A correlation between acute phase proteins and cytokines in patients suffering from mycosis fungoides

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ABSTRACT

Background: Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma and cytokines are involved in the pathogenesis of the disease. To evaluate whether the serum levels of sIL-2R, IL-4, IL-5, IL-10, and INF-gamma reflect the processes in various stages of MF, to measure the acute phase protein concentrations in the serum of the patients, and to determine the glycosylation profile of two proteins.

Material and methods: Serum samples were obtained from 52 patients with MF and from 20 healthy subjects. Concentrations of interleukins IL-4, IL-5, IL-10, INF-gamma, and sIL-2R were measured using ELISA tests. Concentrations of proteins were measured using Laurell rocket electrophoresis.

Results and discussion: An inflammatory reaction exists in all MF patients because the concentrations of acute phase proteins were increased. In more advanced stages of MF, intensive activation of T cells is present, as reflected by the sIL-2R levels and Th2 cytokines outweighing the main Th1 cytokine (IFN-gamma). Increased levels of sIL-2R, IL-4, and IL-10 accompanied the increase in concentrations of acute phase proteins and a decrease in their reactivity with Con A. It appears that the increase of IL-4, IL10, and sIL-2R may be due to the activation of T-helper lymphocytes.

KEY WORDS

cutaneous T-cell lymphoma, cytokines, acute phase proteins, serum

Introduction

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL), characterized by many years' duration and clinical progression from patch to plaque to tumor stage. Erythrodermic variants also exist. The mechanism responsible for the initiation and progression of MF still remains undetermined (1–3). Some environmental and occupational exposures have been implicated as possible causes of CTCL, but subsequent case-control studies failed to support this hypothesis (4). Human T-cell lymphotropic virus type-I (HTLV-I), an etiological factor of adult T-cell leukemia/lymphoma, has been investigated as a cause of MF due to the similarity between the skin lesions of MF and those of adult T-cell lymphoma /leukemia (5), but its possible role was excluded (6–9).

Cytokines are considered to be of major importance for the pathogenesis of MF. Investigations of cytokine production in skin lesions or peripheral blood lympho-

Table 1. Characteristics of the groups of patients suffering from mycosis fungoides and of the healthy controls.

Stage of MF	Number of patients	Mean age (years)	Women N	Women %	Men N	Men %	Mean disease duration (years)
IA + IB	13(IA-3+IB-10)	55.6	7	53.8	6	46.2	7.5
IIA	12	64.3	6	50.0	6	50.0	7.8
IIB	12	63.7	3	25.0	9	75.0	9.8
III + IVA	15(III-9+IV-6)	65.1	3	20.0	12	80.0	8.1
Total	52	62.2	19	36.5	33	63.5	8.2
Healthy controls	20	55.5	10	50.0	10	50.0	—

cytes from patient with cutaneous T-cell lymphomas led to the general concept that a shift in cytokine profile from type Th1 to Th2 accompanies disease progression (10, 11).

The aim of the study was to evaluate whether the serum levels of soluble interleukin 2 receptor (sIL-2R), interleukins 4 (IL-4), 5 (IL-5), 10 (IL-10), and interferon-gamma (INF-gamma) reflect the cellular processes in various stages of MF. Another analysis was performed to measure the acute phase protein concentrations in the serum of the patients under study and to determine the glycosylation profile of two proteins, alpha$_1$-acid glycoprotein (AGP) and alpha$_1$-antichymotrypsin (ACT). Finally, the correlation between the serum concentration of cytokines and acute phase proteins was calculated.

Material and methods

The study group consisted of 52 patients at various stages of the disease. Diagnosis of MF was confirmed by clinical observations, histopathological, and immunocytochemical examinations of skin biopsy and TCRγ gene rearrangement assay. Serum samples were obtained from 52 patients with MF, from 33 to 82 years of age. A blood sample was drawn at admission (prior to any treatment) and left to clot, then the serum was separated and frozen until investigation. After the staging evaluation (12) the patients were classified into four groups: 13 patients with MF stage IA+IB, 12 patients with stage IIA, 12 patients with stage IIB, and 15 patients with III+IVA.

Patients with patches and plaques covering < 10% of the skin surface without lymph node involvement were staged as IA; patients with patches and plaques covering > 10% of the skin surface without lymph node involvement were staged as IB; patients with patches or plaques and clinically abnormal peripheral lymph nodes and pathology negative for CTCL were staged as IIA; patients with patches, plaques, and tumors with or without lymphadenopathy were staged as IIB; patients with generalized erythroderma with or without lymphadenopathy were staged as III; and patients with patches, plaques, tumors, or erythroderma and lymph node pathology positive for CTCL were staged as IVA. None of the patients presented with visceral organ involvement. Sera from 20 healthy subjects, 10 women and 10 men from 20 to 77 years of age, mean age 55.5, served as controls. The demographic data are shown in Table 1.

Concentrations of interleukins IL-4, IL-5, IL-10, and INF-gamma were measured in pg/mL, and sIL-2R in ng/mL, using ELISA tests (mfr: R&D Systems, Minne-

Table 2. Concentrations (mean ± SD) of the analyzed cytokines in patients with various stages of MF.

Cytokine	IA-IB *n* = 13	IIA *n* = 12	IIB *n* = 12	III-IVA *n* = 15
IL-4 (pg/mL)	2.72 ± 1.23	3.28 ± 2.12	11.89 ± 11.48	24.05 ± 7.16
IL-5 (pg/mL)	0 ± 0	62.49 ± 70.75	0 ± 0	0.7 ± 1.1
IL-10 (pg/mL)	2.98 ± 10.73	0 ± 0	72.86 ± 33.4	206.07 ± 22.14
IFN-γ (pg/mL)	73.69 ± 26.6	90.25 ± 11.6	30.33 ± 26.7	18.57 ± 23.05
sIL-2R (pg/mL)	1.32 ± 0.3	1.76 ± 0.63	3.21 ± 0.52	5.77 ± 2.11

Table 3. Correlation of the cytokine concentrations with clinical parameters. The correlation coefficient (Spearman's *r*) is given if $p < 0.05$. The strongest correlations are shown in bold.

Parameter	Age	Sex	Stage of MF
IL-4	0.078	–0.246	**0.766**
IL-5	0.116	–0.127	**0.612**
IL-10	0.332	**–0.537**	**0.665**
IFN-γ	–0.182	0.197	**–0.689**
SIL-2R	0.219	**–0.298**	**0.888**

apolis, MN). Concentrations of selected acute phase proteins (APP): C-reactive protein (CRP), AGP, ACT, alpha$_2$-macroglobulin (A2M), transferrin (Tf), ceruloplasmin (Cp), and haptoglobin (Hp) were measured using Laurell rocket electrophoresis (13). The glycosylation profiles of two selected acute phase proteins (AGP and ACT) were estimated using crossed affinity immunoelectrophoresis with the jack bean lectin Concanavalin A (Con A) as the ligand, using Bog-Hansen's method (14). All experimental procedures were accepted by the Local Ethics Committee. Results were analyzed using STATISTICA 6.0 Software.

Results

Mean serum levels of the investigated cytokines (mean ±SD) are presented in Table 2. Statistically significant differences at different stages of MF were determined by the Kruskall-Wallis test for IL-4 ($p = 0.000$), IL-5 ($p = 0.128$), IL-10 ($p = 0.005$), IFN-gamma ($p = 0.000$), and sIL-2R ($p = 0.000$). The analysis of cytokine concentrations in serum revealed normal IL-4 levels in earlier stages of MF with a tendency to increase at the more advanced stages. IL-5 concentration above the normal value was observed only in stage IIA, and IL-10 concentration increased in all advanced stages. All MF patients showed increased IFN-gamma levels, but the concentration of this interleukin decreased as the MF progressed. Statistically significant correlations of cytokine concentrations with the stage of the disease

Table 4. Concentrations (mean ± SD) of the selected acute phase proteins, measured in sera of patients with MF in various stages. A statistically significant difference ($p < 0.05$) was present between group IIA and all other groups for the given proteins.

Protein	IA-IB*n* = 13	IIA*n* = 12	IIB*n* = 12	III-IVA*n* = 15
CRP (mg/L)	6.31 ± 7.49	3.0 ± 5.51	9.0 ± 10.43	9.2 ± 10.2
AGP (mg/L)	1161 ± 351	821 ± 162	1265 ± 460	1356 ± 447
ACT (mg/L)	450 ± 96	400 ± 82	465 ± 125	604 ± 221
A2M (g/L)	3.06 ± 0.77	3.16 ± 1.16	3.08 ± 1.00	2.97 ± 0.86
Tf (g/L)	2.78 ± 0.87	2.77 ± 0.90	2.68 ± 0.62	2.57 ± 1.06
Cp (mg/L)	517 ± 109	461 ± 71	512 ± 65	507 ± 117
Hp (g/L)	2.37 ± 1.58	1.59 ± 0.57	2.52 ± 1.83	1.88 ± 0.75

Table 5. Mean concentrations of the distinctly glycosylated variants of acid alpha$_1$ glycoprotein (AGP) and alpha$_1$ antichymotrypsin (ACT) in patients with MF and in healthy controls.

Variant	IA+IB *n* = 13	IIA *n* = 12	IIB *n* = 12	III+IVA *n* = 15	Healthy controls
AGP–W0	495	366	627	617	344
W1	503	343	492	560	360
W2	128	86	113	142	90
W3	35	26	32	37	0
ACT–A1	84	86	109	140	100
A2	116	112	133	157	100
A3	107	82	101	121	95
A4	134	114	117	173	105
A5	9	5	5	14	0

were observed, as shown in Table 3. Comparison of the increase in parallel concentrations of soluble interleukin-2 receptor and interleukin 4 in sera of patients in various stages of MF is shown in Figure 1.

Acute phase protein concentrations were shown to differ between stages of MF. Exact data are given in Table 4. It shows that the concentrations of AGP and ACT increase, whereas the concentration of Tf decreases with the progression of MF. The glycosylation profiles of the investigated proteins (AGP and ACT) were also dependent on the stage of MF. A statistically significant difference ($p < 0.05$) was present between group IIA and all other groups for given proteins. Mean concentrations of distinctly glycosylated variants of AGP and ACT are given in Table 5. The participation of distinctly glycosylated variants of antichymotrypsin in its total concentration in patients with MF and the controls is shown in Figure 2, and for acid $alpha_1$-glycoprotein in Figure 3. This representation allowed the depiction of mainly increasing participation of strongly Con A reactive variants in stages IIIA+IV, but also differing patterns in various stages, showing distinct activity of the cytokine network.

Some statistically significant ($p < 0.05$) correlations between the concentrations of cytokines and acute phase proteins were found. Concentration of IL-4 correlated with total AGP concentration as well as its variant W0. Similarly, significant correlation was found between total ACT concentration and its variant A1. Concentration of IL-10 correlated selectively with the variant W1 of AGP; the concentration of sIL-2R correlated with variants W0 and A1; and the concentration of IFN-gamma correlated negatively with concentrations of AGP, W0, W1, ACT, A1, A2, and A3. WO, W1, and A1 are distinctly glycosylated variants of glycoproteins, W0 and W1 of AGP, and A1 of ACT; the higher the number, the stronger affinity to Con A, meaning relatively more biantennary glycans on the glycoprotein

Similar correlations were observed for IL-4, IL-10, and sIL2R, and the opposite for IFN-gamma.

Discussion

The Th1/Th2 cytokine pattern has been studied in peripheral blood T-cells in patients suffering from MF and Sezary syndrome (SS) by using mRNA analysis or enzyme-linked immunosorbent assay to detect the cellular cytokines levels after phytohemaglutinine stimulation of peripheral blood mononuclear cells (10, 15). Vowels et al. (10) showed significantly higher levels of IL-4 and lower levels of IL-2 and INF-gamma in SS patients compared to controls. Similar results in patients with MF stage I and II were reported by Dummer et al. (15). However Saed et al. (16) showed a mixed cytokine mRNA pattern of Th cells similar to healthy controls in patients with the plaque stage of MF. Acute phase proteins may serve as a laboratory marker of cytokine network function: changes in APP level reflect changes in cytokine balance and are easier to follow analytically. An inflammatory reaction clearly exists in all MF patients, as the concentrations of AGP and ACT, typical acute phase reactants, were increased. The lowest intensity of acute phase reaction was shown in stage IIA of MF. An increase in the concentration of AGP in many inflammatory processes has been previously described. The synthesis of AGP or ACT is stimulated mainly by IL-6, suggesting a constant production of this cytokine in all stages of MF except IIA. ACT concentration was related to necrotic processes or traumatic events with massive tissue damage (17–19). In patients with MF the concentrations of AGP and ACT seem to increase in parallel, as if stimulated by the same factor. Changes of the glycosylation profiles were seen in all patients (except those in stage IIA of MF), with the highest intensity for patients with erythroderma, which influenced the mean value for the group III+IVA (20). This suggests that in early stages of MF the inflammatory reaction seems to be acute, and it changes during progression of the disease towards a chronic image.

Clearly in more advanced stages of MF, intensive activation of T-cells is present, as reflected by the sIL-2R levels and Th2 cytokines (IL-4 and IL-10) outweighing the main Th1 cytokine (IFN-gamma). IL-4 serum concentration correlated with increased concentrations of glycosylation variants weakly reactive to Con A, which is typical of chronic inflammatory processes. Increased serum levels of sIL-2R, IL-4, and IL-10 accompanied the increase in concentrations of acute phase proteins and a decrease in their reactivity with Con A. It seems that IL-4, IL10, and sIL-2R may relate to the same feature: the increase in concentrations of the investigated acute phase proteins and the decrease in their reactivity with Con A. It may, however be suggested that they do not cause this change themselves because the correlation coefficients are low. This change may be due to the activation of T helper lymphocytes.

The study by Papadavid et al. showed that cytoplasmic IL-4 expression was an important parameter in the evaluation of the clinical course in patients with CTCL (21). Based on our own study it seems that the serum concentration of IL-4 followed the cellular process. In a case of MF it was demonstrated that the tumor cell clone produced a Th2- type cytokine profile (IL-4, IL-6, and IL-10) whereas reactive clones from T-cells with cytotoxic activity expressed a Th1 cytokine profile (IL-2 and INF-gamma) (22).

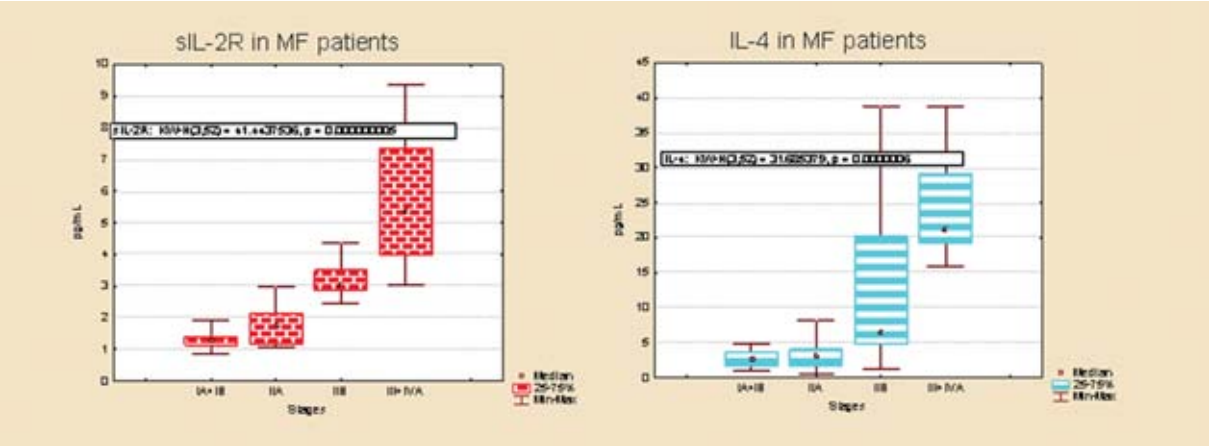

Figure 1. Comparison of parallel increases in concentrations of soluble interleukin-2 receptor and interleukin 4 in sera of patients at various stages of MF.

Our results partially reflect the shift from the Th1 cytokine profile, characteristic of early stages of MF, to Th2, found at the more advanced stages. The changes of immunological parameters observed in patients with MF during progression of the disease may be attributable to a dysregulation of T lymphocyte function: in later advanced stages of the disease more intensive activation of T cells is present and is accompanied by some changes in circulating cytokine levels. Whether it is due to an altered balance between the Th1 and Th2 type response, or to the presence of tumor cells, must be the subject of further investigation.

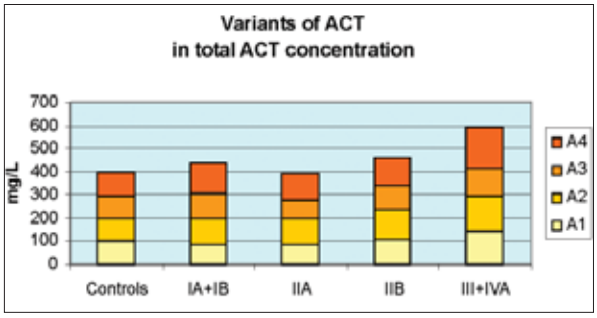

Figure 2. The participation of distinctly glycosylated variants of antichymotrypsin in its total concentration in patients with MF and the controls.

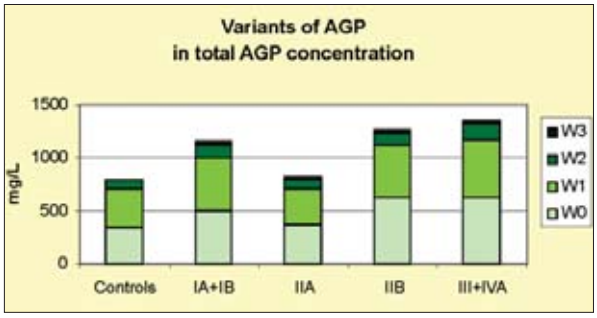

Figure 3. The participation of distinctly glycosylated variants of acid glycoprotein in its total concentration in patients with MF and the controls.

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