Soluble IL2 Receptor Serum Levels and Epidermal Cytokines in Mycosis Fungoides and Related Disorders

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We examined the immune activation in 20 patients with mycosis fungoides, 6 patients with erythrodermia of unknown origin (Pré-Sézary's syndrome), 5 with lymphomatoid papulosis, 4 with parapsoriasis, 2 with Sézary's syndrome, and 2 with actinic reticuloid, by measuring soluble interleukin-2 receptor levels in serum. In Mycosis fungoides we observed normal levels in 3 patients (<500 units/ml), between 500 and 1000 units/ml in 9 patients, and >1000 units/ml in 5 patients. Four of these 5 patients died within one year after this observation, as did 2 patients with Pré-Sézary and Sézary's syndrome, respectively, who had a similarly large increase in sIL2R. Although sIL2R is not a specific parameter for cutaneous T-cell lymphoma, a value above 1000 units/ml is correlated with clinical disease activity and is a serious prognostic parameter. We also studied cytokine activity in epidermal homogenates from 9 patients with Mycosis fungoides and one patient with Sézary's syndrome. We observed interleukin-1-like activity within the normal range for healthy skin. However, we also observed in the same epidermal homogenates a T-lymphocyte chemotactic activity in patients with stage II, but not in stage I. The nature of this activity is not yet fully elucidated, but it may be an important biological factor for the epidermal T-cell accumulation in this disorder. Key words: Actinic reticuloid; Chemotaxis; Epidermis; Interleukin-1; Lymphocytes; Lymphomatoid papulosis; Parapsoriasis; Pré-Sézary; Sézary syndrome.

(Accepted April 15, 1991.)

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Mycosis fungoides (MF) is histologically characterized by accumulations of pleomorphic T-lymphocytes in the skin. The infiltrating T-cells, which show signs of activation, belong to a subset of helper cells, CD45R/2H2− (1). Similar pleomorphic T-cells are also seen in MF-related disorders such as Pré-Sézary (PS) and Sézary's syndrome (SS), parapsoriasis (PA), and lymphomatoid papulosis (LP), although the histological changes are distinct for these disorders and not for MF. The reason for the lymphocyte accumulation and activation is still not understood.

We have evaluated the immune activation in patients with MF and related disorders by measuring soluble interleukin-2 receptor levels (sIL2R) in serum. We have also evaluated lymphocyte chemotactic and thymocyte-activating activity in epidermis of MF patients in order to ascertain whether such activities could be of significance for the epidermotropism and activation of the lymphocytes.

PATIENTS AND METHODS

Patients

Mycosis fungoides (MF): A total of 20 patients were included. All had a clinical and histological diagnosis of MF except stage I, where Pautrier's microbodies were not found. Stage I included 2 men and one woman; stage II with plaque MF included 9 men and 3 women; stage III with plaques and skin tumours included 2 men and one woman; stage IV with lymph node involvement encompassed one man and one woman. The age range for all 20 patients was 51 to 89 years (median 73 years).

All patients received maintenance therapy, consisting of topical nitrogen mustard once monthly during admission. Blood and skin samples were taken before this therapy was given. Some patients were also given low-dose prednisone (<20 mg daily). etretinate or were receiving maintenance chemotherapy (2).

We have indicated in the figures whether or not the patients had clinical signs of disease activity. None were in complete remission. Some had sparse signs of disease, but most had active lesions requiring continued therapy.

Pré-Sézary (PS): This syndrome is an erythrodermia of unknown origin, also called the Red Man Syndrome (3). Six men with a median age of 72 years (range 48-86 years) were included. One was in total remission and had stopped therapy, whereas the remainder were receiving low-dose
Epidermal tissue preparation

Epidermis was obtained from clinically affected and from normal-looking skin, i.e. no signs of disease (including erythrodernia) using the suction blister technique (5). Blister were rinsed and kept in Hank's at -20°C before homogenization. The homogenate was dialysed twice to remove low weight inhibitors and finally ultrafiltered using a Filtren disposable chamber with a cut-off at 3 kDa, thereby concentrating the sample. Samples were stored at -20°C until examination (6).

Interleukin-1 assay

IL-1 activity (ETA/F/IL-1) was measured as described elsewhere (6). Briefly, homogenized EC samples were tested for their enhancement of phytohemagglutinin (PHA) induced proliferation of thymocytes from 6-8-week-old female BALB/c mice (Bomholtgaard, Ry, Denmark). Single cell suspensions of thymocytes were prepared, washed in medium and resuspended. Thymocytes (1 x 10^6/ml) were cultured for 72 h at 37°C in 5% CO2 ambient air, in flatbottomed tissue culture plates (NUNC) with 10 μg/ml of PHA (DIFCO), 2 x 10^-5 M 2-mercaptoethanol and standard IL-1 (human rIL-1α; National Institute for Biological Standards and Control, London) or EC homogenate samples. Cultures were pulsed with 0.5 μCi of tritiated thymidine during the last 24 h of incubation. The proliferation of non-stimulated cultures did not exceed 3% of the maximal cpm of the standard IL-1 containing supernatant. In order to compare IL-1 activity in different samples, we determined the individual activity as described by Lager et al. (7):

Units/ml of test sample = Activity of standard preparation x reciprocal titre of test sample at 30% maximum cpm of standard

reciprocal titre of standard at 30% of maximum cpm

Since the surface area of epidermis contributing to each sample can be calculated, we expressed IL-1-like activity as units/cm^2 epidermis.

Chemotaxis assay

Lymphocyte chemotaxis was assessed using a modification of our ^51Cr assay (8). Briefly, monocyte-depleted T-lymphocyte suspensions were prepared from defibrinated venous blood of healthy donors by using E-AT-1 rosette-forming cells. These cells were labelled with ^51chromium and adjusted to a final concentration of 3.5 x 10^6 cells/ml in medium. Epidermal homogenate was placed in the lower compartment of the chemotactic chamber, separated from the upper compartment containing T lymphocytes by a sandwich, of two filters, an upper polycarbonate filter and a lower nitrocellulose filter, each with a pore size of 5 μm (Nucleopore Corp., Pleasanton, Calif.). After incubation for 90 min at 37°C, the radioactivity of the lower filters was determined in a gamma-counter (LKB, Wallac, Sweden).

The chemotactic activity was expressed as a chemotactic index (CI), which is the ratio of active migratory rate in the presence of chemotactic stimulus to random migration.
In the presence of the medium alone. A CI of 1.20 is the upper 95% confidence limit in persons without skin disorders (6).

RESULTS

The results of sIL2R in serum from patients with MF, PA, LP, and AR are shown in Fig. 1, and from PS and SS patients in Fig. 2. A rather wide variation was found. Three MF patients had levels below 500 units/ml, 9 had between 500 and 1000 U/ml, and 5 had sIL2R above 1000 U/ml. Clinical disease activity and extent of skin involvement correlated with increased sIL2R. Therapy did not directly influence sIL2R, as several patients were treated intensively with chemotherapy and still showed a high sIL2R value.

Four patients with PA (Fig. 1) and 2 with AR (results not shown) had an increase in sIL2R, whereas 5 patients with LP (Fig. 1) had normal or slightly elevated values. The PS and SS patients showed a wide range (Fig. 2). The patients with the highest values had the most active disease.

Four of 5 MF patients with sIL2R levels above 1000 U/ml have died within one year following the investigation (Fig. 1). A further 2 of the 5 patients with PS or SS and sIL2R above 1000 U/ml have also died (Fig. 2). This shows that an sIL2R above 1000 U/ml is a grave prognostic indicator.

Fig. 3 shows the individual results from patients studied for ETA1/IL-1 activity in epidermis. In previous studies we have found that epidermis from healthy persons contains between 60 and 451 U/cm² (mean 190 U/cm²) (n = 9; ref. 6) using exactly the same technique as used in MF patients. Thus, all patients except one had normal IL-1 activity in their skin. Affected skin tended to have higher IL-1 activity than non-affected epidermis, but the difference is not statistically significant (Wilcoxon's test). MF

Fig. 4. Epidermal lymphocyte chemotactic activity (ELCA) in homogenates from 5 MF stage I and 6 MF stage II and 1 SS patient (same patients as in Fig. 3). E-AET rosette purified normal human T-lymphocytes were used as target cells in a ¹⁴C-labelled Boyden chamber technique. A C.L. of 1.20 was the detection limit for T-lymphocyte chemotactic activity.
Table I. Release of interleukin 6 (log₆ U/ml), interleukin 8 (IL-8) (ng/ml), and interferon gamma (IFN-g) (ng/ml) from peripheral blood mononuclear cell cultures stimulated with or without PHA from patients with Mycosis fungoides (n = 7) and control persons (n = 15)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PHA</th>
<th>Mycosis fungoides</th>
<th>Control persons</th>
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<tbody>
<tr>
<td></td>
<td>Median Range</td>
<td>Median Range</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>3.0 2.7-4.0</td>
<td>2.9 2.9-3.7</td>
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<td></td>
<td>-</td>
<td>1.9 0.7-3.5</td>
<td>1.3 0.7-2.7</td>
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<tr>
<td>IL-8</td>
<td>+</td>
<td>105 44.640</td>
<td>96 34.360</td>
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<tr>
<td></td>
<td>-</td>
<td>11.3 3.4-106</td>
<td>19.8 0.2-98</td>
</tr>
<tr>
<td>IFN-g</td>
<td>+</td>
<td>36.9 0.7-120</td>
<td>30.6 0.7-135</td>
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<td>0 0-0.6</td>
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sIL2R is an index of interleukin-2-induced lymphocytic proliferation. It is not disease specific. We have observed increased levels in serum from patients with atopic dermatitis and psoriasis (10, 11). In the present study we found sIL2R levels increased in patients with active and widespread disease. Four of 5 MF patients with sIL2R levels above 1000 U/ml died of their disease within one year. Only one other patient with MF stage IV has died. He had consistently normal levels of sIL2R during a one-year period in which he received intensive systemic chemotherapy. The sIL2R test may therefore be used as an important prognostic parameter, irrespective of the fact that these patients were treated with systemic prednisone and also other forms of therapy.

Fig. 3 shows that patients’ epidermis did not contain more lymphocyte-activating activity (ETAF/IL-1) than epidermis from healthy persons (6). However, MF stage II patients had significant levels of lymphocyte chemotactic activity (ELCF) in affected epidermis (Fig. 4). ELCF is not found in healthy epidermis (8). The ELCF amount in MF corresponds to the amounts found during a cell-mediated immune reaction (12) or an irritant reaction (13) in human skin.

Other studies on cytokines in MF patients have looked at suction blister fluid, skin scrapings and biopsies. Dowd et al. (14) have recently found low but significant levels of IL-1 in suction blister fluid from 3 patients with MF. The amount was comparable to normal skin. Braverman et al. (15) studied interleukin-1 in normal and lesional skin of 10 patients with MF before and/or after electron-beam therapy, using skin biopsies and the mouse thymocyte assay, but they did not find significant amounts of ETAELF-1. Tron et al. (16) have performed immunohistological studies on skin biopsies from MF patients and found an intercellular staining in epidermis of 7 patients and a cytoplasmic distribution in 3 patients, using an anti-human IL-1β antibody. A similar staining of normal skin was either negative or showed weak cytoplasmic reactivity. Lawlor et al. (17) have studied skin exudates in 6 patients with plaque-type MF and found decreased IL-1 levels, increased IL-6 levels, whereas IL-2, TNF-alfa, and GM-CSF could not be detected at all.

Our observations and those of Dowd et al., Braverman et al., and Lawlor et al. (14, 15, 17) indicate low or normal levels of biologically active IL-1 in epidermis from MF patients. One explanation for the low level of IL-1 could be the treatment with
nitrogen mustard, an alkylating drug which will inhibit protein synthesis. We cannot exclude the possibility that previous therapy will reduce the amount of cytokines in the skin, but patients were studied in lesions showing clinical signs of activity. Also, the nitrogen mustard was given one month earlier. The patients studied by Dowd et al. and some of the patients studied by Braverman et al. did not receive therapy and they too showed low IL-1 activity. The nitrogen therapy may therefore be of no importance for our IL-1 results, although this possibility cannot be entirely excluded. Our use of the mouse thymocyte proliferation assay measures not only IL-1, but also IL-6, which may be one reason for finding normal levels of ETAf/IL-1 activity. However, we have recently compared IL-1 activity in LPS-stimulated mononuclear cell supernatants as measured with the C3H mouse thymocyte assay and an ELISA IL-1β assay (18) and found a very close correlation (unpublished).

We observed an increased chemotactic activity in epidermis (ELCF) in 5 of 6 persons with MF stage II, but not MF stage I. One patient with Sézary's syndrome had very high activity in healthy-looking skin, but not in diseased skin (see Fig. 4). ELCF is not found in healthy persons (2), but develops in epidermis of patients exhibiting allergic eczema (12).

The lymphocyte chemotactic activity called ELCF is specific for CD4+ T lymphocytes (19) and can be partly inhibited with anti-IL8 antibodies (Zachariae; unpublished). Its capacity to specifically attract CD4+ T lymphocytes makes it an important biological candidate for epidermal T-cell accumulation — also in MF. It is not present in MF stage I, which does not exhibit Pautrier's microabscesses, or in Sézary's syndrome in diseased skin. However, its presence in normal-looking skin in one Sézary patient indicates that it is not the only factor necessary for the development of epidermal T-cell accumulation. Expression of adhesion molecules is also necessary (20). Actually, Nickoloff et al. (20) have observed that these molecules are not expressed in epidermis of Sézary's syndrome, indicating that the ELCF signal alone is not sufficient for epidermal T-cell accumulation.

We do believe that ETCF/IL-1 does not contribute to the ELCF activity, for the following reasons: We have performed extensive studies on recombinant IL-1-alfa and -beta and its chemotactic activity towards purified T-lymphocytes and observed that IL-1 is not chemotactic, when using the 51Cr-Boyden chamber technique. However, when using the microcell chamber technique, recombinant IL-1 showed T-cell chemotactic properties. Thus, we agree with Bacon & Camp (21) that IL-1 will show T-cell chemotactic activities in vitro, when using the microcell technique, but this assay was not applied in the present study.

Interleukin-8 has recently been described both as a T lymphocyte and neutrophil granulocyte chemotactic cytokine (22). However, we were not able to demonstrate significant amounts of IL-8 in suction blister fluid in 2 patients, but this does not exclude its presence in epidermal homogenate. However, its relevance and relation to ELCF in epidermal homogenate must be further elucidated.

ACKNOWLEDGEMENTS

Clau Zachariae was supported by a grant from the Danish Medical Research Council (grant no. 12-8261). We have obtained financial support from the Institute of Experimental Clinical Research, and the Research Foundation of the University of Aarhus.

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