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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.***

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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, CDw29+; 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 microabcesses 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 maintenace 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

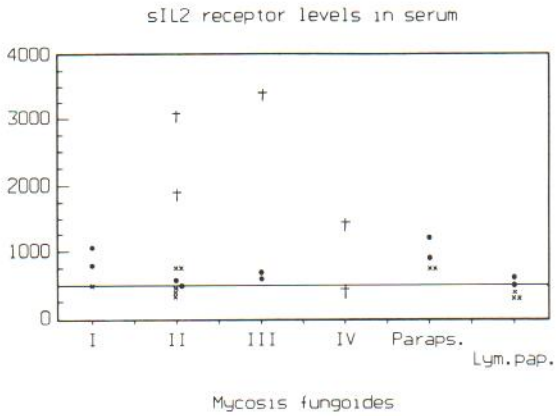

Fig. 1. Soluble interleukin-2 receptor levels (sIL2R) in serum from 17 patients with mycosis fungoides (MF), 4 patients with parapsoriasis (PA), 6 with lymphomatoid papulosis (LP), and 2 with actinic reticuloid (AR). MF patients are marked according to clinical active disease (×, slight activity; ●, moderate to strong disease activity; †, fatal outcome of disease within the next year of observation).

systemic prednisone (<25 mg daily) and topical nitrogen mustard once a month.

Sézary (SS): Two men with SS were studied, one receiving chemotherapy and without clinical signs of relapse, and another who developed the disease and then received chemotherapy.

Parapsoriasis (PA): Four men were included; their ages ranged from 38 to 79 years (median 68 years). They had parapsoriasis *en plaque* and were treated with PUVA. Their disease was still active at the time of the investigation.

Lymphomatoid papulosis (LP): Four men and one woman were included; their ages ranged from 32 to 55 years (median 41 years). Two were given methotrexate 10 mg per week to control their symptoms; 3 were not given treatment, but had minor clinical recurrence of papules from time to time. None have developed lymphoma within their disease period of 2–10 years.

Actinic reticuloid (AR). Two men aged 70 and 73 years were included. They had active disease and received potent topical steroids at the time of investigation.

Soluble interleukin-2 receptor levels in serum

Serum was collected from both patients and controls (healthy laboratory staff) and stored at −20°C until assayed. Soluble interleukin-2 receptors (sIL2R) were determined by an enzyme-linked immunosorbent assay (ELISA) (Cellfree® Interleukin-2 Receptor Test kit, T Cell Sciences Inc., Cambridge, Mass.) according to the manufacturer's instructions. sIL2R concentrations are expressed in Units/ml (U/ml). The detection limit is approx. 50 U/ml and the upper normal range was 500 U/ml (4).

Epidermal tissue preparation

Epidermis was obtained from clinically affected and from normal-looking skin, i.e. no signs of disease (including erythrodermia) using the suction blister technique (5). Blisters were rinsed and kept in Hanks' at −20°C before homogenization. The homogenate was dialysed twice to remove low weight inhibitors and finally ultrafiltrated using a Filtron dispensable chamber with a cut-off at 3 kD, thereby concentrating the sample. Samples were stored at −20°C until examination (6).

Interleukin-1 assay

IL-1 activity (ETAF/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 C_3H/SSI mice (Bomholtgaard, Ry, Denmark). Single-cell suspensions of thymocytes were prepared, washed in medium and resuspended. Thymocytes (1×10^6/ml) were cultured for 72 h at 37°C in 5% CO_2 ambient air, in flat-bottomed tissue culture plates (NUNC) with 10 μg/ml of PHA (DIFCO), 2×10^{-5} M 2-mercaptoethanol and standard IL-1 (human rIL-1α; National Institute for Biological Standards and Control, London) or EC homogenate sample. 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 Luger et al. (7):

$$\text{Units/ml of test sample} = \frac{\text{Activity of standard preparation} \times \text{reciprocal titre of test sample at 30\% maximum cpm of standard}}{\text{reciprocal titre of standard at 30\% of maximum cpm}}$$

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 ^{51}Cr assay (8). Briefly, monocyte-depleted T-lymphocyte suspensions were prepared from defibrinated venous blood of healthy donors by using E-AET rosette-forming cells. These cells were labelled with ^{51}chromium and adjusted to a final concentration of 3.5×10^6 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 migrational rate in the presence of chemotactic stimulus to random migration

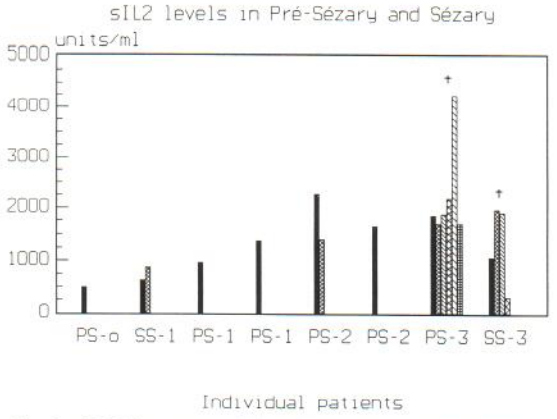

Fig. 2. sIL2R in serum of 6 patients with erythrodermia of unknown origin (Pré-Sézary's syndrome; PS) and 2 patients with Sézary's syndrome (SS) were studied, some twice or more for sIL2R in serum. The suffix "o" indicates no sign of disease activity, "1" slight, "2" moderate, and "3" severe disease activity. † indicates fatal outcome.

in the presence of the medium alone. A CI of 1.20 is the upper 95% confidence limit in persons without skin disorders (8).

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 ETAF/IL-1 activity in epidermis. In previous studies we have found that epidermis from

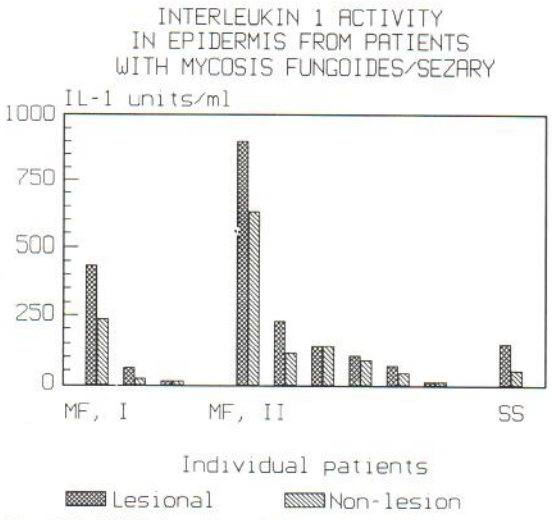

Fig. 3. Individual results of IL-1 activity in epidermal homogenates from 3 patients with MF stage I, 6 MF stage II and 1 SS patient. The IL-1 activity was measured using a mouse thymocyte assay.

healthy persons contains between 60 and 451 U/cm^2 (mean 190 U/cm^2) (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

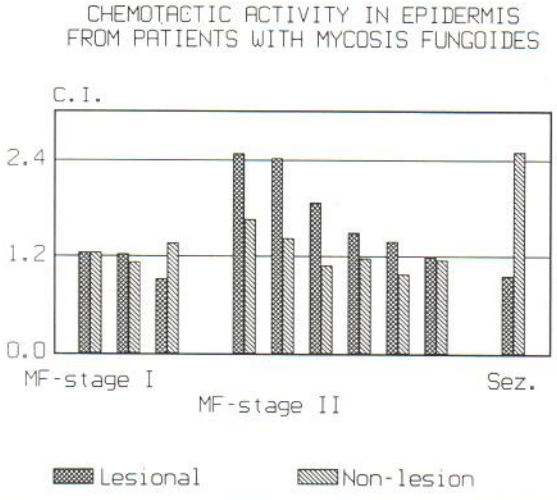

Fig. 4. Epidermal lymphocyte chemotactic activity (ELCF) in homogenates from 3 MF stage I and 6 MF stage II and 1 SS patient (same patients as in Fig. 3). E-AET rosette purified normal human T-lymphocytes were used as target cells in a ^{51}Cr-labelled Boyden chamber technique. A C.I. of 1.20 was the detection limit for T-lymphocyte chemotactic activity.

Table I. *Release of interleukin 6* (log_{10} 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)*

Cytokine	PHA	Mycosis fungoides		Control persons	
		Median	Range	Median	Range
IL-6	+	3.0	2.7–4.0	2.9	2.9–3.7
	−	1.9	0.7–3.5	1.3	0.7–2.7
IL-8	+	105	44–640	96	34–360
	−	11.3	3.4–106	19.8	0.2–98
IFN-g	+	36.9	0.7–120	30.6	0.7–135
	−	0	0–0.6	0	0–1

stage II patients tended to have higher IL-1 levels than MF stage I patients.

ELCF activity was not found in 3 MF stage I patients, but was present in significant amounts in clinically affected skin of 5 of 6 MF stage II patients. ELCF was not present in normal-looking skin, except in small amounts in 2 MF stage II patients and in significant amounts in one patient with SS (Fig. 4).

We have made preliminary studies of IL-8 in suction blister fluid in 2 MF stage II patients and found <50 pg/ml and 50 pg/ml. These values are below what is found in patients with psoriasis and eczema (unpublished).

We have also looked for IL-6, IL-8, and IFN-gamma release from PHA-stimulated peripheral blood mononuclear cells. The results are summarized in Table I. We did not observe significant deviations from control persons.

DISCUSSION

The group of diseases investigated belong to the cutaneous T cell lymphomas, or are closely related disorders. It has recently been demonstrated that sIL2 estimations in 80 patients with various forms of internal lymphomas show a close direct relationship with tumour burden and disease prognosis. Thus, patients with advanced stages of lymphoma had a significantly higher sIL2R value (av. 1.648 U/ml) than less advanced stages (sIL2R = 706 IU/ml). The parameter was also significantly related to fatal outcome of the disease (9).

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 ETAF/IL-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 not be of significance 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 microabcesses, 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 ETAF/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 ^{51}Cr-Boyden chamber technique. However, when using the microwell 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 microwell 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.

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