Cellular Coincidence of Clonal T Cell Receptor Rearrangements and Complex Clonal Chromosomal Aberrations—A Hallmark of Malignancy in Cutaneous T Cell Lymphoma

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Detection of a clonal T cell receptor (TCR) gene rearrangement is used in the diagnosis of primary cutaneous T cell lymphomas (CTCL) whereas chromosomal aberrations serve as a diagnostic tool for leukaemias and nodal lymphomas. To what extent both approaches specify the same cell population remains unknown. We investigated the coincidence of TCR clonality with complex clonal chromosomal aberrations, indicating qualitative alteration of the affected cells, in 17 CTCL patients. Out of 41 skin, blood, and lymph node samples studied, 34 gave results in chromosome and TCR analyses. With 88%, most specimens revealed corresponding results by both techniques (27 of 34 clonal, three of 34 non-clonal). In two patients, analysis of micro-dissected cells demonstrated that neoplastic T cells bear both a dominant TCR rearrangement and a complex chromosomal aberration. The cutaneous clone was found in blood samples of 11 of 12 patients (including early stages), and investigation of follow-up skin and blood samples indicated persistence of the T cell clone in 11 of 14 cases. In conclusion, we show that dominant TCR clones and chromosomal clones converge in all stages of CTCL. These clones disseminate into blood and skin at early disease stages and persist despite therapy. The coexistence of a dominant TCR clone and a clonal chromosomal aberration can thus be used as a hallmark of malignancy.

Key words: chromosome/clone/CTCL/TCR
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Primary cutaneous T cell lymphomas (CTCL) represent a heterogeneous group of non-Hodgkin lymphomas whose pathogenesis is poorly understood. During the past decades, up to 3-fold increases in CTCL incidence have been observed in the western world (Weinstock and Horm, 1988; Siegel *et al*, 2000; Vakeva *et al*, 2000). The most common CTCL is mycosis fungoides (MF), which is difficult to diagnose in its early presentation since it may clinically and histologically resemble eczema or parapsoriasis (Siegel *et al*, 2000).

In the latter connection, detection of a clonal T cell receptor (TCR) gene rearrangement has become widely used. By means of PCR and high-resolution electrophoresis, a TCR clone can be demonstrated in skin biopsies of up to 90% of MF cases (Trainor *et al*, 1991; Bottaro *et al*, 1994), and its detection at the initial diagnosis has been found to be an independent negative predictive marker of treatment response (Delfau-Larue *et al*, 1998). Moreover, the cutaneous TCR clone is detectable in the peripheral blood already in about 40% of patients with early MF (stages IA–IIA) (Theodorou *et al*, 1995; Muche *et al*, 1997; Fraser-Andrews *et al*, 2000).

However, detection of a TCR clone does not unanimously mean malignancy, and the presence of additional different clonal TCR gene rearrangements in skin and lymph node samples of about one-third of MF patients may be confusing (Vega *et al*, 2002). Clonal T cell expansions have been identified in a substantial portion of blood samples derived from healthy individuals and patients with benign diseases, as well as in skin specimens from benign dermatoses (Posnett *et al*, 1994; Kolowos *et al*, 1997; Muche *et al*, 1999; Lukowsky *et al*, 2000). Here, the accumulation of T cell clones was found to be age-dependent and started earlier as well as was more pronounced in the CD8-positive fraction (Wack *et al*, 1998).

Besides TCR rearrangements, the detection of clonal chromosomal aberrations designates a clonal T cell expansion. In CTCL, a variety of aberrations have been found in the vast majority of patients and even in some patients with large-plaque parapsoriasis (LPP) (Whang-Peng *et al*, 1979;

Abbreviations: CGH, comparative genome hybridization; CTCL, cutaneous T cell lymphoma; FFA, fluorescence fragment analysis; FISH, fluorescent *in situ* hybridization; LPP, large-plaque parapsoriasis; MF, mycosis fungoides; PUVA, psoralen UV-A treatment; SKY, spectral karyotyping; SS, Sézary's syndrome; TCR, T cell receptor

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Karenko *et al*, 1997). Moreover, their detection may precede relapse or progression of the disease (Whang-Peng *et al*, 1979; Karenko *et al*, 1997). Chromosomal clones, especially with complex somatic alterations, indicate qualitative and functional alteration of the affected cells, and occur very rarely in blood and normal tissue of healthy adults (Johnson *et al*, 1998; Broberg *et al*, 2001; Rubes *et al*, 2002; Karenko *et al*, 2003).

The correlation of TCR clonality and chromosomal clonality has been previously described by Bagot *et al* (1998), showing trisomy 7 in a CTCL-derived tumor cell line by fluorescent *in situ* hybridizations (FISH) in combination with the detection of three clonally rearranged TCRβ segments in the cell line and in corresponding skin and blood samples, but not in a corresponding cytotoxic cell line. However, the significance of solitary trisomy 7 as a marker of malignancy is controversial, as it has also been observed in some non-malignant tissues and may correlate with age (Broberg *et al*, 2001). Since the coexistence of clonal chromosomal abnormalities and TCR clones had not been investigated in a large series of samples *in vivo*, we explored them in 41 samples derived from skin, blood, or lymph nodes of 17 patients suffering from CTCL.

Results and Discussion

Out of the 41 CTCL samples, 34 could be analyzed for chromosome aberrations and 40 were positive by PCR for TCRγ rearrangements. The majority of the 34 specimens, which were successfully studied by both approaches, revealed corresponding results for TCR and chromosomal clonality (88%, 27 of 34 clonal, three of 34 non-clonal, Table I). Whether the same cell bearing the clonal TCR rearrangement also had clonal chromosomal aberrations was further demonstrated in two patients (#15 and #16). Both showed clonal trisomy 8 as part of a very complex karyotype, which thus could be used as a selection marker for the chromosomally clonal cells. Here, identical clonal fragment lengths and TCRγ sequences were detected in DNA extracted from the whole tissue sample and from single, trisomy 8-positive cells micro-dissected from touch preparations of the lymph node (#15) or skin (#16) samples (Fig 1). Out of the nine control samples taken from healthy volunteers and psoriasis patients, none was found to bear a TCR or chromosomal clone (Table I). Thus, at least in CTCL, the occurrence of TCR clonality is strongly associated with the occurrence of complex clonal aberrations of chromosomes or, vice versa, complex chromosomal aberrations arise predominantly in clonally expanded T lymphocytes.

All of the divergent specimens were clonal by TCR analysis only (patients #01, 04, 05, 14) and were derived from peripheral blood. Since the TCR clones found in two of them (#04 and #14) differed from the primary TCR clone, this discrepancy may be due to the occurrence of reactive clonal T cells as previously described in the peripheral blood of CTCL patients (Delfau-Larue *et al*, 2000; Beylot-Barry *et al*, 2001) or to the evolution of subclones from a dominant clone as suggested by Vega *et al* (2002). However, as indicated by the detection of chromosomal clonality in a follow-up sample of #01, sensitivity limits of G-banding and MFISH or tumour cell death prior to chromosomal analysis *in vitro* may also be an explanation for this observation. Interestingly, in patient #06, showing a chromosomal clone and TCR clone in the skin, chromosomal analysis of two available blood samples identified a single cell in the earlier blood sample to bear the same aberrations as observed in the skin, whereas TCR analysis revealed no clonality.

Corresponding skin and blood specimens were available in 12 of 17 CTCL patients. Here, FFA revealed identical TCR clones in the skin and blood (and lymph node of #15) of nine patients, whereas in three patients a different (#04, #10) or no blood clone (#06) was detected. When successful, chromosomal analysis confirmed the TCR data in the nine above-mentioned patients and identified one additional patient (#04) to bear identical clones in the skin and blood. Since identical clones were found in CTCL stages IA–IVB, previous TCR data demonstrating an early dissemination of the T-cell clone in both skin and blood (Muche *et al*, 1997; Karenko *et al*, 1997, 1999, 2001; Fraser-Andrews *et al*, 2000; Beylot-Barry *et al*, 2001) are now for the first time confirmed by parallel chromosomal analyses.

Analysis of follow-up samples revealed persistence of the initial TCR/chromosomal clone in 11 of 14 patients despite therapy and even if temporary complete clinical remission was achieved (Table I). This long-standing persistence of initial T cell clones stands in line with previously published data (Vega *et al*, 2002; Poszepczynska-Guigne *et al*, 2003), and indicates that clonal cells were not targeted by current therapies nor were they sensitive to therapy-induced apoptosis as currently suggested (Kacinski and Flick 2001; Nikolova *et al*, 2002).

In conclusion, our data show that dominant TCR clones and chromosomal clones converge in all stages of CTCL. These clones disseminate into blood and skin at early disease stages and persist despite current therapeutic modalities. As proven in two cases at the single cell level, neoplastic T lymphocytes bear both a dominant TCR rearrangement and microscopically detectable chromosomal aberrations as a hallmark of malignancy.

Material and Methods

Patients and samples Forty-one blood, skin, and lymph node biopsy samples were obtained, after written informed consent and in the context of routine examinations, from 17 CTCL patients (three female, mean age 52 y, range 19–79 y, and 14 male, mean age 55 y, range 20–87 y): 16 with MF or Sezarý's syndrome (SS), and one with subcutaneous panniculitis-like CTCL (s.c. CTCL). Patients were diagnosed and treated according to the EORTC classification (Willemze *et al*, 1997) at the Department of Dermatology, Helsinki University Hospital, and samples were taken prior to and between any therapy (psoralen UV-A treatment (PUVA), interferon-α, electron beam, or combinatorial chemotherapy) during a follow-up, of 2 mo to 4 y (Table I). Nine additional blood samples from voluntary donors (healthy individuals, $n=5$; patients with psoriasis vulgaris, $n=4$) served as controls. The study was approved by the Ethical Review Board of Helsinki University Hospital. Detailed information about the karyotypes or therapy given and response to therapy during follow-up have been previously published for the following patients: #01(Karenko *et al*, 1997, 1999), #03, #04, #08 (Karenko *et al*, 1997, 2001, 2003; Karenko *et al*[2]), #06 (Karenko *et al*, 2001, 2003; Karenko *et al*[2]),

Table I. Outcome of TCR and chromosomal analysis

Diagnosis	Patient	Source	Date	TCR clone	Chromosomal clone	Clinical outcome (therapy given)[a]
MF IA	#01	sk	04/99	np	nd	
		bl	01/95	c[b]	nc	
		bl	06/99	c[b]	c	CR (PUVA)
	#02	sk	01/97	c	nd	
		bl	04/96	oc[c]	c	CR (PUVA)
	#03	sk	10/01	c	nd	
		bl	03/98	c	c	CR, relapses (PUVA, EB)
MF IB	#04	sk	06/00	c	c	
		bl	04/93	c[d]	nc	
		bl	08/00	c[d]	c	PD (PUVA)
	#05	sk	09/95	c	nd	
		bl	08/96	c	nc	CR (EB)
	#06	sk	07/97	c	c	
		sk	03/98	c	nd	
		bl	08/97	nc	nc[e]	
		bl	03/98	nc	nc	PD (EB)
MF IIA	#07	bl	08/00	nc	nc	PD (multiple therapies)
MF IIB	#08	sk	08/93	c	c	
		sk	08/93	c	c	
		bl	05/93	c	c	PD/DOD
	#09	sk	07/98	c	c	
		bl	09/98	oc[c]	c	PD/DOD
	#10	sk	08/01	c	nd	
		bl	04/99	c[d]	nd	
		bl	03/01	c[d]	c[f]	PD/DOD
MF IVA	#11	sk	03/98	c	c	
		bl	03/98	c	c	
		bl	12/99	c	c	PD/DOD
	#12	bl	10/00	c[b]	c	
		bl	03/01	c[b]	c	PD/DOD
SS	#13	bl	02/00	c	c	CR (chemo)
	#14	bl	11/93	c	c	
		bl	03/96	c[g]	nc	CR (chemo)
	#15	sk	01/98	c	c	
		ln	01/98	c	c	
		bl	03/98	c	c	PD/DOD
	#16	sk	02/97	c	c	
		bl	02/97	c	c	PD/DOD
s.c. CTCL	#17	sk	03/99	c	c	
		bl	03/99	oc[c]	c	
		bl	06/99	oc[c]	c[f]	CR (chemo)
Healthy controls	#18	bl	—	nc	nc	
	#19	bl	—	nc	nc	
	#20	bl	—	nc	nc	
	#21	bl	—	nc	nc	
	#22	bl	—	nc	nc	
Psoriasis vulgaris	#23	bl	—	nc	nc	
	#24	bl	—	nc	nc	
	#25	bl	—	nc	nc	
	#26	bl	—	nc	nc	

bl, blood; sk, skin; c, clonal (if not otherwise indicated, clones were identical in all samples from a given patient); nc, non-clonal; oc, oligoclonal; nd, not determined; np, no PCR amplification.

[a]CR, complete response; EB, electron beam therapy; PD, progression; DOD, died of CTCL after multiple forms of therapy; chemo, poly/monochemotherapy.

[b]Skin sample not available.

[c]Up to four additional clones detectable.

[d]Predominant clone not identical with cutaneous clone.

[e]One cell with the aberrations corresponding to the skin clone found.

[f]Later sample analyzed for chromosomal clonality.

[g]Different TCR clone in follow-up sample.

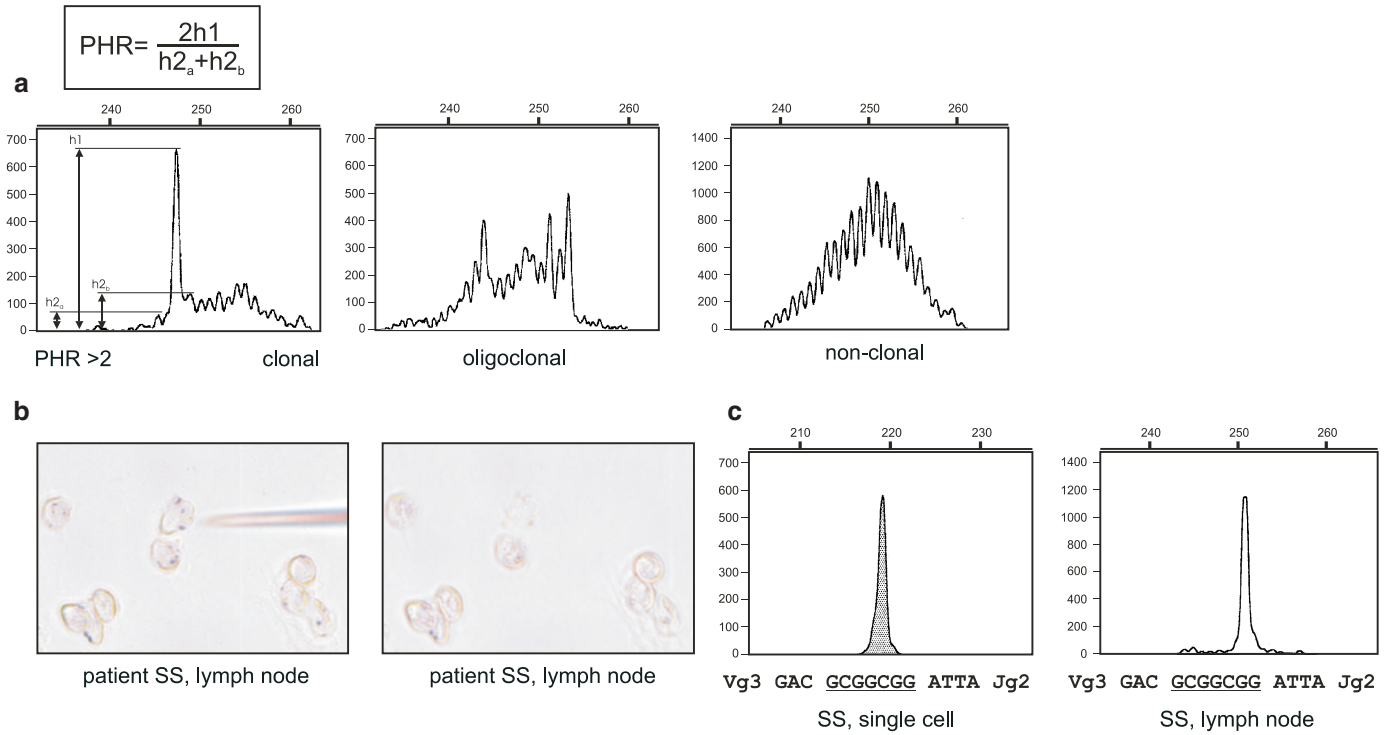

Figure 1
Assignment of clonality by fluorescence fragment fnalysis (FFA). (*a*) peak height ratio (PHR) is calculated to objectively assign T cell clonality. A PHR >2 is defined as clonal, the occurrence of >2 peaks with PHR >2 is regarded as oligoclonal, and Gaussian distribution of the peaks is regarded as non-clonal. (*b*) micro-dissection of a trisomy 8-positive lymphocyte from the lymph node of patient SS (bright spot: chromosome 1 centromere; dark spot: chromosome 8 centromere). (*c*) FFA profiles and TCRγ sequence of a trisomy 8-positive single cell dissected from the the lymph node (left) and of the entire lymph node sample (right) of patient SS correspond (due to a nested PCR in single cells, clonal fragment lengths differ by 32 base pairs).

#09–13 (Karenko *et al*[2]), #15–17 (Karenko *et al*, 1999, 2001, 2003; Karenko *et al*[2]).

Clonal chromosomal abnormalities These were sought with standard G-banding, comparative genomic hybridization (CGH), and FISH techniques (multi-fluor FISH, MFISH; spectral karyotyping, SKY; centromere-specific techniques). In G-banding, a minimum of 20 blood lymphocyte karyotypes and, if no clone was detected, up to 100 mitoses were examined. A chromosomal clone was defined, according to the International System for Human Cytogenetic Nomenclature (ISCN), as the same numerical aberration observed in at least three cells or the same structural aberration in at least two cells (Mitelman 1995; Karenko *et al*, 1997). In other words, a chromosomal clone was assumed if at least 3% of the lymphocytes studied showed the same numerical aberration or at least 2% an identical structural aberration. For CGH, DNA was extracted from blood lymphocytes and, after micro-dissection of frozen skin sections, from epidermotropic lymphocytes. All DNA copy-number changes were interpreted as clonal (Karenko *et al*, 1999). SKY and MFISH were performed from blood lymphocytes as recommended (SKY kit, Applied Spectral Imaging, Migdal HaEmek, Israel; 24XCyte-kit, Metasystems, Altlussheim, Germany). A clone was defined as above. Centromere-specific FISH was carried out on interphase as well as metaphase chromosomes (Karenko *et al*, 1997, 2001).

TCR rearrangement studies These studies were performed blinded to the results of the chromosomal analyses as described (Lukowsky *et al*, 2002). In brief, TCRγ rearrangements were amplified by two PCR and labelled amplicons were subjected to fluorescence fragment analysis (FFA) on an ABI 310 PRISM CE sequencer (PE Applied Biosystems, Weiterstadt, Germany). TCR clonality was assessed according to peak-height ratios as clonal, oligoclonal, or non-clonal (Fig 1*a*). Clonal fragment lengths were recorded for comparison of blood and skin samples. Lymphocytes trisomic for centromere 8 (this aberration serving as a marker) were detected enzymatically by *in situ* hybridization, micro-dissected from tissue touch preparations (Karenko *et al*, 2001) of patient #15 and #16, forwarded to TCRγ PCR, and subsequently analyzed by FFA and TCRγ sequencing as described (Gellrich *et al*, 2000).

[2]Karenko *et al* (2003), submitted.

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