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Coincidence of increased soluble interleukin-2 receptors, diminished natural killer cell activity and progressive disease in cutaneous T-cell lymphomas

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In 24 patients with cutaneous T-cell lymphomas (CTCL) the clinical course was documented by determination of a tumour burden index (TBI) on entering the study and six months later. In addition to the first TBI, soluble interleukin-2 receptor levels (sIL-2R) in the serum were determined using an ELISA technique in 23 patients. In 18 patients natural killer cell (NK) activity was assessed by a 4 h chromium-51 release assay. Statistical analysis revealed a negative correlation between NK activity and an increase of TBI as well as NK activity and sIL-2R. Furthermore, there was a strong positive correlation between the increase of TBI and sIL-2R. sIL-2R and NK activity might be prognostic factors in CTCL patients.

Keywords: cutaneous T-cell lymphomas, soluble interleukin-2 receptor, natural killer cell activity, prognostic factors.

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders originating in the skin. In general, the term designates a clinically, histologically and phenotypically variable spectrum of diseases which are slowly progressive and show striking interindividual differences [1].
An increased risk of second malignancy [2] and a decrease in natural killer cell activity [3, 4] indicate imbalanced immunofunctions in these patients. Recently, we and others [5, 6] reported elevated serum levels of soluble interleukin-2 receptors (TAC-protein) in some of these patients. Yet, the biological relevance of this phenomenon is unclear.
In the present study we analysed the interactions of NK activity, sIL-2R and the clinical outcome in CTCL patients.

Table I. ***Patients characteristics: sIL-2R, NK activity, and clinical course described by △ TBI***

Pat. (n = 24)	Stage TNM[19]	Histol. diagnosis	Pretreatment	therapy during follow-up period (6 months)	sIL-2R (U/ml) (n = 23)	Spec. lysis (%) (n = 18)	△ TBI (n = 24)
GE	IIA	low-grade peripheral	e.b.r e. ster.	e. ster.	1690	5.0	18
HE	IIB	low-grade peripheral	e. ster.	e. ster.	385	13.0	5
WA	IA	low-grade peripheral	e. ster.	e. ster.	405	16.5	0
BA	IIA	low-grade peripheral	e. ster.	e. ster.	330	4.0	0
HO	IIB	high-grade peripheral	MTX, IFN-α PUVA	MTX, e. ster.	538	30.7	0
ST	IIB	low-grade peripheral	BCNU, topical, e. ster.	BCNU topical e. ster.	3000	6.4	30
TI	III	low-grade peripheral	e. ster.	BCNU topical	1320	4.0	12
KA	IIA	low-grade peripheral	e. ster., IFN-α	e. ster.	3570	6.0	20
KAI	IIB	high-grade peripheral	PUVA, e. ster.	e. ster.	10700	6.0	20
KE	IIA	low-grade peripheral	e. ster.	e. ster.	1190	21.9	20
SCH	IA	low-grade peripheral	e. ster.	e. ster.	950	10.5	0
HO	IVB	low-grade peripheral	PUVA, e. ster. BCNU, MTX	e. ster., MTX	9230	n.d.	40
RE	IIA	high-grade peripheral	e. ster.	e. ster.	720	15.0	0
TR	IA	low-grade peripheral	PUVA, e. ster.	IFN-α, e. ster.	800	42.3	0
GEI	IIB	high-grade peripheral	e. ster.	e. ster.	570	n.d.	0
BET	IIA	low-grade peripheral	none	PUVA	7330	2.3	20
HER	IA	low-grade peripheral	PUVA, e. ster.	e. ster.	1110	6.9	0
KÜ	IB	low-grade peripheral	e. ster.	e. ster. prednimustine	650	24.7	2
VA	IIB	low-grade peripheral	e. ster.	e. ster.	300	22.5	0
GR	IA	low-grade peripheral	e. ster.	BCNU topical	790	n.d.	0
DO	IVA	low-grade peripheral	PUVA, e. ster.	e. ster.	12300	n.d.	40
KUN	IVA	low-grade peripheral	PUVA, e. ster.	e. ster.	1190	n.d.	40
YI	IA	low-grade peripheral	PUVA, e. ster.	e. ster.	510	n.d.	0
WAL	IA	high-grade peripheral	none	e. ster.	n.d.	19.8	0

MTX: methothrexate, BCNU: carmustine, IFN-α: interferon-α2a, e. ster.: external steroids, e.b.r.: electron beam radiation, n.d.: not done.

Patients and methods

Patients

Twenty four patients with histologically proven CTCL in different stages were investigated. Before entering the study, systemic treatment had been stopped for at least two weeks. Physical examinations were done on day 0 and repeated six months later (for all 24 patients). Staging procedures and histological classification were done according to the recommendations for staging and therapy of cutaneous lymphomas of the European Organization for Research and Treatment of Cancer, cutaneous lymphoma project group [7]. On day 0, peripheral blood mononuclear cells for ^{51}Cr release assay were taken in 18 patients, and serum samples were collected in 23 patients. Patients' characteristics including pretreatment and therapy during the follow-up period are summarized in *Table I*. Therapy in the follow-up period was applied according to the clin-ical necessities.

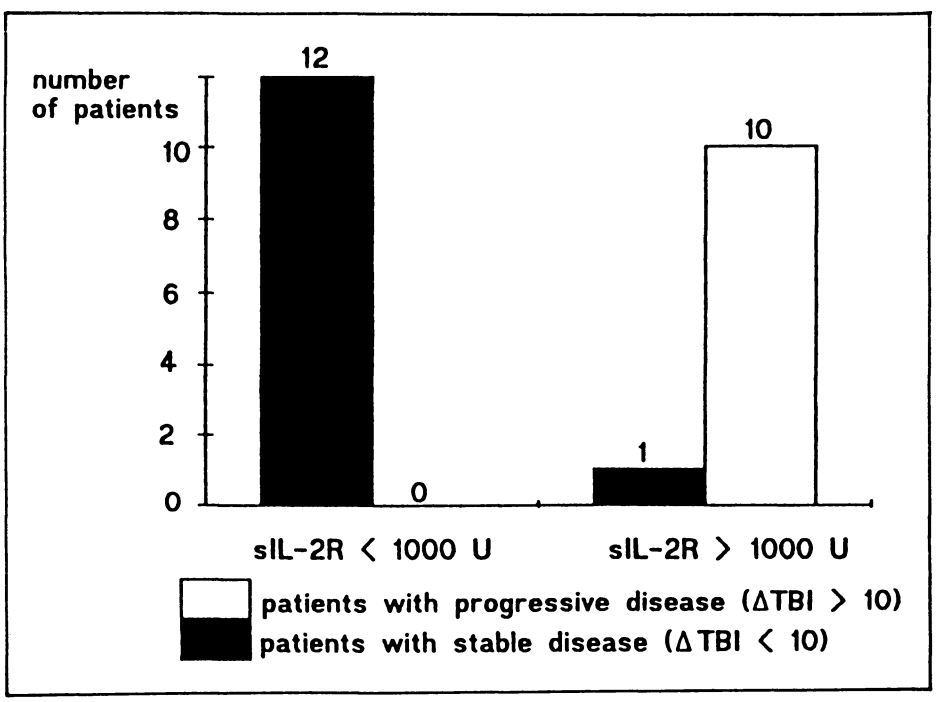

Figure 1. ***Clinical outcome according to soluble interleukin-2 receptor serum levels.***

Clinical course

In order to estimate the cutaneous tumour mass in a semiquantitative manner, a tumour burden index was introduced. Physical examinations were done by one investigator, only. The percentage of involved skin was documented using the rule of the nines. Speci-fic skin lesions were differentiated in patches (flat lesions, diameter larger than 1 cm), plaques (flat or slightly elevated lesions with increased consistency) and tumours (large nodular lesions, diamter larger than 1 cm) [7]. The extension of tumours, patches and plaques was noted and the tumour burden index (TBI) was calculated as:

	tumour	plaque	patch
TBI =	x% × 4 +	x% × 2 +	x% × 1

x% = involved skin in %

Under these conditions the maximal achievable TBI was 400. In our patients the TBI was 41 ± 59 (mean ± standard deviation, minimal 2, maximal 280).

The clinical course of progressing patients was determined by the difference of the tumour burden index (△ TBI) at day 0 and six months later.

It was calculated as:

$\triangle$ TBI = $TBI_6 - TBI_0$;

TBI_6 = after six months, TBI_0 = TBI day 0.

Patients with a stable disease or regression -0.3492, $P < 0.05$). A strong positive correlation was found between sIL-2R and

Soluble interleukin-2 receptors

Soluble interleukin-2 receptors (sIL-2R) in the serum were determined by sandwich ELISA (T Cell Sciences Inc., Cambridge, USA) [8]. The test employs two non-competing murine monoclonal antibodies to the α-chain of human IL-2R. It was done according to the manufacturer's instructions. The microtiter plates were read at 490 nm using a Dynatech MR 700 microplate reader. Units of sIL-2R were calculated from a standard curve constructed on the basis of a supernatant from phytohemagglutinin-stimulated peripheral blood mononuclear cells. Interassay variation was 5%.

Natural killer cell activity

Peripheral mononuclear cells (PMC) were separated by Ficoll-Hypaque centrifugation. NK activity was determined in a 4 h ^{51}Cr release assay as previously described [9], using the NK-sensitive erythroblastoma cell line K562 as target cells. The effector:target ratio was 40 : 1. Spon-taneous release was assessed by incubating labelled K 562 in medium alone, and total release was evaluated by a solution of SDS. After harvesting the supernatants using a Skatron filterstick system, radioactivity was measured in a Pharmacia gamma-counter.

Specific lysis (SL) was calculated as:

$$SL(\%) = \frac{\text{Mean experimental release} - \text{mean spontaneous release}}{\text{Mean total release} - \text{mean spontaneous release}} \times 100$$

Statistical tests

A computerized correlation analysis was performed using a new statistical programme (MEDAS) developed by Grund C., Haubitz I. and Rausche A. Institute for Biostatistics, University of Würzburg. Correlation between sIL-2R and NK activities was analysed by the calculation of Spearman's correlation coefficient, ρ. Correlation between △TBI and sIL-2R and NK activity, respectively, was determined by the calculation of Kendal's correlation coefficient, τ.

Results

The results of sIL-2R, NK activity and clinical course are summarized in *Table I*. Statistical analysis of our data revealed correlations between NK activity, sIL-2R and disease progression, which was described by the △TBI.

There was a negative correlation between NK activity and △TBI (τ = 0.3492, $P < 0.05$). A strong positive correlation was found between sIL-2R and △TBI ($\tau = 0.6091$, $P < 0.0005$). No correlation between staging and sIL-2R or staging and NK activity was observed. NK activity and sIL-2R showed a negative correlation ($\rho = -0.5423$, $P < 0.03$).

Analysing the reliability of sIL-2R as a prognostic factor, we suggest that a △TBI of at least 10 should be considered as a significant change. Moreover, sIL-2R should exceed the upper normal range by a factor of 2 (> 1000 U/ml). Under these conditions the specificity of sIL-2R for tumour progression was 90.0% (n = 11). There was no false-positive value, indicating high sensitivity *(Fig. 1)*.

Discussion

Recent reports of successful transplantation of cutaneous lymphomas on natural killer cell-depleted severe combined immunodeficient (SCID) mice [10] as well

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as an enhanced risk of developing secondary neoplasias [2] point to impaired immunofunctions in CTCL patients. Analysing NK activity and clinical course in these patients we found a correlation between the decrease of NK activity and the progression of the disease. We conclude that NK activity might be a prognostic factor for CTCL patients.

Elevated levels of sIL-2R were found in the serum of patients suffering from lymphoproliferative disorders [5, 6, 11, 12] as well as in the serum of patients suffering from inflammatory skin diseases [13]. Regarding the strong correlation between tumour progression in CTCL patients and sIL-2R, we suggest that this soluble protein may be of high prognostic value. This is consistent with the detection of sIL-2R as an unfavourable prognostic sign in other lymphoproliferative disorders such as Hodgkin's disease [11] and lymphoblastic lymphomas [12].

From an immunological point of view, the negative correlation between sIL-2R and NK activities offers interesting perspectives. Under physiologic circumstances, membrane-bound and soluble forms of the TAC protein are observed after T-cell stimulation, for example by antigen presenting cells and interleukin 1. As a consequence, the stimulated T lymphocytes produce interleukin-2 [14]. In CTCL patients this mechanism seems to be disturbed. In some CTCL patients, interleukin 1 [15] might enhance expression of TAC protein, albeit, without additional induction of interleukin-2, which is able to restore diminished NK activity [16] and is known to be a potent stimulating cytokine for NK activity [9]. This discordance could be an important factor for tumour spreading. We propose, that sIL-2R might be responsible for the decreased NK activity by neutralizing interleukin-2 [17, 18] ■

Zusammenfassung

Bei 24 Patienten mit cutanem T-Zell Lymphom (CTCL) wurde der klinische Verlauf mittels eines "Tumor burden index (TBI)" bei Beginn der Beobachtung und sechs Monate später dokumentiert. Zusammen mit dem ersten TBI wurde der lösliche Interleukin-2 Rezeptor (sIL-2R) im Serum mittels ELISA und die natürliche Killerzell- (NK-) Aktivität der peripheren mononukleären Zellen mittels "Cr-51 Release Assay" bestimmt. Die statistische Auswertung der drei Parameter ergab eine negative Korrelation zwischen NK-Aktivität und Zunahme des TBI, sowie zwischen NK-Aktivität und sIL-2R. Außerdem fand sich eine hochsignifikante Korrelation zwischen sIL-2R und Zunahme des TBI. sIL-2R und NK-Akivität sind möglicherweise von prognostischer Bedeutung bei Patienten mit CTCL.

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Abbreviations

CTCL: cutaneous T-cell lymphoma.
SCID: severe combined immune deficiency.
sIL-2R: soluble interleukin-2 receptor.
NK: natural killer cell.
TBI: tumour burden index.