participation in the pathogenesis of these illnesses.

Measurement of soluble IL-2R in patients with DH may help determine the presence of immune activation in this illness, and elucidate the relationship between immune T-cell activation and both the cutaneous and gastrointestinal manifestations of this disease. In this study, soluble IL-2R levels were measured in the sera of 30 patients with DH and compared to those of healthy HLA-B8/DR3 individuals including those with HLA-B8 and/or HLA-DR3/DQw2. In addition IL-2R levels were compared to the levels of IgA antibodies directed against the dietary antigen bovine β-lactoglobulin. Results of these studies indicate that DH is characterized by elevated levels of soluble IL-2R that are not directly correlated with skin disease; a correlation with IgA antibodies to β-lactoglobulin, however, suggests a role of an ongoing mucosal immune response in causing these elevations.

MATERIALS AND METHODS

Cross-Sectional Study Groups The sera of 30 patients with DH were studied cross-sectionally (23 male, seven female). All patients had typical clinical and histologic features of DH, and each had granular deposits of IgA at the dermal-epidermal junction of normal-appearing skin. Two patients had active skin disease with blisters on extensor surfaces at the time of serum sampling; these patients were taking no medications and were not on dietary restrictions. Twenty-seven patients had their skin disease controlled with medication, using either dapsone alone (25–300 mg/d, 22 patients), dapsone plus a gluten-free diet (two patients), sulfapyridine 500–1000 mg/d (two patients), or topical corticosteroids (one patient). One patient's skin disease was controlled only on a gluten-free diet. Although two treated patients reported mild gastrointestinal symptoms, no clinical evidence of malabsorption was present in any of the DH patients studied. The two untreated patients with active skin disease did not report any gastrointestinal complaints.

Two control groups, comprised of sera from HLA-typed healthy individuals, were used. Thirty-one individuals typed as HLA-B8 and/or HLA-DR3/DQw2 formed one control group. The second control group consisted of ten healthy non–HLA-B8/DR3 individuals.

Longitudinal Study Groups Sequential serum samples, obtained at times of clinically active and inactive skin disease, were available in four of the 30 patients with DH, and were assayed for soluble IL-2R levels. In one patient serum samples were available before the onset of clinically symptomatic gastrointestinal disease, as well as after the institution of a gluten-free diet. In addition, serial serum samples from seven patients with DH, all of whom were on dapsone, a normal diet, and had inactive skin disease, were analyzed. For comparison, the sera of two patients with bullous pemphigoid, obtained when their skin disease was active (blisters involving greater than 50% of body surface area) and when no active blistering was present, were also studied.

Soluble IL-2R Assays Soluble IL-2R levels were measured in each serum sample using a commercially available kit (T Cell Sciences, Cambridge, MA). This assay is a sandwich enzyme-linked immunosorbent assay (ELISA) in which the coating antibody is anti-Tac and the peroxidase-conjugated antibody is 7G7/B6, a monoclonal antibody that recognizes a site on the IL-2R distinct from both the Tac and the IL-2 binding sites [18]. Sera were stored at −70°C until use, and the assay was performed according to manufacturers' instructions. Values of soluble IL-2R are reported as units/ml (U/ml), and based on comparison to a standard curve generated from supplied standards. All values represent the average of duplicate measurements. In a typical experiment individual duplicate measurements varied 4.4 ± 0.5% (mean ± SEM) from the average of the duplicate values.

IgA Anti-Bovine β-Lactoglobulin The levels of IgA antibovine β-lactoglobulin antibodies were determined using an ELISA as previously described [8]. Briefly, bovine β-lactoglobulin was adsorbed to ELISA plates at a concentration of 0.1 mg/ml in a carbonate-bicarbonate buffer, pH 9.3, for 16 h at 4°C. After washing, serial dilutions of a standard sera containing IgA anti–β-lactoglobulin antibodies (12,800 U/ml) were added in duplicate as were the test sera, all diluted in PBS with 0.05% Tween-20. After 2 h at 37°C, the plates were washed with PBS-Tween and an affinity-purified, heavy-chain–specific, peroxidase-conjugated goat anti-human IgA reagent (Tago, Burlingame, CA) was added for 2 h at 37°C. The plates were again washed and developed with O-phenylenediamine. The concentration of IgA anti–β-lactoglobulin antibodies in the test sera was determined utilizing the linear portion of the standard curve generated using our standard serum.

RESULTS

Sixteen of the 30 (53%) patients with DH had soluble IL-2R measurements that exceeded the upper 95% confidence limit of the HLA-B8/DR3 control group (upper 95% confidence interval = 651 U/ml) compared to only one of 31 HLA-B8/DR3 normal subjects (p < 0.0001, Fisher's exact test) and 1 of 10 non–HLA-B8/DR3 normal subjects (p = 0.0018, Fisher's exact test) (Fig 1). In addition, the group of 30 patients with DH studied cross-section-
Table I. Soluble IL-2R Measurements in the Sera of Patients with Dermatitis Herpetiformis and Bullous Pemphigoid at Times of Active and Inactive Skin Disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease*</th>
<th>Date of Serum Sample</th>
<th>Status of Skin Disease</th>
<th>Soluble IL-2R (U/ml)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DH</td>
<td>4/8/88</td>
<td>Inactive</td>
<td>569</td>
<td>Dapsone 75 mg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/17/89</td>
<td>Active</td>
<td>764</td>
<td>Dapsone 75 mg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/7/89</td>
<td>Active*e</td>
<td>1165</td>
<td>Dapsone 100 mg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10/20/89</td>
<td>Inactive</td>
<td>1001</td>
<td>Dapsone 150 mg/d GFD</td>
</tr>
<tr>
<td>2</td>
<td>DH</td>
<td>2/12/87</td>
<td>Active</td>
<td>293</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/30/89</td>
<td>Inactive</td>
<td>152</td>
<td>Dapsone 25 mg/d GFD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9/7/89</td>
<td>Inactive</td>
<td>210</td>
<td>Dapsone 25 mg/d GFD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10/5/81</td>
<td>Inactive</td>
<td>352</td>
<td>Dapsone 100 mg/d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/23/81</td>
<td>Active</td>
<td>526</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/8/85</td>
<td>Inactive</td>
<td>360</td>
<td>Dapsone 100 mg/d</td>
</tr>
<tr>
<td>3</td>
<td>DH</td>
<td>6/15/87</td>
<td>Active</td>
<td>295</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/14/89</td>
<td>Inactive</td>
<td>342</td>
<td>Dapsone 200 mg/d</td>
</tr>
<tr>
<td>4</td>
<td>BP</td>
<td>8/10/86 ,</td>
<td>Active</td>
<td>745</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>BP</td>
<td>7/2/87</td>
<td>Inactive</td>
<td>309</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>BP</td>
<td>3/9/87</td>
<td>Active</td>
<td>1219</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/22/88</td>
<td>Inactive</td>
<td>506</td>
<td>Prednisone, 20 mg, qod</td>
</tr>
</tbody>
</table>

* DH, dermatitis herpetiformis; BP, bullous pemphigoid.

*a Onset of gastrointestinal symptoms, minimal skin disease.
* GFD, gluten-free diet.

ally had a mean soluble IL-2R level of 744 ± 381 U/ml, with a range of 293–2096 U/ml, significantly higher than those of the 31 HLA-B8/DR3 control individuals (mean soluble IL-2R level = 388 ± 160 U/ml) (p < 0.0001, Student t test, two tailed). The mean soluble IL-2R level of the patients with DH was also significantly higher than that of 10 non-HLA-B8/DR3 control individuals (397 ± 201 U/ml; p = 0.002). There was no significant difference in soluble IL-2R levels between the HLA-B8/DR3 control and the non-HLA-B8/DR3 control groups (p = 0.77).

Two patients with DH had active skin lesions (10–20% body surface involvement) at the time of serum sampling. One had a normal level of soluble IL-2R level (293 U/ml) whereas the other patient had an elevated IL-2R level (797 U/ml). Of the two patients with mild gastrointestinal symptoms, the soluble IL-2R levels were 366 U/ml and 746 U/ml. There was no correlation between soluble IL-2R level and dapson dose (r = 0.28; p = 0.13) in 23 patients for whom dapson dose was available. In the three patients treated with a gluten-free diet, the soluble IL-2R levels were 746 U/ml, 638 U/ml, and 880 U/ml. It is important to note that both of the patients on a gluten-free diet with elevated levels of IL-2R (746 U/ml and 880 U/ml) continued to require medication (dapsone) to control their skin disease.

To further assess the relationship between soluble IL-2R levels and the presence of active skin disease, soluble IL-2R levels were measured on sequential sera obtained from four DH patients at times of active and inactive skin lesions (Table I). In two of the four DH patients, soluble IL-2R levels were increased at the time of active skin disease, relative to times when their skin disease was controlled (Table I, patients 2 and 3). However, there was no apparent relationship between soluble IL-2R levels and the activity of the skin disease in the other two patients with DH (Table I, patients 1 and 4). In patient 1, increases in soluble IL-2R levels occurred around the time of development of symptomatic GSE and improved with the institution of a gluten-free diet, although the patient continued to require dapsone therapy to control his skin disease. In patient 4, there was little variability in soluble IL-2R levels despite marked differences in skin disease activity at two determinations. In contrast, sequential sera of both patients with bullous pemphigoid demonstrated decreases in soluble IL-2R of greater than 50% when their skin disease was controlled (Table I).

Serial serum samples from seven patients with DH taken while the patients were on a normal diet and their skin disease was controlled by dapsone showed no significant change in the IL-2R levels (Table II).

IgA Anti-β-Lactoglobulin Sera of 27 of the patients with DH were assayed for both soluble IL-2R levels and IgA antibodies against bovine β-lactoglobulin. Eleven of 27 (41%) patients with DH had elevated levels of IgA anti-β-lactoglobulin antibodies (upper 95% confidence interval, 29 normal subjects, 8 U/ml). Elevated levels of soluble IL-2R (> 651 U/ml) were found in 14 of these 27 (52%) patients. Elevated IL-2R levels were present in 10 of 11 (91%) patients with IgA anti-β-lactoglobulin antibodies, but in only 4 of 16 (25%) patients lacking IgA anti-β-lactoglobulin (p = 0.001, Fisher's exact test, 2 tailed) (Table III). In addition, a positive correlation was seen between the levels of IL-2R and IgA anti-β-lactoglobulin (r = 0.596, p = 0.01, Spearman rank order correlation).

**DISCUSSION**

In this study of 30 patients with DH, soluble IL-2R levels were found to be increased in approximately 50% of patients. In addition, the mean soluble IL-2R level was found to be significantly higher in the patients with DH (744 U/ml) when compared to that of both HLA-matched and non–HLA-matched controls. The level of IL-2R elevation seen in our patients with DH was comparable to soluble IL-2R levels reported in the sera of patients with other immunemediated diseases, including rheumatoid arthritis and SLE [20,21,25,26]. The presence of high levels of soluble IL-2R in this patient group was not related, however, to the presence of active skin lesions, dapson dose, or gastrointestinal symptoms. The presence of serum IL-2R elevations in many DH patients without active skin lesions indicates that this measure does not uniformly reflect

Table II. Mean Serum IL-2R Levels in Patients with Dermatitis Herpetiformis on a Normal Diet and Dapsonole

<table>
<thead>
<tr>
<th>Patient</th>
<th>N</th>
<th>IL-2R Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5*</td>
<td>1000 (20.3)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>385 (7.2)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>499 (11.5)</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>595 (30.3)</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>845 (45.5)</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>420 (36.9)</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>1136 (58)</td>
</tr>
</tbody>
</table>

* Number of serial serum samples analyzed.
* Mean (SEM) serum IL-2R levels units/ml.
Table III. Correlation of Presence of Elevated Levels of IgA Anti-β-Lactoglobulin and Soluble IL-2R in Patients with DH

<table>
<thead>
<tr>
<th>Elevated IL-2R</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal IL-2R</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

* Number of patients.

the status of the cutaneous disease and is not secondary to the cutaneous inflammation that occurs in these patients. Sera from four patients with DH were studied at times of active and inactive skin disease and showed no consistent relationship between the activity of the skin lesions and the level of soluble IL-2R. In addition, soluble IL-2R levels remained stable in patients with DH who had no active skin disease while on a normal diet and dapsone (Table II). In contrast, soluble IL-2R levels in two patients with bullous pemphigoid decreased dramatically with control of their skin disease.

The absence of an apparent association between soluble IL-2R levels and active skin lesions in DH suggests that other immunologic features of these patients influence serum IL-2R levels. Patients with DH have a high frequency of the HLA-B8, -DR3, -DQw2 haplotype [1,4,27]. A number of specific immunologic abnormalities have been described in healthy HLA-B8/DR3 individuals, including elevated CD4:CD8 T-cell ratios, decreased T-cell Fe receptors, defective immune clearance mechanisms, and increased spontaneous B-cell immunoglobulin secretion and enhanced primary antibody responses [28–32]. These immunoregulatory abnormalities raise the possibility that the elevated soluble IL-2R levels in patients with DH may be secondary to the strong HLA associations. Comparison of the soluble IL-2R levels in patients with DH and in healthy HLA-B8/DR3 individuals revealed a significant difference, however, indicating that the elevated soluble IL-2R measurements in these patients are disease related, and do not reflect genetically determined differences in levels of immune activation. In addition, soluble IL-2R levels were similar in the HLA-B8/DR3 and non–HLA-B8/DR3 control groups.

The elevated levels of IL-2R seen in our patients with DH might also be related to increased levels of activated T cells in the circulation. Clegg and co-workers have evaluated the level of HLA-DR–bearing T cells in patients with DH and found no differences between patients with DH and normal HLA-B8/DR3 control subjects [33]. In addition, Hall and co-workers have found no evidence of elevated levels of HLA-DR–positive peripheral blood mononuclear cells in patients with DH [34]. These data suggest that the elevated IL-2R levels we observed are not secondary to an increased frequency of circulating activated T cells.

Although soluble IL-2R levels did not correlate with the presence of skin disease there did appear to be a clinical correlation with gastrointestinal disease. In one patient a marked increase in IL-2R level occurred coincidently with the onset of gastrointestinal symptoms and began to fall with institution of a gluten-free diet. In addition, soluble IL-2R levels were elevated in two of three patients on a gluten-free diet, both of whom required dapsone, in addition to a gluten-free diet, to control their skin disease. The patient with a normal IL-2R level, however, was on a gluten-free diet alone. Finally, in the two patients with DH treated with GFD, a decrease of the soluble IL-2R levels was noted when compared to serum obtained before the initiation of the GFD (Table I: patient 1, 14% decrease; patient 2, 28% decrease). This degree of change, although modest, is similar to that seen in patients with isolated GSE following gluten challenge and subsequent resumption of a GFD [24] and was not seen in serial serum samples from patients with DH who were on a normal diet (Table II).

Serum IL-2R levels in patients with DH also correlated with both the presence and level of IgA anti-β-lactoglobulin antibodies. Recently, we have demonstrated that the presence of IgA antibodies in the serum is an accurate reflection of the mucosal immune response in intestinal secretions [35]. The correlation between soluble IL-2R levels and IgA anti-β-lactoglobulin antibodies supports the hypothesis that many patients with DH have an ongoing mucosal immune response in the gut associated with in vivo T-cell activation. It is possible that the variable levels of IL-2R found in our patients with DH may reflect the sensitivity to and/or the amount of dietary gluten they ingest.

The precise relationship between the mucosal immune response in the gut and the skin disease in patients with DH is not known. The skin disease is characterized by the presence of polymorphonuclear leukocytes (PMN) in the dermal papillary tips, with a relative absence of mononuclear cells [2,36]. In contrast, the small intestinal histologic findings in both DH and celiac disease is characterized typically by an infiltrate of T and B lymphocytes and plasma cells [5,12–14]. T cells, predominantly CD8, are found in the epithelial layer, whereas both CD4+ and CD8+ T cells comprise the lymphocytic infiltrate in the lamina propria [12–14]. Demonstration of gluten-specific T suppressor abnormalities in these illnesses suggests a functional importance of the gut mucosal T lymphocytes [37–40]. In addition, the altered immunosuppressive effects of these T cells may be one factor underlying the enhanced antibody production of intestinal B cells [39,41]. These data all support the hypothesis that the gastrointestinal tract is a primary site of immunologic activity in dermatitis herpetiformis.

The manner in which the gut and skin interact to result in the typical PMN-rich skin lesions of DH is, however, unclear. Recently, Hendricks and co-workers have demonstrated that PMN can bind in vitro to the IgA deposits in DH skin only after the PMN have been treated with the cytokine granulocyte-macrophage colony-stimulating factor [42]. This result suggests that in DH, PMN that have been activated by cytokines may be then able to bind to the IgA in skin. Our demonstration of significantly elevated levels of IL-2R in the serum of patients with DH and the strong correlation of these levels with independent measures of the mucosal immune response suggest that the mucosal immune response in the gut could be a source of cytokines that activate PMN.

In this study, we have found that elevated levels of soluble IL-2R are commonly present in patients with DH, although not associated with the severity of the patient’s skin disease or dapsone therapy. A correlation with the magnitude of IgA anti-β-lactoglobulin antibodies in the skin was observed however. These data offer further evidence supporting the presence of an active, ongoing immune response in patients with DH, even in the absence of clinically active skin or gastrointestinal disease.

REFERENCES


