

ORIGINAL ARTICLE

Paucity of FOXP3⁺ cells in skin and peripheral blood distinguishes Sézary syndrome from other cutaneous T-cell lymphomas

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Cutaneous T-cell lymphomas (CTCL) are mainly comprised of two variants: mycosis fungoides (MF) with CD4⁺ tumor cells confined to the skin and the leukemic Sézary syndrome with tumor cell spread to the blood. In this study, we investigated cutaneous expression of the regulatory T-cell (T_{reg}) marker FOXP3 in 30 CTCL patients. Immunohistochemical analysis revealed significantly lower numbers of CD4⁺FOXP3⁺ cells within the dermal lymphomononuclear infiltrate of Sézary patients (16% FOXP3⁺ cells of CD4⁺ cells) in contrast to MF (43% FOXP3⁺ cells ($P<0.05$)) and rare types of CTCL (45% FOXP3⁺ cells). Furthermore, CD4⁺FOXP3⁺ T cells were also markedly reduced in the CD4⁺ population within the peripheral blood of Sézary patients compared to controls as determined by fluorescence-activated cell sorter, quantitative PCR and functional analyses. The data support the conclusion that the neoplastic cells in CTCL do not express the T_{reg} marker FOXP3. Our data also identify Sézary syndrome as, to our knowledge, the first reported neoplastic disease with a clear reduction in T_{reg} numbers within the CD4⁺ population. This lack of T_{reg} might account for the more aggressive nature of Sézary syndrome compared with other CTCL.

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Introduction

Cutaneous T-cell lymphomas (CTCL) represent a heterogeneous group of rare lymphoproliferative disorders of skin homing T cells with an estimated annual incidence of 0.65/100 000.¹ The most common variant, mycosis fungoides (MF), is confined to the skin and characterized by patches, plaques and/or tumors occurring at different stages of the disease.² In contrast, the more aggressive Sézary syndrome is defined by erythroderma, generalized lymphadenopathy and circulating tumor cells in the peripheral blood (>1000 cells/mm³).³ Cutaneous T-cell lymphomas represent monoclonal proliferations of CD4⁺ T cells that produce Th2 cytokines⁴ and show resistance to interferon- γ .⁵ One study examined the CD25 expression of

CTCL tumor cells in 17 CTCL patients showing a median of 20% CD25⁺ tumor cells in skin samples, which was comparable to skin specimens from patients with chronic dermatitis (5–20%).⁶ Unfortunately, detailed results on CD25 expression in MF and Sézary patients were not supplied, as all patients were summarized in the CTCL group. Another group could show that tumor cells of Sézary patients failed to upregulate CD25 following incubation with α -CD3 antibody.⁷

Advanced CTCL patients often show features of immunosuppression with a fatal outcome owing to an increased susceptibility to various infections. This immunosuppression could be explained by either a replacement of reactive CD4⁺ and CD8⁺ cells by the tumor or by an immunosuppressive nature of the tumor. The latter hypothesis is supported by a recent study by Berger *et al.*⁸ suggesting that CTCL is a malignant proliferation of regulatory T cells (T_{reg}). This group demonstrated in an *in vitro* model that under certain circumstances, CTCL tumor cells could express FOXP3, currently the best marker to identify T_{reg}.⁹ Such FOXP3⁺ CTCL cells show characteristics of T_{reg} function: for example, production of interleukin-10 and transforming growth factor- β as well as suppression of effector T-cell responses.^{9,10} Another group suggested that adult T-cell leukemia/lymphoma could possibly originate from human T lymphotropic virus type-1-infected T_{reg}.¹¹ In healthy individuals, T_{reg} are usually rare, representing only less than 5% of peripheral blood mononuclear cells, and can be identified by very high CD25 expression (CD25^{high}) and expression of the transcriptional repressor scurf (FOXP3).^{9,12,13}

However, it remains unclear if FOXP3⁺ CTCL cells exist *in vivo* and if FOXP3 might serve as a CTCL tumor cell marker. We analyzed skin samples from 30 CTCL patients including MF, Sézary syndrome, small-/medium-sized pleomorphic CTCL and lymphomatoid papulosis for the presence of FOXP3⁺ cells. FOXP3, CD4, CD25 and CD95 expression was further analyzed in peripheral blood of patients with Sézary syndrome together with apoptosis assays of fluorescence-activated cell-sorted (FACS) Sézary CD4⁺ T cells.

Patients, materials and methods

Patients

Thirty patients with different types of CTCL diagnosed according to the WHO-EORTC classification of cutaneous lymphomas¹ and the criteria of the international society of cutaneous

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lymphomas³ were included in the study (Table 1). As controls, we investigated skin biopsy specimens from patients with chronic dermatitis ($n=15$), psoriasis ($n=3$), atopic dermatitis ($n=10$) and blood samples from healthy donors ($n=8$). Informed consent was obtained from all subjects before inclusion. The study was conducted according to the ethical guidelines at our institution and the Helsinki Declaration, and approved by the ethics committee II of the Ruprecht-Karls-University of Heidelberg, Germany.

Histology and immunohistochemistry

Skin biopsies were taken at first presentation of the patients before the onset of treatment and were fixed in formalin. Hematoxylin and eosin, CD4 (clone 1F6, Novocastra, Newcastle upon Tyne, UK) and CD25 (clone 4C9, Dianova, Hamburg, Germany) stainings were performed as described.¹⁴ For FOXP3 staining, we used 4 μ m sections, FOXP3 IgG1 antibody (Ab) 236A/E7 (AHB, GR) or mouse IgG1 isotype control (Dako, Glostrup, Denmark) and followed the published protocol.¹⁵ All specimens were blinded and analyzed by two independent investigators (CDK, NP) as described.¹⁶ Each investigator evaluated the proportion of positively stained cells, expressed as a fraction of the total lymphocytic infiltrate, and the percentage of CD4⁺ and FOXP3⁺ cells for each case was calculated from the values of six different areas.

Cell isolation and sorting

Peripheral blood lymphocytes were obtained from healthy individuals and Sézary patients and separated by Ficoll gradient

centrifugation. After staining with phycoerythrin-labeled α -CD25 Ab (Miltenyi Biotech, Auburn, CA, USA) and fluorescein isothiocyanate-labeled α -CD4 Ab (BD Pharmingen, Heidelberg, Germany), CD4⁺CD25⁻ (T_{eff}), CD4⁺CD25⁺ and CD4⁺CD25^{high} (T_{reg}) cells were sorted with a FACS-Diva™ cell sorter (BD Biosciences) as described.¹³

Cell surface staining

Cells were stained with PE-labeled α -CD25, PE-Cy5- or FITC-labeled α -CD4 Ab and FITC-labeled α CD95 Ab or their respective isotype control Ab and analyzed with a FACScan cytometer with at least 10 000 cells counted.¹³ The agonistic monoclonal α CD95 Ab (α Apo-1) was purified from hybridoma supernatants by Protein A (Sigma-Aldrich, St Louis, MO, USA) affinity purification.¹⁷

Cell culture and cytotoxicity assays

Freshly isolated T cells were cultured in IL-2 (100 IU) containing ex vivo-15™ medium (Cambrex, Verviers, Belgium) supplemented with 1% Glutamax (Invitrogen, Karlsruhe, Germany). For apoptosis induction, T cells were stimulated with a 1:10 dilution of a leucine zipper-tagged CD95 ligand (CD95L).^{17,18} Annexin V Alexa Fluor 488 was purchased from Molecular Probes (Eugene, OR, USA) and propidium iodide (PI) was obtained from Sigma-Aldrich (St Louis, MO, USA). Unstimulated cells were incubated with CD95L-free control medium yielding spontaneous apoptosis of 15–30% in 20 h. Cell death was assessed by annexin V/PI costaining and forward-to-side-scatter profile. Specific cell death was calculated as described previously.¹⁷

Table 1 Patient characteristics regarding clinical data, histological evaluation of the skin biopsies and molecular biological data

No.	Diagnosis	Gender	Age (years)	CD8 (%)	CD4 (%)	FOXP3 (%)	TCR-R skin
1	S-/m-p CTCL	F	83	10	85	37	PC
2	S-/m-p CTCL	M	67	<5	49	15	MC ^a
3	LP	M	26	10	42	28	MC
4	LP	M	48	ND	55	28	MC
5	LP	F	61	30	67	23	ND
6	LP	M	61	20	41	19	MC
7	MF IIa	M	73	7	76	23	MC
8	MF Ib	F	95	ND	43	17	MC ^a
9	MF Ib	M	65	15	54	50	MC
10	MF Ib	F	75	20	83	48	ND
11	MF IIa	M	50	10	44	43	MC
12	MF Ib	M	32	30	72	25	MC
13	MF IIa	M	61	ND	31	21	MC
14	MF Ib	M	71	10	18	8	ND
15	MF IIa	M	71	ND	65	33	MC
16	MF Ib	F	83	ND	41	16	PC
17	MF IIa	F	81	25	78	18	ND
18	MF Ib	F	64	<5	57	34	MC
19	MF IIa	M	35	30	67	31	MC
20	MF IIb	M	15	<5	58	23	MC
21	MF Ib	M	61	10	85	17	MC
22	MF IIa	F	34	5	64	19	MC
23	Ss IVa	F	83	33	67	19	MC ^a
24	Ss IVa	F	55	5	88	7	MC
25	Ss IVa	F	71	<5	57	9	ND
26	Ss IVa	M	51	<5	46	4	MC ^a
27	Ss III	M	78	20	83	15	MC ^a
28	Ss III	M	69	ND	73	13	MC ^a
29	Ss III	F	60	15	69	5	PC
30	Ss III	M	64	<5	ND	ND	ND

Abbreviations: F, female; LP, lymphomatoid papulosis; M, male; MC, monoclonal; ND, not determined; PC, polyclonal; S-/m-p CTCL, small-/medium-sized pleomorphic cutaneous T-cell lymphomas; TCR-R, T-cell receptor rearrangement.

^aIndicates an identical TCR-R in the skin and blood.

(% Experimental cell death – % spontaneous cell death) / (100% – % spontaneous cell death) × 100.

RNA preparation and quantitative reverse transcription-PCR

Total RNA was isolated using the Absolutely RNA Microprep kit (Stratagene, Heidelberg, Germany) and cDNA was prepared using random oligo(dT) primers (Invitrogen).¹³ FOXP3 mRNA expression was quantified by detection of incorporated SYBR[®] Green using the ABI Prism 5700 sequence detector system (Applied Biosystems, Foster City, CA, USA). The relative expression level was determined by normalization to glyceraldehyde phosphate dehydrogenase with results presented as fold expression of T_{eff} mRNA levels. FOXP3 primer sequences were as follows: 5'-AGC TGG AGT TCC GCA AGA AAC-3' (forward) and 5'-TGT TCG TCC ATC CTC CTT TCC-3' (reverse).

PCR conditions and fluorescent fragment analysis (GeneScan)

DNA was extracted from formalin-fixed and paraffin-embedded skin biopsies and peripheral blood samples using a QIAmp tissue kit (QIAGEN GmbH, Hilden, Germany). To analyze the skin and blood samples for T-cell receptor (TCR)- γ rearrangement, we used the previously published novel oligonucleotide consensus primers covering the V γ 1–8 segments (V γ 1_{1–8} as outer and V γ 2_{1–8} as inner primers)¹⁹ and the J region (JGT_{1/2}, JGT₃),²⁰ and for TCR- β rearrangement, consensus primers covering the V β 1 to V β 65 segments and the primers J β FS1A/J β FS1 and J β FS2A/J β FS2 for the J region.²¹

Statistical analysis

Data are presented as individual values and as median. The Wilcoxon signed-rank test was used to compare the skin infiltrates of the different patient groups. The test was considered significant when *P* is less than 0.05.

Results and discussion

FOXP3 staining of paraffin-embedded lymphoid tissues and skin biopsies

In order to identify FOXP3⁺ T_{reg}, we first established a FOXP3 staining protocol using various paraffin-embedded lymphoid tissues, for example, tonsils and sorted CD4⁺CD25^{high} cells (T_{reg}) from the peripheral blood of healthy donors. The specificity of the anti-FOXP3 Ab was demonstrated by comparing non-transfected with FOXP3-transfected Jurkat cells and sorted CD4⁺CD25[–] with CD4⁺CD25^{high} cells. A positive staining for FOXP3 expression was selectively observed in the FOXP3 transfected Jurkat (data not shown) and the CD4⁺CD25^{high} cells (Supplementary Figure S1). To exclude nonspecific crossreactivity of the FOXP3 Ab with different cells and structures present in the skin, we analyzed normal skin sections, which were all negative (data not shown). The mouse IgG1 isotype control Ab gave negative results in all experiments (data not shown and Figures 1e and j). This allowed specific detection of FOXP3⁺ T_{reg} in paraffin-embedded skin samples as recently shown for other lymphoid tissues.¹⁵

Cutaneous FOXP3 expression in various types of cutaneous T-cell lymphomas

In this study, we have investigated the presence of FOXP3⁺ cells in the skin of 30 patients with MF, rare variants of CTCL and Sézary syndrome (Table 1). The diagnosis was based on the clinical features, histology, immunohistochemistry and molecular studies of TCR rearrangement. In all skin specimens, the lymphocytic infiltrate was predominated by CD3⁺ T cells mixed with only single CD20⁺ B cells (data not shown). With the exception of three patients, a monoclonal T-cell population could be demonstrated in the skin by either TCR- β or TCR- γ rearrangement, which is in accordance with other studies (Table 1).^{19,21,22}

In patients with MF, the cutaneous lymphomononuclear cell infiltrate was primarily found in the upper dermis, predominated

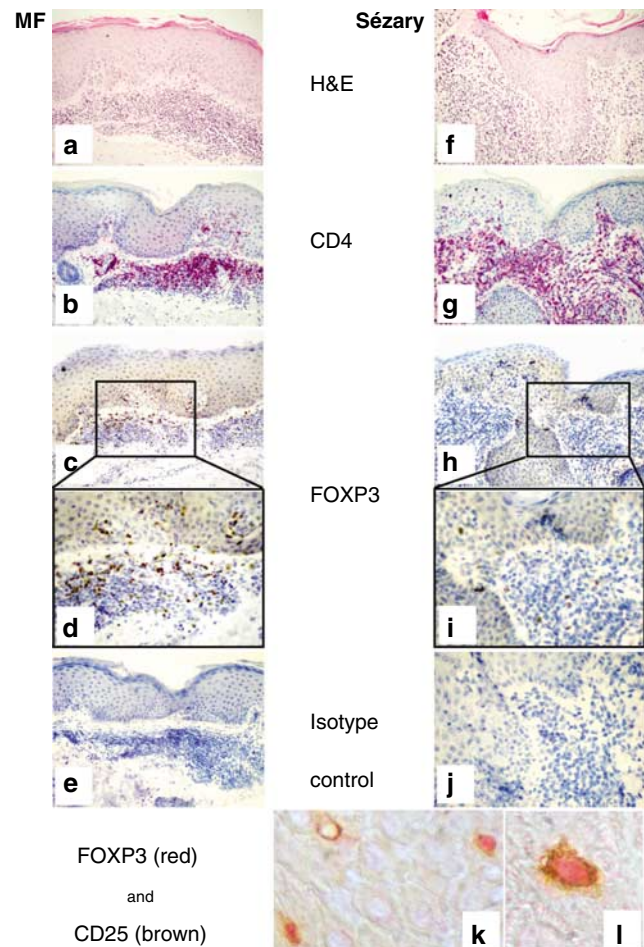


Figure 1 Hematoxylin and eosin (H&E), CD4 and FOXP3 stainings of skin specimens from patients with mycosis fungoides (MF) (a–e) and Sézary syndrome (f–j). (a) In MF, H&E staining shows a typical band-like lymphomononuclear cell infiltrate in the upper dermis with epidermotropism of single lymphocytes. (b) Most of the dermal and epidermal infiltrate is composed of CD4⁺ cells. (c and d) FOXP3⁺ cells are readily detectable within the MF skin infiltrate. (e) Application of the isotype control IgG1 antibody shows no unspecific staining. (f) Routine H&E histology of a skin specimen from a patient with Sézary syndrome reveals a diffuse dermal lymphomononuclear cell infiltrate with less epidermotropism. (g) The vast majority of the dermal infiltrate consists of CD4⁺ cells. (h and i) Only rare FOXP3⁺ cells are seen in skin specimens of Sézary syndrome. (j) Staining with the isotype control IgG1 antibody is also negative. (k and l) Double stainings demonstrate cutaneous lymphocytes which stain for both FOXP3 (red nuclear staining) and CD25 (brown surface staining), respectively.

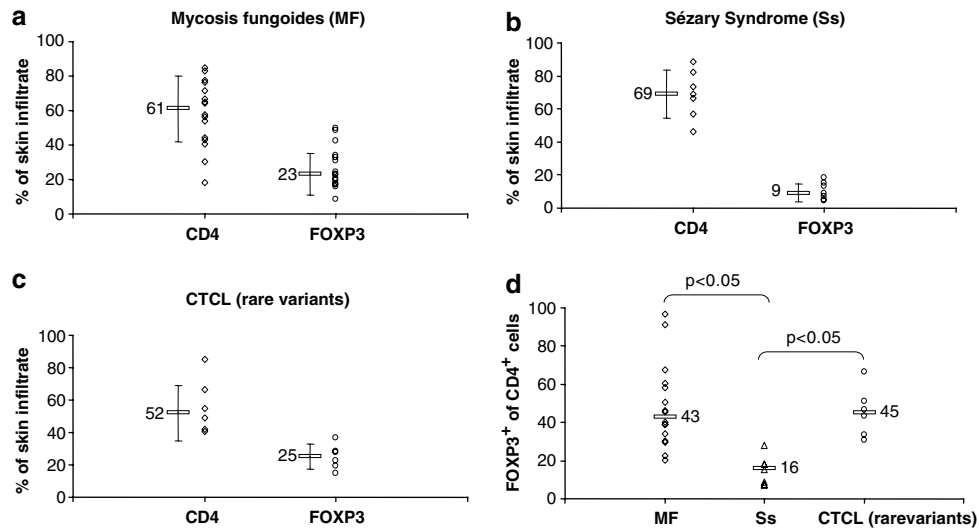


Figure 2 FOXP3⁺ cells are reduced in the skin of patients with Sézary syndrome. (a) In the skin samples of patients with mycosis fungoides (MF) 61% (median) of the skin-infiltrating lymphocytes are CD4⁺ and 23% are FOXP3⁺. (b) Sixty-nine percent of skin-infiltrating lymphocytes in patients with Sézary syndrome are CD4⁺ and 9% FOXP3⁺. (c) Patients with rare variants of CTCL show a median percentage of 25% of FOXP3⁺ cells in their skin lesions. (d) For each skin specimen investigated, the percent of FOXP3⁺ cells of total skin CD4⁺ cells was calculated. The numbers indicate the median value for each diagnosis investigated. FOXP3⁺ cell numbers are significantly reduced in Sézary syndrome compared to MF ($P < 0.05$).

by CD4⁺ cells with a median of $61 \pm 19\%$ of total leukocytes (Table 1 and Figures 1a, b and 2a). Only a minority of CD8⁺ cells ranging from <5 to 30% of the lymphocytic skin infiltrate were seen in the skin samples (Table 1). FOXP3⁺ ($n = 16$) and CD25⁺ ($n = 4$) cells were readily detected within the dermal infiltrate of MF skin samples (23 ± 12 and $23 \pm 5\%$, respectively), whereas staining with the isotype control IgG1 antibody was negative (Supplementary Figure S1 and Figures 1c, d and 2a). FOXP3 is expressed on CD4⁺ cells²³ and we found a median of 43% of CD4⁺ cells expressing FOXP3 by calculating the percentage of FOXP3⁺ cells of all CD4⁺ cells for each skin specimen investigated (Figure 2d). By morphological criteria, these CD4⁺ FOXP3⁺ cells are small in size and do not look like typical MF tumor cells. In rare CTCL types, we found an almost identical distribution of FOXP3⁺ ($n = 6$) and CD25⁺ ($n = 1$) cells as in MF (Table 1 and Figures 2c and d). This is in concordance with recent observations in other tumor entities also showing enhanced T_{reg} numbers in the tumor mass²³ or the draining lymph node of malignant melanoma.²⁴ In patients with Sézary syndrome, the cutaneous infiltrate was also primarily composed of CD4⁺ cells ($52 \pm 24\%$ of leukocytes) (Figures 1f, g and 2b). However, FOXP3⁺ ($n = 7$) and CD25⁺ ($n = 3$) cells were similarly rare within the dermal infiltrate of Sézary skin samples (9 ± 5 and $10 \pm 3\%$ of leukocytes, respectively). Most of the FOXP3⁺ cells were also CD25⁺, as demonstrated by double staining (Figures 1k and l). However, a few cells within the cutaneous infiltrate were CD25⁺ and FOXP3⁻, and possibly represent activated T cells.²⁵ We conclude that FOXP3 expression is much more reliable for the detection of T_{reg} than CD25. Overall, only 16% of CD4⁺ cells expressed FOXP3 in Sézary syndrome compared to 43% in MF ($P < 0.05$) (Figures 1g, h and 2b, d). Interestingly, FOXP3⁺ cell numbers in a given patient were remarkably constant over time in MF (no. 11), Sézary syndrome (no. 26) and one patient with small-/medium-sized pleomorphic CTCL (no. 2) when investigated at multiple time points (Supplementary Figure S2A).

In control patients with chronic dermatitis and psoriasis ($n = 18$) and atopic dermatitis ($n = 10$), we obtained similar high

counts of FOXP3⁺ cells (38 ± 24 and $33 \pm 17\%$ of CD4⁺, respectively) as in MF and rare CTCL. These data are in line with a recent FACS analysis showing 15–35% putative T_{reg} (CTLA-4⁺CD25⁺) in psoriatic lesional skin²⁶ and fit to published CD25 expression levels in the skin of patients with psoriasis and atopic dermatitis.²⁷ FOXP3 has been described as a specific marker for cells with regulatory potential in mice (natural and induced T_{reg}), and other studies have indicated that human FOXP3⁺ cells in tissues represent T_{reg}.^{23,28} Thus, the accumulation of CD25⁺/FOXP3⁺ cells in biopsies of inflamed skin is likely to represent T_{reg}. Two-color immunohistochemical studies demonstrated FOXP3 staining mainly on CD25⁺ cells (Figures 1k and l). It is interesting to note that T_{reg} accumulate in disease-specific organ tissues to a similar proportion of approximately 30% of CD4⁺ cells in inflammatory diseases (rheumatoid arthritis,²⁸ leishmania²⁹) and tumors (ovarian cancer,²³ melanoma²⁴). In contrast, human blood from healthy controls usually contains less than 5% T_{reg} and from patients with atopic dermatitis 7%.^{13,30} The factors governing this accumulation are not clear yet. Further investigations will clarify whether T_{reg} are dysfunctional in inflammation as shown for psoriasis²⁶ and multiple sclerosis³¹ or if the sustained immune response overcomes the suppressive capability of T_{reg}. In cancer, increased numbers of T_{reg} probably contribute to immune suppression; conversely, depletion of T_{reg} can facilitate tumor rejection.^{23,32}

Characterization of FOXP3⁺ cells in the blood of Sézary patients

The suggestion that CTCL is a malignant proliferation of T_{reg}⁸ is consistent with our observation of high T_{reg} numbers in the skin of MF and rare types of CTCL but in contrast with the specifically low FOXP3⁺ cell numbers seen in Sézary syndrome. A possible explanation might be a shift of the tumor load from the skin to the blood in the leukemic variant of CTCL. Therefore, we investigated the status of T_{reg} in the blood of patients with Sézary syndrome. T_{reg} can be identified by a very high CD25 expression

in blood. We found that CD4⁺CD25^{high} cells were markedly reduced in all patients with Sézary syndrome compared with healthy controls (Table 2 and Figures 3a and b). Multiple time-point analyses in one Sézary patient (no. 24) showed consistently low numbers of CD4⁺CD25^{high} cells during the course of the disease (Supplementary Figure S2B). Further sorted CD25⁺ cells (combined CD25^{int} and CD25^{high} cells) showed much lower levels of FOXP3 mRNA than in healthy controls (Figure 3c). This indicates a loss of T_{reg} in the CD4⁺CD25⁺ cell pool of Sézary patients. Other sites of inflammation or tumor cell accumulation in our Sézary patients were excluded by

computer tomography scans. We have recently shown that CD95 expression and sensitivity to CD95L-induced apoptosis is a unique feature of T_{reg} within the CD4⁺ cell population.¹³ In contrast, the CD25⁺ cells of Sézary patients only displayed intermediate CD95 receptor expression rates and intermediate sensitivities to CD95L-induced apoptosis (Figures 4a and b). Thus, blood and skin of Sézary patients contain very few cells with characteristics of T_{reg}. Furthermore, the four CTCL lines (HH, Hut78, Myla, Seax) tested in our lab did not show specific nuclear FOXP3 staining and had no FOXP3 mRNA expression (data not shown). In Sézary syndrome, increasing CD4⁺ tumor

Table 2 Hematological data of patients with Sézary syndrome established at the time of this study

No.	LDH (0–248 U/l)	WBC (4200–10200/μl)	CD3 (600–2800/μl)	CD4 (400–1800/μl)	CD8 (160–1100/μl)	CD4/CD8 (0.9–3.5)	CD4 ⁺ CD7 [–] (%)	Sézary cell count (/μl)	TCR-R blood	CD4 ⁺ CD25 ^{high} (% of CD4 ⁺)
23	209	15 000	12 200	11 980	244	49	60	7560	MC ^a	ND
24	204	5900	1960	1840	98	18.8	91	2048	MC	0.08
25	246	6000	660	338	258	1.3	7	760	ND	0.43
26	297	5400	1070	880	18	4.8	72	935	MC ^a	0.36
27	286	11 300	1590	841	698	1.2	69	1403	MC ^a	2.7
28	274	9400	3380	3308	68	49	85	3020	MC ^a	2.84
29	248	10 100	4460	4150	268	15.5	4	4230	ND	0.3
30	175	22 200	10 900	9573	761	12.6	82	9102	ND	1.4

Abbreviation: LDH, lactate dehydrogenase; MC, monoclonal; ND, not determined; PC, polyclonal; TCR-R, T-cell receptor rearrangement; WBC, white blood cell count.

Ranges of normal values and units used are indicated in parentheses.

^aIndicates an identical TCR-R in the skin and blood.

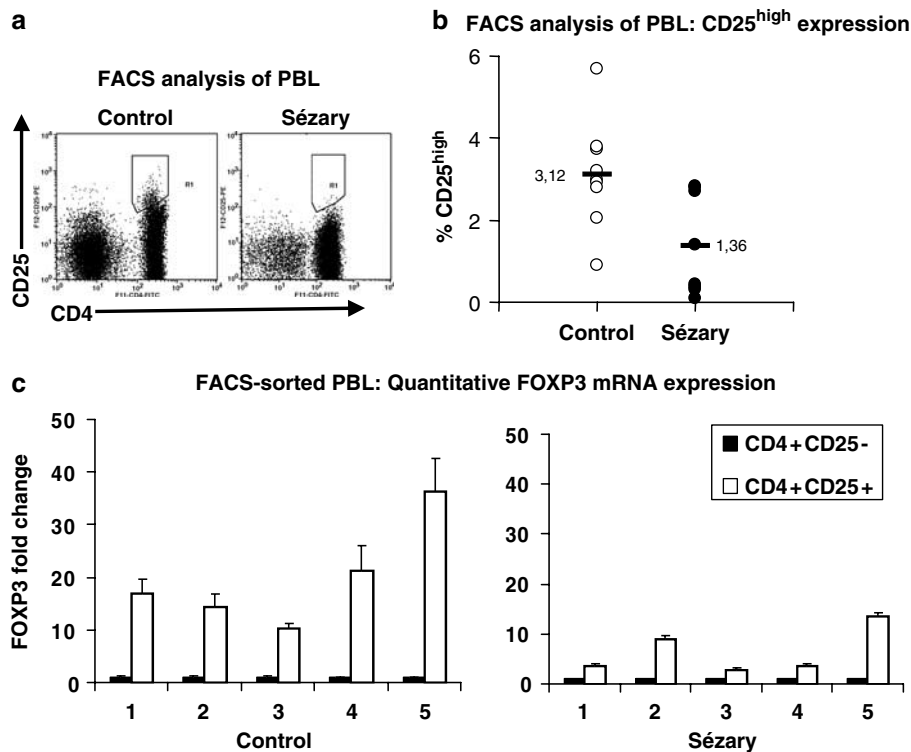


Figure 3 T_{reg} are markedly reduced in the peripheral blood of patients with Sézary syndrome. (a) Representative fluorescence-activated cell sorting (FACS) analysis of peripheral blood stained for CD4 and CD25. Gate R1 indicates CD4⁺CD25^{high} cells. (b) The percentage and median of CD4⁺CD25^{high} cells are shown for healthy controls and Sézary patients, which were always investigated in parallel in the same experiment. (c) FOXP3 mRNA levels were determined by quantitative reverse transcription-PCR in FACS-sorted peripheral blood CD4⁺CD25[–] vs CD4⁺CD25⁺ cells (including CD24^{int} and CD25^{high} cells) from healthy controls and Sézary patients. All experiments were performed in triplicate.

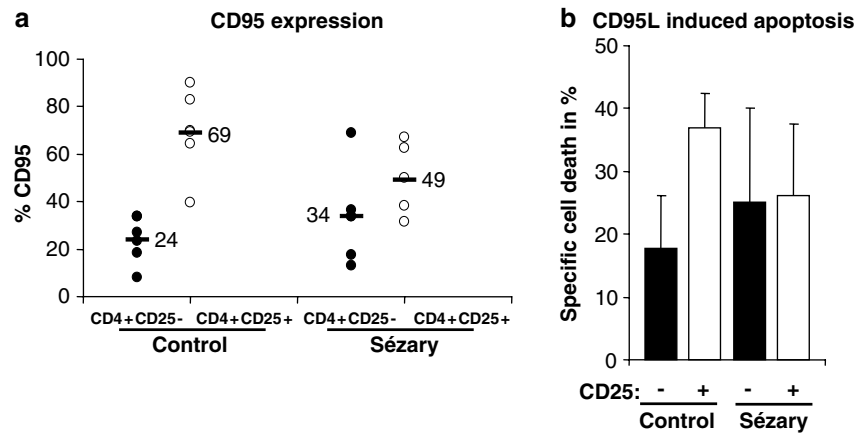


Figure 4 Peripheral blood CD4⁺CD25⁺ cells from patients with Sézary syndrome do not show the characteristic of T_{reg} of high CD95 expression and induction of CD95 ligand (CD95L)-induced apoptosis. **(a)** Evaluation of CD95 expression of CD4⁺CD25⁻ vs CD4⁺CD25⁺ cells by three-color FACS analysis in healthy controls and Sézary patients. Each dot represents a single patient or control and the line shows the median for each group. **(b)** Determination of specific apoptosis induced by soluble CD95L treatment of CD4⁺CD25⁻ vs CD4⁺CD25⁺ cells over 24 h. Mean values and s.d. are shown.

cell numbers of up to 95% of the total leukocytes probably displace T_{reg}. In the majority of our Sézary syndrome patients, there is also a marked reduction of CD8⁺ cells seen in the peripheral blood and in the skin. All skin specimens from Sézary patients with very low numbers of FOXP3⁺ cells also had low numbers of CD8⁺ cells (Table 1). This strongly indicates that expansion of the CD4⁺ tumor cell population reduces the relative numbers of CD8 cells and T_{reg}. Interestingly, T_{reg} do not accumulate, as seen for various malignancies.^{23,24} These data also provide convincing evidence that the tumor cells do not express FOXP3. In line with this interpretation, preliminary data demonstrate a polyclonal TCR rearrangement in FACS-sorted CD4⁺CD25^{high} (T_{reg} gate) cells of patients with Sézary syndrome (data not shown). As we did not detect a dominant T-cell clone within this cell fraction, these data speak against the presence of tumor cells within the CD25^{high} T_{reg} population. Furthermore, Sézary tumor cells do not express CD25.⁷ Nevertheless, as there is no unique CTCL tumor cell marker available, although unlikely, we cannot exclude the possibility that a few tumor cells have gained FOXP3 expression.

Conclusion

Our data collectively suggest that CTCL tumor cells are not of T_{reg} origin. Rather the increased numbers of FOXP3⁺ cells denotes an accumulation of T_{reg} in indolent CTCL (MF). This conclusion is also supported by the fact that inflammatory skin diseases accumulate similar numbers of T_{reg} in the skin. One may speculate that T_{reg} not only suppress the anti-tumor immune response, but also have the potential to suppress tumor growth in MF. Thus, the lack of T_{reg} in Sézary syndrome might account for the more aggressive course seen in these patients. Furthermore, preliminary results have demonstrated a trend towards a worse outcome for patients with rare variants of CTCL and MF with lower numbers of T_{reg} found in their skin lesions (data not shown). Finally, we would like to stress that to the best of our knowledge Sézary syndrome is the first reported malignancy with a clear reduction in T_{reg} cell numbers within the CD4⁺ population. Therapeutically, this loss of T_{reg} could possibly be counteracted by extracorporeal photopheresis, which was recently shown to induce antigen-specific T_{reg}.³³ FOXP3 quantification might be a useful surrogate marker for the

aggressive Sézary syndrome and serve as a prognostic marker for tumor progression.^{34,35}

Acknowledgements

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