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Stage-dependent Expression of CD7, CD45RO, CD45RA and CD25 on CD4-positive Peripheral Blood T-lymphocytes in Cutaneous T-cell Lymphoma

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Flow cytometric analysis of T-cell surface markers in peripheral blood has revealed abnormal patterns in patients with cutaneous T-cell lymphomas (CTCL). Here we investigated CD7, CD25, CD45RO and CD45RA expression on CD4+ T-lymphocytes in patients with CTCL stage I/II and III/IV and in patients with severe inflammatory skin diseases (ISD), as well as in healthy controls. Only late stage CTCL (III/IV) showed a lymphocytosis with a distinct surface marker pattern: CD3+, CD4+, CD8−, CD7−, CD45RO+, CD45RA−. Early stage CTCL (I/II) showed normal lymphocyte counts, a normal T-helper cell expression of CD7, CD45RO and CD45RA, and a slightly increased percentage of CD4+ CD25+ lymphocytes, which was also found in ISD. It is concluded that flow cytometric analysis of the above T-cell surface markers may be useful in the diagnosis of patients with late stage CTCL. However it does not allow us to distinguish patients with early stage CTCL from patients with ISD or controls.

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Cutaneous T-cell lymphoma (CTCL) comprises a range of lymphoproliferative disorders characterized by cutaneous infiltration of malignant T-lymphocytes. The clinical manifestations most commonly seen are mycosis fungoides (MF) and Sézary syndrome (SS), which are now accepted to be different phases/manifestations of the same disease. Cell surface immunophenotyping has shown CTCL to mostly be a malignant proliferation of lymphocytes expressing T-helper cell (CD2+, CD3+, CD4+, CD8−) phenotype (1). Skin infiltrating T-cells show a helper/inducer phenotype (CD45RA, CD29w+) and a deficiency of the pan T-cell marker CD7 and Leu 8 (2, 3). In leukemic CTCL circulating malignant T-helper cells are characterized by a CD7 deficiency (4) and in most cases also show a helper/inducer phenotype (CD45RO+, CD45RA−) (5). In early stage CTCL a reduced percentage of naive T-helper cells (CD4+CD45RA+) has been reported, whereas the percentage of CD4+CD7− lymphocytes was found to be normal (6, 7).

The aim of our study was to investigate whether peripheral blood T-cells in early stage CTCL show an abnormal phenotype, which may be used for diagnostic purposes. We therefore investigated CD7, CD25, CD45RO and CD45RA expression on CD4+ T-lymphocytes in patients with early stage (I/II) and late stage (III/IV) CTCL according to the NCI classification 1979 (8) and in patients with severe inflammatory skin diseases (ISD) (e.g. atopic eczema, psoriasis), as well as in healthy controls.

MATERIALS AND METHODS

Nineteen patients with clinically active CTCL were investigated. The diagnosis of CTCL was based on clinical findings, histology, immunohistology and in selected cases on analysis of the TCR-gamma-chain rearrangement (which was kindly performed by Dr. M. Tiemann, Lymphknotenregister, Institut für Hämatopathologie, University of Kiel). The clinical staging was based on the NCI classification of 1979 (8) and included the estimated body surface involvement, lymphnode status, chest X-ray, CT-scan of pelvis and abdomen, ultrasound of abdomen, bone marrow – and peripheral blood analysis. In case of lymphadenopathy a lymphnode biopsy was performed. Patients with early stage CTCL (5 females, 5 males; age: 64.7 ± 10.6 years) received topical steroids ($n=7$), PUVA ($n=2$) or X-ray therapy ($n=1$). Only one patient received no therapy at the time of blood collection. Patients with late stage CTCL (4 females, 5 males; age: 70.4 ± 11.8 years) received topical steroids only ($n=4$) or in combination with systemic therapy with IFN-α ($n=1$), PUVA ($n=1$), methotrexate ($n=1$) or trofosphamid and prednisolone ($n=2$). Seven women and 8 men (age: 64 ± 18.1 years) with severe ISD were investigated: 7 with atopic eczema, 6 with psoriasis, 2 with generalised contact eczema. The body surface involvement in all patients was >50% (as estimated by the "rule of nine"). The control group consisted of 8 female and 7 male healthy, adult volunteers from the hospital staff (age: 51.6 ± 8.8 years).

Peripheral venous blood (2 ml) was collected in EDTA containers. Leukocyte counts were obtained using a coulter counter. Standard procedure for obtaining "lysed whole blood" and staining of cells with mAb was performed according to the Becton Dickinson protocol using FACS brand lysing solution (Becton-Dickinson, Heidelberg), PBS, PBS 1% paraformaldehyde and the following mAb (either PE- or FITC-conjugated): anti-CD3, anti-CD4, anti-CD7, anti-CD8, anti-CD25, anti-CD45RO (Becton-Dickinson, Heidelberg) and anti-CD45RA (Immunoquality Products). The flow cytometric analysis was performed on a FACScan using Simulset software version 2.3.2. (Becton-Dickinson, Heidelberg). Results are expressed as percent lymphocytes of total lymphocytes. Statistical analysis of unpaired data was performed using the Mann Whitney U-test (StatView 512). Data are presented as mean $\pm$ standard deviation and as range.

RESULTS AND DISCUSSION

The results of this study (Table I) demonstrate that in contrast to late stage CTCL (III/IV) peripheral blood lymphocytes from patients with early stage CTCL (I/II) do not differ from healthy controls with regard to their CD4+CD7−, CD4+CD45RO+ or CD4+CD45RA+T-cell subpopulations. This disagrees with the results of Gilmore et al. (6), who reported a significant reduction of naive T-helper cells (CD4+, CD45RA+) in CTCL independent of disease activity, duration or treatment. It was suggested to reflect an increased conversion of naive T-cells into memory T-cells in the peripheral blood or skin. In contrast to our investigation, the authors did not distinguish between early and late stage CTCL, which may account for the observed difference. The total number of CD4+ CD3+ lymphocytes was found to be

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Table I. *Leukocyte counts, lymphocyte percentage and lymphocyte subpopulations in controls, patients with severe inflammatory skin diseases (ISD) and patients with cutaneous T-cell lymphoma (CTCL) stage I/II and III/IV (NCL classification 1979 [8])*

	Controls $n=15$	ISD $n=15$	CTCL I/II $n=10$	CTCL III/IV $n=9$
Leukocytes/µl	7420±2020 (4600–10700)	10270±1870* (7600–13600)	6990±1720† (4400–10200)	15410±8140* (6700–3700)
% Lymphocytes	33.3±7.8 (17–45)	22.3±7.6* (10–36)	24.0±7.7* (14–36)	37.3±14.5+ (10–55)
CD4+ CD3+Lymphocytes/µl	1220±400 (590–2040)	1250±430 (560–1970)	730±230*† (440–1240)	5590±4780*† (1340–17060)
% CD4+ CD3+Lymphocytes	49.6±7.6 (36–59)	56.3±10.5 (38–76)	47.1±13.6 (29–71)	90.7±6.9*† (77–99)
Ratio CD4;CD8	2.21±0.64 (1.2–3.8)	3.13±1.95 (0.8–6.3)	2.01±1.05 (0.4–3.7)	26.7±25.5*† (9.6–92)
% CD4+CD7−Lymphocytes	6.3±3.2 (4–16)	13.6±6.* (4–27)	9.6±11.8† (2–42)	70.9±26.9*† (26–96)
% CD4+ CD45Ro+Lymphocytes	32.6±5.8 (23–41)	39.6±8.7* (24–56)	29.7±10.1† (20–54)	82.8±15.2*† (46–96)
% CD4+ CD45RA+Lymphocytes	20.6±9 (5–33)	20.1±12.9 (4–57)	12.2±11.8 (5–31)	9±10.4*† (1–34)
% CD4+CD25+Lymphocytes	7.5±5.3 (2–16)	24.2±14.8* (7–53)	16.1±7.6* (6–29)	28.4±24.9* (6–80)

* significant difference to control group ($p<0.05$); † significant difference to ISD group ($p<0.05$)

slightly, though significantly, reduced in CTCL I/II as compared to ISD or controls, possibly reflecting an increased shift of T-helper lymphocytes from the peripheral blood to the skin compartment. The percentage of IL-2-receptor (CD25) positive T-helper cells was significantly increased in CTCL I/II as compared to controls. In CTCL III/IV percentage and total number of CD25+ T-helper cells was even more increased. This may reflect an increased number of activated circulating lymphocytes and may provide an explanation for the increase of soluble IL-2-receptor in the sera of patients with CTCL, which was found to correlate with the clinical severity of CTCL (9). The percentage of CD25+ T-helper cells in CTCL, however, was not significantly different from patients with severe ISD. CD25 expression thus does not make it possible to distinguish between ISD and CTCL.

Patients with ISD showed a slight but significant increase of the CD7− helper T-lymphocytes in the peripheral blood. Recently Moll et al. (10) reported an accumulation of CD7− helper T-cells in skin lesions of various ISD, including atopic eczema and psoriasis. The lack of CD7 expression was regarded as a stable characteristic in a major subset of skin infiltrating lymphocytes. An increased percentage of peripheral blood CD7− helper T-cells in ISD may thus reflect an increased number of T-lymphocytes bound for skin infiltration or an increased number of lymphocytes returning from the skin compartment during normal lymphocyte trafficking. In contrast to our results Moll et al. (10) reported a normal percentage of CD7− helper T-cells in peripheral blood of patients with atopic eczema or psoriasis. This difference may be explained by the patient selection (we only included patients with severe manifestation of the disease, i.e. body surface involvement >50%) and the age of the patients (mean age 64.2±18.1 years), since the number of circulating CD4+CD7− lymphocytes correlate with the age of the donor (10).

Patients with late stage CTCL (III/IV) showed a T-helper lymphocytosis with a rather homogeneous pattern of surface markers: CD3+, CD4+, CD7−, CD8−, CD45RO+, CD45RA−. Notably 3 of 9 patients showed normal leukocyte and lymphocyte counts, as performed by automated analysis (Coulter counter). Two patients who showed only an incomplete deficiency of CD7 on CD4+ lymphocytes (26% and 27%, respectively) were clinically and immunologically not different from the rest of the group. The CD7 deficiency is characteristic of leukaemic stage CTCL and allows one to exclude other forms of CD4+ lymphocytosis (e.g. acute lymphoblastic leukemia) (4). However, it is not one single parameter which allows the diagnosis, but the combination of a CD4+ lymphocytosis, an increased CD4: CD8 ratio, a deficient CD7, a dominant CD45RO and a reduced CD45RA expression on CD4+ lymphocytes together with the clinical symptoms.

At the time of blood collection all late stage CTCL patients received either topical steroids only or topical steroids in combination with systemic therapy. When patients who only received topical treatment ($n=4$) and patients who received systemic treatment ($n=5$) were compared, no major differences could be observed. However, it is quite likely that different therapeutic modalities may have influenced the results (e.g. systemic corticosteroids induce leucocytosis, methotrexate induces lymphocytopenia). Due to the small number of patients in this study, we were not able to evaluate the effect of different systemic therapies on the lymphocyte subpopulations. Two patients with late stage CTCL in complete remission (one diagnosed in 1987, stage IVb T4 N3 Bl Ml; the second diagnosed in 1990, stage IVa T4 N3 Bl Mo) showed normal T-lymphocyte subpopulations with a normal expression of the above surface markers (data not shown). This indicates that the analysis of the above T-helper cell surface markers may prove to be a good parameter for patient follow-up. Whether or not this pattern correlates with disease activity of late stage CTCL or therapeutic modalities is currently under investigation.

In summary, analysis of T-cell surface markers (CD7,

CD45RO, CD45RA, CD25) in peripheral blood does not allow us to distinguish patients with early stage CTCL from patients with ISD or controls. In our daily routine the analysis of the above T-cell surface markers, however, proves to be a very helpful and fast diagnostic tool in the differential diagnosis of late stage CTCL (e.g. Sézary syndrome) versus erythrodermic ISD (e.g. erythrodermic psoriasis).

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