Variable CD7 Expression on T Cells in the Leukemic Phase of Cutaneous T Cell Lymphoma (Sézary Syndrome)

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CD7, a molecule normally expressed on 90% of normal $CD4^+$ T cells, is often deficient on the malignant T cells of cutaneous T cell lymphoma. To investigate the clinical and biologic implications of CD7 expression, blood lymphocytes from 42 patients with the leukemic phase of cutaneous T cell lymphoma (CD4/CD8 ratio of 10 or more with evidence of a T cell clone in the blood) were analyzed for level of expression of CD7 by flow cytometry. CD7 expression by cells did not clearly segregate into two distinct subgroups that are either CD7 positive or CD7 negative as generally thought; however, nine of 17 patients with a predominantly $CD4^+CD7^+$ tumor population on early studies became $CD4^+CD7^-$ over time whereas the converse situation was not observed. In addition, of three patients with evidence of large tumor cells in the blood coexisting with smaller cells, discordant CD7 expression was observed in one instance. In lymph node specimens, the percentage of cells expressing CD7 and other T cell markers did not correlate with histologic evidence of involvement. CD7 expression on blood lymphocytes also did not correlate with patients' survival nor to serum IgE levels or blood eosinophil counts, a finding suggesting that this marker does not identify functional cell subsets that produce serum interleukin-4 or -5, respectively. We speculate that the level of CD7 expression on malignant T cells may be the effect of sustained antigen stimulation *in vivo* analogous to what has been proposed to occur with normal T cells during aging. ***Key words: CD4/flow cytometry/Th2. J Invest Dermatol 117:654–662, 2001***

Sézary syndrome (SS) is an erythrodermic and leukemic variant of cutaneous T cell lymphoma (CTCL) (Lutzner *et al*, 1975; Diamandidou *et al*, 1996). Traditionally, the diagnosis of SS is based on evidence of increased numbers of atypical lymphocytes with cerebriform nuclei (Sézary cells) in the blood (Winkelmann and Linman, 1973; Flandrin and Brouet, 1974). The malignant T cells of SS have been characterized to be mature "memory" helper T cells [T cell receptor (TCR)-$\alpha\beta^+$, $CD2^+$, $CD3^+$, $CD4^+$, $CD5^+$, $CD8^-$, $CD45RO^+$] that are often deficient in CD7 expression (Haynes *et al*, 1981; Bogen *et al*, 1996; Harmon *et al*, 1996; Bernengo *et al*, 1998), occasionally exhibit decreased expression or loss of one or more pan T cell markers (Harmon *et al*, 1996; Bernengo *et al*, 1998), and are clonal by molecular genetics techniques (Weiss *et al*, 1989; Bakels *et al*, 1991; Whittaker *et al*, 1991; Zelickson *et al*, 1991; Weinberg *et al*, 1995; Russell-Jones and Whittaker, 1999) or by chromosome analysis (Whang-Peng *et al*, 1976; Nowell *et al*, 1982; Johnson *et al*, 1985; Thangavelu *et al*, 1997).

Most patients with SS develop erythroderma and Sézary cytemia shortly after the onset of the disease, but the salient features of SS may occur later in the course of disease following a prodrome of pruritus, nonspecific dermatitis, erythroderma without definite hematologic involvement, or in patients previously diagnosed to have mycosis fungoides (MF) (Clendenning *et al*, 1964; Schein *et al*, 1976; Buechner and Winkelmann, 1983; Bowen *et al*, 1995). In addition, occasional patients with extensive plaque or tumor phase MF have large numbers of Sézary cells in their blood (Flandrin and Brouet, 1974; Schein *et al*, 1976), but such patients are not usually designated as SS because of the absence of erythroderma. In this study, we include all patients with CTCL who manifest the blood findings of SS (defined herein as numerous Sézary cells in the blood, increased numbers of T cells with high CD4/CD8 ratio, and evidence of T cell clonality).

CD7 is a 40 kDa glycoprotein member of the immunoglobulin gene superfamily that is expressed on 85–90% of normal adult peripheral blood T cells (almost all $CD8^+$ T cells and approximately 90% of $CD4^+$ T cells) (Reinhold *et al*, 1993). It also is expressed on most natural killer cells (Rabinowich *et al*, 1994). Within the $CD4^+$ T cell population, CD7 is expressed on almost all $CD45RA^+$ "naive" T cells, and divides the $CD45RA^-$ "memory" T cells into $CD7^+$ and $CD7^-$ subsets (Reinhold *et al*, 1993). Reinhold *et al* (1996; Reinhold and Abken, 1997) have provided evidence that normal $CD4^+CD7^-$ cells are derived from $CD4^+CD7^+$ precursors by differentiation during the late immune response. Functionally, CD7 is thought to be involved in signal transduction (Lazarovits *et al*, 1994; Leta *et al*, 1995) or integrin-mediated adhesion (Shimizu *et al*, 1992). The natural ligand of CD7 is still unknown.

The atypical lymphocytes present in tissue infiltrates and blood of CTCL are frequently deficient in CD7 expression, and this has

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Abbreviations: CTCL, cutaneous T cell lymphoma; SS, Sézary syndrome.

been used for diagnostic purposes (Wood *et al*, 1986; Harmon *et al*, 1996; Bergman *et al*, 1998). The percentage of $CD4^+CD7^-$ cells also correlates well with Sézary cell counts in some series (Vonderheid *et al*, 1994; Laetsch *et al*, 2000). It is unclear whether the increase in $CD4^+CD7^-$ cells in CTCL is the result of an aberrant loss of CD7 expression by malignant T cells or the result of neoplastic expansion of a cell population that is $CD4^+CD7^-$. In support of the latter hypothesis, a small population of $CD4^+CD7^-$ cells with cerebriform nuclei has been identified in the blood of normal individuals (Matutes *et al*, 1983).

Th1 and Th2 lymphocyte subsets based on cytokine secretion profiles were first described in mice by Mosmann *et al* (1986), and subsequently in human T cell clones by Romagnani (1991). $CD4^+$ T helper lymphocytes and other immune cells that produce interleukin (IL)-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-β), and IL-12, preferentially favor development of cellular immune responses; these Th1-like cells are referred to collectively as type 1 cytokine producing cells (Abbas *et al*, 1996; Lucey *et al*, 1996). $CD4^+$ T helper and other cells that produce IL-4, IL-5, IL-6, IL-10, and IL-13, provide help for antibody production; these Th2-like cells are referred to as type 2 cytokine producing cells. In this regard, IL-4 and IL-13 facilitates the isotype switch by B cells to produce IgE, and IL-5 activates eosinophils. In humans, the Th1 and Th2 cell dichotomy is not as apparent as in mice because the majority of T cell clones can produce IFN-γ together with IL-4 or IL-10. These Th0 cells have an intermediate cytokine profile with release of IL-2, IL-4, IL-5, and IFN-γ, and might represent a transient state before Th1 or Th2 cell differentiation (Autran *et al*, 1995).

In most cases of SS, malignant cells on stimulation produce lower IL-2 and IFN-γ and higher IL-4 or IL-5, i.e., a cytokine profile that typifies the Th2 subset of T helper cells (Vowels *et al*, 1992; Borish *et al*, 1993; Saed *et al*, 1994; Tendler *et al*, 1994), and this presumably contributes to the immunodeficiency, hypereosinophilia (via IL-5), and elevated serum IgE levels (via IL-4 and IL-13) that often occurs in SS (Rook and Heald, 1995). In one case of SS, however, in which the tumor cell immunophenotype was $CD4^+CD7^+$, the cells produced the Th1 cytokines IL-2 and IFN-γ, but not IL-4 (Yagi *et al*, 1996). It was suggested that cases of SS with the $CD4^+CD7^+$ phenotype may be examples of the Th1 subset.

In this study, we present our experience with SS and related leukemic expressions of CTCL in terms of the clinical importance of CD7 expression on circulating malignant T cells.

MATERIALS AND METHODS

Single color flow immunophenotyping of peripheral blood lymphocytes was performed using a panel of murine monoclonal antibodies purchased from Becton-Dickinson (San Jose, CA) and Ortho Diagnostics (Raritan, NJ): T cell markers CD2 (anti-Leu5), CD3 (anti-Leu4), CD4 (anti-Leu3), CD5 (anti-Leu1), CD7 (anti-Leu9), CD8 (anti-Leu2); B cell markers CD19 (anti-Leu12), CD20 (anti-Leu16), anti-κ, anti-λ; activation markers HLA-DR, CD25 (anti-IL2Rα), CD71 (anti-transferrin receptor), CD38 (anti-OKT10); and natural killer cell-associated markers CD16 (anti-Leu11), CD56 (anti-Leu19), and CD57 (anti-Leu7). For data analysis, the percentage of $CD16^+$ cells was used to signify the percentage of natural killer cells in samples. Quantitative Sézary cell counts (number of Sézary cells per 100 lymphocytes on blood smear), chromosome analysis, Southern blot and polymerase chain reaction/denaturing gradient gel electrophoresis molecular studies were performed using methods that were previously reported (Nowell *et al*, 1982; Vonderheid *et al*, 1985; Weinberg *et al*, 1995). Other laboratory tests were performed using standard procedures.

Patients Forty-two patients with CTCL and evidence of blood involvement were studied (**Table I**). Patients were selected on the basis of immunophenotypic evidence of an expanded $CD4^+$ T cell population with high CD4/CD8 ratio (≥ 10) on at least one study, blood findings recently advocated to be a useful diagnostic criterion for SS (Willemze *et al*, 1997); however, for patient nos 13 and 41, the tumor cell phenotype was $CD2^+CD3^+CD4^-CD8^-$ (the cells of patient 41 also reacted with antibody directed against the αβ TCR complex and not γδ TCR determinants, indicating that these cells were not derived from TCR-$\gamma\delta^+$ T cells). The malignant T cell phenotype of four other patients was $CD2^-CD3^+CD4^+CD8^-$ (nos 4, 9, 32, and 42) and $CD2^+CD3^-CD4^+CD8^-$ in one patient (no. 25). All patients had extensive skin involvement, which was manifested as generalized or nearly generalized erythroderma in 35 patients, disseminated plaques in five patients (nos 5, 13, 15, 27, and 29), diffuse brownish hyperpigmentation with associated sarcoidal granulomatous inflammation in patient 11 (Gregg *et al* 2000), and mild erythema with features of granulomatous slack skin disease in one patient (no. 20). Of the 35 patients diagnosed to have SS, the erythroderma and characteristic blood findings were preceded by lesions more typical of MF in four patients (nos 18, 25, 36, and 39) and, therefore, may be considered to be examples of secondary SS rather than classic SS in which erythroderma begins *de novo*. Lymph node (LN) biopsies were performed on 33 patients; however, flow immunophenotyping was available on only 24 specimens (**Table II**). Two LN specimens were obtained at different times during the course of disease in two patients (nos 9 and 21), and in patient 39, flow studies were performed on a fine needle aspirate. LN were classified from LN1 to LN4 based on the number of atypical lymphoid cells in paracortical zones and the degree of distortion of LN architecture as described by Sausville *et al* (1985). The pathologic diagnosis was dermatopathic lymphadenopathy (LN1–2 rating) in six LN specimens, lymphoma (LN3–4 rating) in 17 specimens, and granulomatous inflammation in the LN from patient 11 with sarcoidal granulomatous features in the skin. In the 14 nodal specimens with evidence of partial or diffuse effacement of architecture by malignant cells (LN4 rating), the morphologic appearance of the malignant cells was a small cerebriform cell predominant in seven LN, mixed small–large cell pattern in four LN, and a large cell predominate pattern in three LN.

Thirty-six of the patients were Caucasian (15 men, 21 women) and six were African-American (two men, four women). The median age at the time of study was 72.5 y (range, 31–90 y), and the median duration from onset of disease to the time of study was 38 mo (range, 4–334 mo). All patients had quantitative Sézary cell counts exceeding 15% of lymphocytes on blood smears (median, 45%; range, 17–92%) with an absolute Sézary count that exceeded 1000 per mm^3 in 26 (62%) patients. Evidence of T cell clonality was shown in the blood by Southern blot, polymerase chain reaction or chromosome analysis in 22 of 27 (81%), 22 of 25 (88%), and 40 of 42 (95%) studied, respectively. The presence of a T cell clone was shown by at least one method in all patients, and by two or more methods in all but three patients (nos 3, 28, and 33). All 39 patients screened for antibody against type I human T lymphotropic virus had a negative test result, including the six African-American patients (testing not performed on patients 8, 29, and 39).

To confirm our results, we also analyzed the expression of CD7 by circulating malignant T cells from patients with SS studied at the University of Turin (Bernengo *et al*, 1998). At this center, the diagnosis of SS is based on the presence of generalized erythrodermic CTCL and Sézary cell counts > 10% of blood leukocytes and absolute counts > 1000 cells per mm^3. For comparison with our patients, 27 patients with a $(CD3^+CD4^+)/(CD3^+CD8^+)$ ratio ≥ 10 were selected for analysis.

Statistical analysis Because some of the data did not have a normal distribution, i.e., failed the normality test of Kolmogorov–Smirnov, the p-values of nonparametric tests are reported herein for most analyses, but in some instances when nonparametric testing showed a marginal level of significance, significant differences among groups could be confirmed with parametric tests using transformed data that had a normal distribution, e.g., log (%CD57) and log (%CD38) instead of raw %CD57 and raw %CD38, respectively. To adjust for the contribution of $CD7^+$ non-$CD4^+$ cells on total CD7 expression, CD7 values were corrected by subtracting the percentage of $CD8^+$ T cells and $CD16^+$ natural killer cells from the percentage of CD7 for each case, and the level of CD7 expression by $CD4^+$ cells was estimated by dividing the corrected CD7 value by CD4. Two patients with $CD4^-$ tumor cells were excluded from this calculation. The validity of this calculation as an estimate of the level of CD7 expression by $CD4^+$ cells was shown by the strong inverse correlation between the calculated CD7 level with the percentage of $CD4^+CD7^-$ cells that were directly measured by two-color immunophenotyping in seven of the more recently studied patients in this series (rho = –0.991, $p < 0.001$) and in 12 patients studied at the University of Turin (rho = –0.732, $p < 0.001$). The correlation between number of $CD7^+$ cells and other continuous laboratory measurements was examined using the Spearman rank order correlation test. Differences in laboratory studies between patients grouped according to relatively low (< 50%) and high (≥ 50%) CD7 expression on $CD4^+$ lymphocytes were tested using

Table I. Concurrent laboratory data in patients with the leukemic phase of CTCL[a]

PT	A/R/S	DX	DUR[b]	Stage (TN rating)	WBC	LY	EO	SZ	CD3	CD4	CD8	CD7	IgE	LDH	LS[c]
1	87/W/F	SS	24	III (T4N1)	84.6	70	4[f]	64	92	73	2.0	6	—	607	D
2	59/B/M	SS	36	III (T4N1)	13.5	54	5[f]	44	82	88	1.0	6	527	327	D
3	88/W/F	SS	10	IVa (T4N3)	33.1	56	1[f]	30	91	89	5.0	7	2	386	D
4	70/W/F	SS	27	IVa (T4N3)	8.2	44	4[f]	26	92	87	8.0	8	131	296	A
5	74/B/F	MF	11	IVa (T2N3)	8.6	42	2	43	92	89	4.0	9	489	389	D
6	60/W/F	SS	40	III (T4N0)	11.0	15	1	63	70	67	6.0	14	85	297	D
7	64/W/F	SS	26	IVa (T4N3)	4.7	20	6[f]	28	94	90	4.0	14	8	292	D
8	84/W/F	SS	239	IVa (T4N3)	14.7	43	0	74	95	92	3.0	14	312	246	D
9	62/W/M	SS	7	III (T4N1)	5.2	41	2	50	89	89	4.0	16	112	203	D
10	68/W/M	SS	53	III (T4N0)	6.2	27	1	71	90	88	3.0	17	587	184	D
11	69/W/M	SS[e]	15	IVa (T4N3)	15.2	65	2[f]	50	90	96	1.0	20	27	201	D
12	44/W/F	SS	127	III (T4N1)	5.9	44	2	70	92	87	6.0	20	235	235	A
13	73/W/F	MF	10	Ib (T2N0)	10.9	30	5[f]	19	92	10	7.0	22	2	198	D
14	52/W/M	SS	92	IVa (T4N3)	107.6	95	1[f]	91	90	99	0.5	27	5	680	D
15	83/W/M	MF	29	IVa (T2N3)	11.1	77	0	92	95	96	2.0	28	4	265	D
16	31/W/F	SS	89	IVa (T4N3)	7.0	11	1	50	88	82	8.0	32	765	204	A
17	56/W/F	SS	54	III (T4N1)	10.1	18	2	35	79	68	5.0	36	5	200	D
18	59/W/M	SS[e]	83	IVa (T4N3)	7.0	32	1	35	73	76	4.0	55	5	335	D
19	83/W/F	SS	9	III (T4N1)	10.7	8	0	37	88	83	6.0	44	9	307	A
20	72/W/M	GSS[d,e]	334	IVa (T2N3)	10.2	11	49[f]	46	89	84	4.0	47	—	291	D
21	65/W/F	SS	25	III (T4N1)	10.2	37	3[f]	45	75	76	5.0	48	1347	427	D
22	75/W/M	SS	31	III (T4N1)	16.7	52	3[f]	72	82	84	1.0	52	242	308	D
23	90/W/F	SS	58	IVa (T4N3)	8.2	26	6[f]	38	78	74	5.0	55	208	211	D
24	79/W/M	SS	30	III (T4N1)	8.5	45	2	58	90	82	3.0	56	227	183	D
25	81/W/F	SS[e]	4	III (T4N1)	8.0	14	0	17	13	85	5.0	58	1	277	D
26	70/W/F	SS	34	III (T4N0)	32.9	88	0	43	92	93	2.0	60	1	650	D
27	72/W/F	MF	42	IVa (T2N3)	16.0	32	1	33	92	90	4.0	63	197	168	D
28	74/B/F	SS	67	III (T4N1)	12.2	22	7[f]	19	67	63	6.0	65	39	314	A
29	86/W/M	MF	50	IVa (T2N3)	8.4	20	1	41	71	66	6.0	67	3	287	D
30	60/B/M	SS	88	IVa (T4N3)	35.0	82	4[f]	43	83	84	1.0	68	113	272	D
31	73/W/F	SS	117	III (T4N0)	15.3	29	4[f]	34	88	83	8.0	71	5244	184	A
32	80/W/F	SS	56	III (T4N0)	9.3	38	2	41	83	79	8.0	72	8	289	D
33	80/W/M	SS	10	IVa (T4N3)	10.4	57	1	85	82	88	1.0	77	13	602	D
34	49/W/M	SS	45	III (T4N1)	4.4	31	1	61	88	81	8.0	81	29	338	A
35	60/B/F	SS	97	IVa (T4N3)	5.9	50	3	27	83	76	7.0	83	153	195	D
36	54/B/F	SS[e]	128	IVa (T4N3)	4.7	22	4	21	67	55	0.5	85	42323	258	D
37	79/W/F	SS	9	IVa (T4N3)	7.4	24	0	57	89	80	8.0	86	23	363	D
38	66/W/M	SS	207	IVa (T4N3)	4.9	22	1	57	89	85	5.0	87	175	225	D
39	78/W/M	SS[e]	36	IVa (T4N3)	26.7	73	2[f]	53	98	92	6.0	87	—	—	D
40	73/W/F	SS	39	III (T4N0)	9.6	39	1	48	85	82	8.0	90	38	214	A
41	79/W/M	SS	12	III (T4N1)	22.6	60	1[f]	29	95	7	3.0	97	293	212	D
42	84/W/F	SS	19	IVa (T4N3)	26.6	69	2	92	96	97	1.0	99	1	276	A

[a]Data correspond to time that CD4/CD8 ratio was 10 or more on flow immunophenotyping of blood lymphocytes. Patients ranked according to percentage of CD7 expression by lymphocytes. Normal ranges for reference laboratory: White cell count (WBC) = 4.8–10.8 K per μL; Lymphocyte (LY) and eosinophil (EO) count on WBC differential count = 19–48% and 0–7%, respectively; Percentage of normal lymphocytes expressing CD3, CD4, and CD8 = 68–82%, 35–55%, and 19–37%, respectively; IgE = 14–122 U per liter; LDH = 0–220 U per liter; A/R/S, age, race, and sex of patient; SS, Sézary syndrome; MF, MF with Sézary cytemia; GSS, Sézary syndrome with features of granulomatous slack skin disease; DUR, duration of disease; NR, PR, or CR, no response, partial response, or complete response; LS, last status; A, alive, D, deceased; LDH, lactic dehydrogenase.

[b]Months from onset of disease manifestations to flow study.

[c]Status as of August 2000.

[d]Sézary syndrome with sarcoidal tissue reaction.

[e]Sézary syndrome preceded by MF.

[f]Absolute eosinophil count greater than 250 mm^3.

the nonparametric Mann–Whitney test, but also the parametric Student's T-test when appropriate. The Fisher's exact test and Pearson χ^2-square test for 2×2 tables and $R \times C$ tables, respectively, were used as measures of association among categorized clinical and laboratory parameters. Kaplan–Meier and the Cox proportional hazards regression model were used to test for variables that have prognostic significance. Log-rank and Gehan tests were used to detect survival differences between groups. Statistical software used for data analysis were SYSTAT for Windows, Version 8 (SYSTAT, Evanston, IL), SigmaStat for Windows, Version 2 (Jandel Scientific Software, San Rafael, CA), and EGRET (Statistics and Epidemiology Research, Seattle, WA).

RESULTS

The percentage of $CD4^+$ cells in our patients with leukemic phase CTCL was increased above the normal range for single color flow immunophenotyping for our laboratory (i.e., 35% to 55%) in all patients excluding two patients with a $CD3^+CD4^-CD8^-$ tumor cell phenotype. The mean percentage of $CD4^+$ cells for the $CD4^+$ cases was 83.0% ± 9.7% (mean ± SD; range, 55–99%), and the mean percentage of $CD8^+$ cells for all cases was correspondingly low (4.4% ± 2.5%, mean ± SD). Of interest, the percentage of $CD3^+$ cells was increased above the normal laboratory range (i.e., 68–82%) in only 30 of 41 (73%) patients, excluding one patient with an aberrant loss of CD3 (mean %CD3 ± SD, 86.2% ± 8.1%; range, 67–98%). This suggests that as the proportion of malignant $CD4^+$ cells increases in the blood of SS, normal $CD4^+$ and $CD8^+$ cells decrease in tandem before the total $CD3^+$ cell population increases. The percentage of natural killer cells in these patients as assessed by CD16 and CD57 was also low (mean %CD16 ± SD, 4.6 ± 3.9

Table II. Comparison of flow immunotyping of blood and LN from patients with the leukemic phase of CTCL

					Percentage of lymphocytes					
PT	Tissue	Date	DX[a]	PCR	CD2	CD3	CD4	CD8	CD7	CD16
2	Blood	3/21/90	13.5/54/44	+	90	82	88	1	6	3
	LN	3/30/90	LN3	+	67	57	59	4	23	1
3	Blood	2/5/91	15.2/43/46	+	95	92	80	15	24	5
	LN	2/7/91	LN4 (M)	+	63	74	71	5	9	5
4	Blood	5/15/96	8.2/44/26	+	45	92	87	8	8	2
	LN	5/23/96	LN4 (S)	+	42	51	65	12	11	0
5	Blood	3/9/95	8.6/42/43	+	79	92	89	4	9	4
	LN	3/17/95	LN4 (L)	ND	33	48	66	5	ND	5
9	Blood	4/23/91	5.2/41/50	–	22	89	89	4	16	3
	LN	4/24/91	LN4 (S)	–	22	61	57	6	23	2
	Blood	11/18/94	15.0/53/32	+	16	96	96	2	2	0
	LN	11/30/94	LN4 (M)	+	18	30	27	18	4	1
10	Blood	5/16/95	5.3/16/12	+	81	70	24	49	45	17
	LN	5/22/95	LN4 (L)	ND	78	85	81	59	5	7
11	Blood	11/24/93	15.2/65/50	+	98	90	96	1	20	0
	LN	12/16/93	GI[b]	+	69	68	66	4	46	3
16	Blood	8/23/96	6.3/50/83	+	ND	95	93	2	9	ND
	LN	8/23/96	LN4 (M)	+	43	24	15	4	14	1
18	Blood	9/6/90	7.0/32/35	+	76	73	76	4	55	6
	LN	9/7/90	LN4 (S)	+	62	68	65	2	55	0
19	Blood	5/28/96	6.6/16/39	+	54	70	68	7	60	10
	LN	4/30/96	LN1	+	56	48	43	5	40	2
20	Blood	4/6/92	14.3/12/35	+	84	38	59	12	55	22
	LN	4/14/92	LN4 (S)	+	91	74	87	8	53	1
21	Blood	2/4/92	10.2/37/45	+	79	75	76	5	48	3
	LN	1/7/92	LN2	+	48	45	46	3	45	0
	Blood	7/25/95	12.7/51/34	+	ND	88	87	1	40	5
	LN	7/7/95	LN2	+	40	43	32	4	37	0
23	Blood	2/11/97	7.2/19/32	+	78	66	60	10	47	ND
	LN	4/9/97	LN1	+	69	68	69	4	27	ND
27	Blood	5/8/90	16.0/32/33	+	93	92	90	4	63	2
	LN	5/30/90	LN4 (S)	+	73	78	83	3	65	0
28	Blood	5/31/95	12.2/22/19	–	77	67	63	6	65	8
	LN	6/27/95	LN2	–	49	74	69	2	28	1
29	Blood	2/17/98	5.2/36/16	+	70	46	42	8	83	44
	LN	2/19/98	LN4 (L)	–	92	90	93	2	37	5
33	Blood	5/30/95	10.4/57/85	–	83	82	88	1	77	2
	LN	6/21/95	LN4 (S)	ND	63	67	88	2	59	0
37	Blood	7/22/98	8.7/33/nd	+	92	88	82	1	88	ND
	LN	7/24/98	LN4 (S)	ND	ND	70	74	1	70	ND
38	Blood	9/20/93	4.9/22/57	+	62	89	85	5	87	4
	LN	9/21/93	LN4 (M)	ND	58	61	63	4	53	2
39	Blood	7/2/96	26.7/73/53	+	ND	98	92	6	87	ND
	LN	7/3/96	c/w MF[c]	–	ND	93	93	2	65	ND
41	Blood	7/10/98	22.6/60/29	+	96	95	7	3	97	2
	LN	8/7/98	LN1	+	82	82	31	9	42	1
42	Blood	6/5/97	26.6/69/92	+	20	96	97	1	99	1
	LN	7/3/97	LN3	+	32	92	87	2	93	0

+, positive; –, negative.

[a]DX refers to routine diagnostic data obtained on tissue samples: For blood, the numbers are the total leukocyte count, percentage of lymphocytes on the differential count, and the number of Sézary cells per 100 lymphocytes; for LN, the LN rating is based on the classification scheme of Sausville *et al* (1985), and the cytologic appearance of malignant infiltrate in effaced (LN4) nodes is shown in parenthesis where S, M, and L refer to small cell, mixed cell, and large cell patterns as previously described (Vonderheid *et al*, 1992).

[b]Sarcoidal granulomatous inflammation.

[c]Study on LN based on fine needle aspiration.

and mean %CD57 ± SD, 7.4 ± 10.1) and below the normal laboratory range in 14 of 23 (61%) and 20 of 23 (87%) cases, respectively. The percentage of blood lymphocytes expressing CD7 in our cases ranged widely from 6% to 99% (median, 53.5%; mean ± SD, 48.8% ± 29.6, **Table I**). A plot of CD7 expression against the expected normal distribution did not demonstrate a significant deviation (**Fig 1**); however, the distribution of CD7 expression did deviate from a normal distribution if the analysis was restricted to 32 patients with classic SS, i.e., exclusion of patients without generalized erythroderma or patients with secondary SS that developed in patients with MF (Kolmogorov–Smirnov normality test, $p = 0.04$). Nevertheless, a bimodal distribution of cells that were either $CD7^-$ or $CD7^+$ was not apparent for either circumstance. When correlated directly against other clinical or laboratory parameters using the Spearman correlation test, the number of $CD7^+$ cells correlated weakly to the number of $CD16^+$ cells (39 cases, rho = +0.361, $p = 0.02$), $CD57^+$ cells (31 cases, rho = +0.402, $p = 0.03$), and $CD38^+$ cells (31 cases, rho = +0.448, $p = 0.01$).

Because CD7 is also expressed on essentially all normal $CD8^+$ T cells and natural killer cells, and because a significant positive association was found between the number of $CD7^+$ cells and the number of $CD16^+$ and $CD57^+$ cells that are markers expressed on natural killer cells, we investigated the extent to which these non-

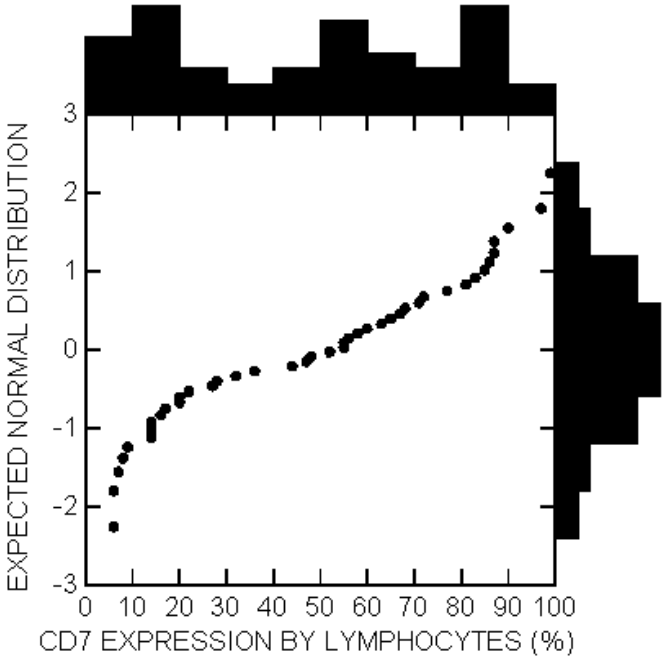

Figure 1. Distribution of CD7 expression in leukemic phase CTCL studied at MCP Hahnemann University. The percentage of CD7 expression by blood lymphocytes of 42 patients with leukemic phase of CTCL does not deviate significantly from the expected value of a normal distribution (Kolmogorov–Smirnov distribution, 0.127; p = 0.09).

$CD4^+$ cells may have contributed to the observed CD7 results. When raw CD7 values were corrected by subtracting the percentage of $CD8^+$ and $CD16^+$ cells from the percentage of CD7 for each case, excluding the two patients that were $CD4^-$, the correlation between raw CD7 and corrected CD7 values remained very strong (n = 37, rho = 0.985, $p < 0.001$). Of interest, the corrected CD7 values continued to correlate positively to the percentage of $CD16^+$ (n = 37; rho = + 0.437, p = 0.007), $CD38^+$ (n = 30; rho = 0.479, p = 0.008), and $CD57^+$ (n = 30; rho = 0.405, p = 0.03) cells.

The patient population was also divided into two cohorts based on the estimated level of CD7 expression by $CD4^+$ cells: a group of 19 patients with relatively low CD7 expression defined as calculated CD7 expression on less than 50% of $CD4^+$ cells, and a group of 23 patients with more than 50% CD7 expression (**Table III**). The four patients whose SS was preceded by typical MF (patients 18, 25, 36, and 39) had leukemic cells that expressed CD7. Comparison of various clinical and laboratory studies between these two groups revealed that patients with lower CD7 levels had significantly higher percentages of $CD4^+$ and $CD5^+$ cells, and lower $CD16^+$ and $CD38^+$ cells. Because studies showing an increased production of IL-4 and IL-5 by malignant T cells in SS *in vitro* were performed on cells with a $CD4^+CD7^-$ phenotype (Vowels *et al*, 1992; Borish *et al*, 1993; Saed *et al*, 1994; Tendler *et al*, 1994; Dummer *et al*, 1996) and one case with a $CD4^+CD7^+$ phenotype had a Th1 cytokine profile (Yagi *et al*, 1996), we wondered whether serum IgE level (via IL-4) and eosinophil count (via IL-5) would correlate to the level of CD7 expression on $CD4^+$ cells in our patients. In our series, serum IgE and absolute eosinophil counts were increased over reference laboratory values in 44% and 40% of studied patients, respectively. No significant difference in median serum IgE level or eosinophil counts, however, was found between patients grouped according to level of CD7 expression (**Table III**). Moreover, no correlation was found between the calculated CD7 expression on $CD4^+$ cells and either serum IgE (rho = –0.028, $p > 0.8$) or absolute eosinophil count (rho = –0.092, $p > 0.5$).

Table III. Comparison of laboratory results on patients with the leukemic phase of CTCL. According to estimated level of CD7 expression by $CD4^+$ lymphocytes

	Expression of CD7 by $CD4^+$ cells[a]		
Marker/test[b]	Low (n = 19)	High (n = 23)	p-value[c]
Duration (mo)	48.9 ± 56.4	69.1 ± 74.6	0.193
Lymphocyte count (K per μL)	12.8 ± 25.4	6.9 ± 8.6	0.879
Eosinophil count (per μL)	435 ± 765	491 ± 1037	0.820
Sézary count (%)	53.1 ± 21.6	44.5 ± 18.7	0.172
CD2 (%)	79.2 ± 21.0	78.0 ± 20.6	0.600
CD3 (%)[d]	88.6 ± 6.3	84.2 ± 9.1	0.088
CD4 (%)[e]	85.9 ± 8.9	80.5 ± 9.9	0.045
CD5 (%)	89.9 ± 5.9	83.3 ± 11.8	0.033
HLA-DR (%)	11.8 ± 9.2	20.2 ± 19.2	0.212
CD25 (%)	2.4 ± 3.6	6.8 ± 9.3	0.058
CD16 (%)	2.9 ± 3.3	6.0 ± 4.0	0.007
CD38 (%)	9.1 ± 6.7	19.3 ± 15.7	0.020
CD71 (%)	7.6 ± 6.9	10.7 ± 15.8	0.984
CD57 (%)	6.9 ± 13.1	7.8 ± 6.0	0.084
IgE (U per liter)	197 ± 242	2402 ± 9219	0.833
LDH (U per liter)	307 ± 134	299 ± 124	0.784

[a]The level of CD7 expression within the $CD4^+$ cell population is calculated from one-color flow immunophenotyping as: (%CD7 – %CD8 – %CD16) ÷ %CD4 ×100. Low and high level of CD7 expression is defined as < 50% CD7 (n = 19) and > 50% CD7 (n = 23) expression by $CD4^+$ cells, respectively.
[b]Values expressed as mean ± 1 SD.
[c]Difference tested by Mann–Whitney test.
[d]One patient with $CD3^-$ phenotype excluded.
[e]Two patients with $CD4^-$ phenotype excluded.

Similar results were found with Sézary patients studied at the University of Turin except that the distribution of CD7 percentages among 27 samples did vary significantly from an expected normal distribution (**Fig 2**). No difference from a normal distribution was found, however, if the values were transformed to log(CD7) ($p > 0.2$). The percentage of lymphocytes expressing CD7 ranged from 2% to 93% (median, 23.0%; mean ± SD, 33.8% ± 32.1%). When compared with American patients, the Italian patients with SS had a significantly higher percentage of $CD3^+$ and $CD4^+$ cells, and a lower number of $CD7^+$ cells (Mann–Whitney test, $p < 0.001$). The difference in the CD4/CD8 ratio and the percentage of $CD8^+$ and natural killer cells between the two series was not significant. Consequently, the estimated level of CD7 expression by $CD4^+$ cells was markedly lower for Italian patients than for our patients (Mann–Whitney test, $p < 0.001$). Indeed, low CD7 expression on less than 50% of the $CD4^+$ cells was observed in 20 of 27 (74%) patients in the Italian series *vs* 18 of 42 (43%) of our series (Fisher's exact test, $p < 0.001$). These findings suggest that the patients studied at the University of Turin may have had more advanced disease than our patients. The fact that only 62% of our cases had an absolute Sézary cell count ⩾ 1000 supports this interpretation. These differences, however, remained significant even if the analysis was restricted to the 26 patients with absolute Sézary cell counts ⩾ 1000 cells per mm^3 in our series. The reason for the observed difference in CD3, CD4, and CD7 percentages between the two patient populations is unclear, but may reflect differences in diagnostic criteria and patient selection at each center.

CD7 in LN Flow immunophenotyping was obtained on 24 LN samples from 22 patients (**Table II**). Histologic evidence of LN involvement (LN3 or LN4 rating) was found in 14 (58%) of the specimens; however, no significant difference was observed between histologically involved *vs* uninvolved specimens in terms of the percentage of various antibody-positive cells, including

$CD7^+$ cells. This result indicates that quantitation of T cell subsets may not be a reliable way to determine the degree of histologic involvement in LN. Moreover, comparison with blood samples obtained at or near the time of the LN sampling indicated that a significantly strong correlation exists between the percentage of $CD2^+$ cells (rho = 0.68) and $CD7^+$ cells (rho = 0.77), but not for $CD3^+$ and $CD4^+$ cells. Also a strong correlation was found between the estimated level of CD7 expression on $CD4^+$ cells (rho = 0.66). This finding suggests that the distribution of malignant T cells in peripheral LN and the blood in SS is comparable.

Serial observations Multiple flow studies that were obtained at least 6 mo apart were available on 30 patients. In 20 patients the CD4/CD8 ratio was greater than 10 on the initial flow study, and in the remaining 11 patients the CD4/CD8 ratio was subsequently recorded as 10 or more 6–30 mo after the initial study. For patient 3, high numbers of $CD8^+$ cells on the initial flow study masked the tumor phenotype, but the $CD4^+CD7^-$ phenotype became apparent and the CD4/CD8 ratio exceeded 10 on later blood studies with progression of disease and also by immunophenotyping on an involved LN obtained as part of the initial staging evaluation (**Table IV**). In other patients with high CD4/CD8 ratios, the number of $CD8^+$ cells generally remained less than 10% throughout the clinical course of disease, but in five patients, the numbers consistently increased to above 15%; this contributed to an increase in CD7 percentage making the assessment of changes in CD7 expression within the $CD4^+$ population more difficult. For this reason calculation of the level of CD7 expression on $CD4^+$ was found to be useful to estimate the percentage of $CD4^+CD7^+$ cells when dealing with single parameter results.

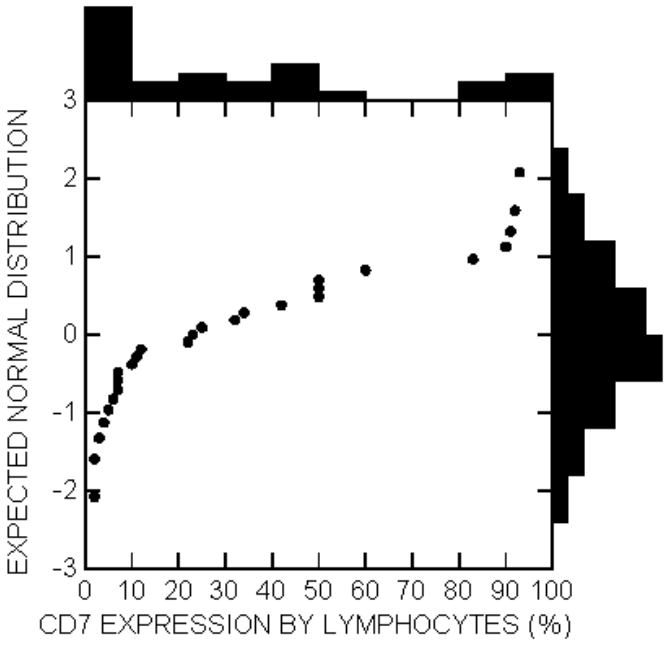

Figure 2. Distribution of CD7 expression in leukemic phase CTCL studied at the University of Turin. Compared with the results shown in Fig 1, the distribution of CD7 expression by blood lymphocytes of 27 patients with Sézary syndrome with CD4/CD8 ratio = 10 who were studied at the University of Turin shows a greater proportion of cases with low CD7 expression (Kolmogorov–Smirnov distribution, 0.127; p = 0.009).

The calculated level of CD7 expression by $CD4^+$ cells changed from $CD7^+$ to $CD7^-$ in some patients over time (**Table IV**). Of 17 patients with evidence of CD7 expression on more than 50% of $CD4^+$ cells initially, subsequent studies showed an eventual predominance of $CD4^+CD7^-$ cells in nine patients, often associated with clinical evidence of disease progression. The remaining eight patients retained the $CD7^+$ phenotype. Conversely, none of the 13 patients with CD7 on less than 50% of $CD4^+$ cells on initial studies developed a $CD4^+CD7^+$ cell phenotype. No significant difference in duration of follow up was found among these three subgroups of patients.

In patients 29, 34, and 37, a discrete subset of large $CD4^+$ cells, representing 17%, 19%, and 41% of cells in the lymphocyte gate, respectively, were detected on the basis of forward scatter (**Fig 3**). For two patients (nos 34 and 37), the phenotype of these cells was the same as the smaller cells (both $CD7^+$), whereas for patient 29 the large cell component was $CD4^+CD7^-$ and the small cell component was $CD4^+CD7^+$. In one other patient (no. 4), a small subpopulation of $CD4^+CD8^+CD7^-$ cells was detected in one study, but then disappeared following a course of chemotherapy.

With regard to survival, the clinical and laboratory parameters that correlated significantly to survival on univariate analysis were T and N ratings ($p = 0.002$ and $p = 0.019$, respectively), age ($p = 0.017$), total leukocyte count ($p < 0.001$), percentage and absolute lymphocyte count ($p = 0.041$ and $p < 0.001$, respectively), absolute eosinophil and Sézary cell counts ($p = 0.034$ and $p = 0.002$), but not their percentages, and serum lactic dehydrogenase ($p = 0.029$). None of the lymphocyte markers, including CD7 expression ($p > 0.7$) correlated significantly with survival in this subset of patients.

DISCUSSION

The patients in this series were selected to meet recently recommended flow immunophenotypic criteria for blood involvement in SS, namely an increase in $CD4^+$ cells with a CD4/CD8 ratio of 10 or more with evidence of a T cell clone in the blood

Table IV. Selected examples of serial single color immunophenotyping in two patients with Sézary syndrome[a]

PT	Date	Lymphocyte count (per mm^3)	Percentage of lymphocytes						Level CD7 by CD4 (%)[b]
			Sézary	CD3	CD4	CD8	CD7	CD16	
3	11/12/90	1288	20	86	38	57	73	7	17
	2/5/91	6536	46	92	80	15	24	5	10
	6/26/91	18536	30	91	89	5	7	4	2
21	7/21/92	1350	47	67	73	3	63	ND	79
	6/23/93	810	33	59	57	3	50	4	78
	6/1/94	1026	33	66	60	6	56	10	76
	6/25/96	4840	49	71	61	2	19	1	27
	5/26/98	5880	48	97	98	1	2	2	10

[a]Patient 3 initially had high number of $CD8^+$ cells that masked a $CD4^+CD7^-$ tumor population that became more evident with disease progression; patient 21 showed change from $CD4^+CD7^+$ predominant phenotype to a $CD4^+CD7^-$ phenotype.

[b]Level of CD7 expression by $CD4^+$ cells calculated as: (%CD7 – %CD8 – %CD16) ÷ %CD4 ×100.

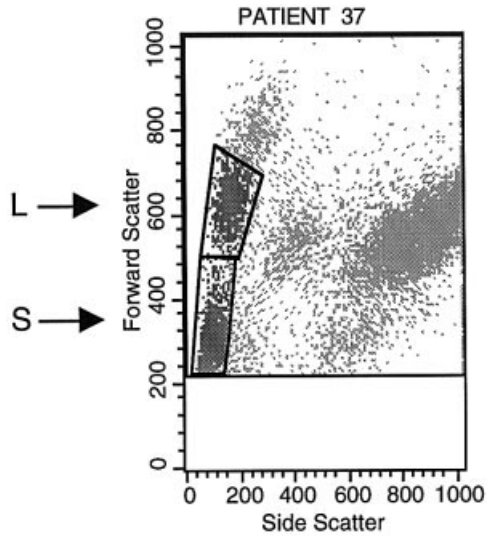

Figure 3. Dot plot shows two lymphocyte populations in Sézary syndrome. Two populations of malignant lymphocytes are identified on the flow scattergram for patient 37 (small cells in the box designated by the S arrow, large cells in the box designated by the L arrow). The large cell component represents 40.6% of all lymphocytes.

(Willemze *et al*, 1997). Our observations indicate that CD7 expression on malignant T cells varies widely among cases of leukemic CTCL and do not support the prevailing concept that SS cases occur as two distinct subgroups, i.e., $CD4^+CD7^+$ or $CD4^+CD7^-$, that arise as the consequence of an expanded subset of $CD4^+$ cells.

Haynes *et al* (1981) using the antibody 3A1, were first to report that malignant T cells of SS were often CD7 negative; however, since then a $CD4^+CD7^+$ phenotype has been reported in approximately one-third of cases of SS (Bogen *et al*, 1996; Bernengo *et al*, 1998). With the availability of two-color immunophenotyping, Harmon *et al* (1996) found that the percentage of $CD4^+CD7^-$ cells ranged widely (9–92%) for 18 patients with SS defined as Sézary cell count of more than 1000 per mm^3. In the series reported by Jakob *et al* (1996), the percentage of $CD4^+CD7^-$ cells ranged from 26% to 96% ($70.9 \pm 26.9\%$, mean $\pm$ SD) for nine patients with CTCL at stage III/IV (all with CD4/CD8 ratio > 10). Of three patients with a CD4/CD8 ratio more than 10 studied by Bogen *et al* (1996), the percentage of $CD4^+CD7^-$ cells was 70%, 90%, and 95%. Dummer *et al* (1996) found the percentage of $CD4^+CD7^-$ cells ranged from 30% to 65% (mean, 51%) in eight patients with SS defined by an absolute Sézary cell count of more than 1000 per mm^3 and an elevated CD4/CD8 ratio. Scala *et al* (1999) used three-color immunophenotyping to study six patients with SS, all with absolute Sézary cell counts exceeding 2000 per mm^3. The mean percentage of $CD3^+CD4^+CD7^-$ cells was 88.7% with a large SD of 23.6%. Finally, Rappl *et al* (2001) found that CD7 was uniformly lost on more than 98% of $V\beta^+$ cells in seven patients with SS defined on the basis of Sézary cell counts exceeding 1000 per mm^3. These observations and our findings indicate that the level of CD7 expression by malignant T cells varies greatly among cases of SS defined with other criteria.

Several explanations may account for the CD7 variability observed among patients with SS. The first possibility is that the expanded $CD4^+$ T cell population in SS consists of a heterogeneous mixture of normal and malignant cells, and that the apparent level of CD7 expression in a given patient reflects the relative numbers of these cells. According to this hypothesis, if malignant T cells in a particular case are uniformly CD7 deficient, then a relatively high level of CD7 expression in the blood can occur only when normal $CD4^+CD7^+$ cells are markedly increased relative to the malignant cell population. In this regard, patient 29, who had 66% $CD4^+$ and 67% $CD7^+$ cells overall (calculated level of CD7 on $CD4^+$ cells was 85%), had evidence of a small population (17% of the total lymphocytes) of abnormal large $CD4^+CD7^-$ lymphocytes by forward scatter. This observation indicates that $CD4^+CD7^-$ malignant cells potentially may be masked by large numbers of nonmalignant $CD4^+CD7^+$ cells. In this regard, it is worth noting that patient 3 is an example of a patient with SS in which the malignant $CD4^+CD7^-$ cells in the blood were initially masked by large numbers of reactive $CD8^+$ cells. Conversely, if malignant T cells uniformly express CD7, then a low level of CD7 overall can occur only when relatively high numbers of normal $CD4^+CD7^-$ cells are present in the blood. Lacking a specific marker of tumor cells such as anti-idiotypic antibody (Bigler *et al*, 1983), we can provide no direct evidence by flow cytometry to indicate that such a circumstance may occur. The quantitative Sézary cell count, which provides an inexact measure of blood tumor burden, correlated poorly with both raw (rho = – 0.064) and corrected (rho = – 0.101) CD7 percentages even though it did correlate significantly with the percentage of $CD4^+$ cells (rho = 0.36, $p = 0.023$). If malignant $CD4^+$ T cells were expressing CD7 uniformly among our patients, then a significant correlation between Sézary cell counts and CD7 percentages as well might be expected. The lack of a significant correlation supports the interpretation that CD7 is not expressed uniformly on malignant cells.

An alternative explanation to account for CD7 variability among Sézary patients is that CD7 expression by individual malignant T cells is not uniform, and that the overall level of CD7 expression in a particular patient reflects the relative numbers of tumor cells that may be $CD7^+$ or $CD7^-$. There is some support for this interpretation. First, Ginaldi *et al* (1996) performed quantitative measurements on CD7 molecule expression in five patients with SS. In two patients CD7 was undetectable, and in the other three cases that expressed CD7, the mean number of CD7 molecules per cell ranged widely. Indeed, the mean number of CD7 molecules per cell in SS was lower than the range for normal T cells in one case and above the range in one case. Secondly, for cases of SS reported in the literature in which both a Vβ antibody and CD7 were used to characterize malignant cells, the level of CD7 expression on $V\beta^+$ cells varied in some cases. An excellent correlation between observed Vβ and CD7 reactivity was found in 10 $CD7^-$ cases reported by Bogen *et al* (1996) and Rappl *et al* (2001), and the $CD7^+$ case reported by Yagi *et al* (1996); however, Dummer *et al* (1999) reported that CD7 was expressed in at least 35% of $V\beta^+$ cells from three patients with SS who had high percentages of $CD4^+CD7^-$ in the blood. Thus, CD7 expression seems to vary on malignant T cells in at least in some instances.

What biologic phenomenon underlies the apparent variable expression of CD7 by malignant T cells in SS and other leukemic forms of CTCL? One possibility to be considered is that the level of CD7 expression is related to the activation state of the cells. When normal T cells are stimulated *in vitro*, the level of CD7 expression increases in T cells that express CD7 either strongly and weakly whereas neither CD7 nor CD7mRNA is induced in normal $CD4^+CD7^-$ cells (Reinhold *et al*, 1993). In this regard, Kadin *et al* (1984) noted that CD7 expression remained low on $CD7^-$ malignant T cells of two patients with SS that were cultured in the presence of phorbol ester for 5 d. Yagi *et al* (1996), however, observed that a subpopulation of $V\beta17^+CD7^+$ Sézary cells decreased expression of CD7 when stimulated with concanavalin A, but not staphylococcal enterotoxin B. These authors proposed that Sézary cells are continuously stimulated *in vivo* to express high levels of CD7, but an alternative explanation needs to be considered. Reinhold *et al* (1996; Reinhold and Abken, 1997) observed that when normal $CD4^+CD7^+$ T cells are stimulated repeatedly via CD2 or CD3 *in vitro*, the number of $CD7^-$ cells increases, suggesting that the $CD7^+$ to $CD7^-$ conversion occurs in a subset of $CD4^+$ T cells as a differentiation event in the late immune response. It also is of interest that the cerebriform nucleus that

characterizes Sézary cells can be induced in normal lymphocytes via stimulation of the TCR/CD3 complex and to a lesser extent CD2 (Reinhold *et al*, 1994).

Normal $CD4^+CD7^-$ cells, which are absent in cord blood, increase progressively during life (Kukel *et al*, 1994). The increase in $CD4^+CD7^-$ cells occurs with an increase in the proportion of $CD45RO^+RA^-$ "memory" T cells that also express activation markers ($HLA\text{-}DR^+$, $CD25^+$, and $CD71^+$) and CD57 within the $CD4^+CD7^-$ population. Because malignant T cells typically have the $CD45RO^+$ phenotype, one might expect to find a higher proportion of cells expressing activation markers in patients with relatively low levels of CD7 expression on $CD4^+$ cells. In this regard, Scala *et al* (1999) found a high level of expression of the activation markers CD60 and CD27 on $CD4^+$ cells from six patients with SS. Of interest, three of these patients showed a bimodal distribution of CD3 into $CD3^{bright}$ (normal) and $CD3^{dim}$ (atypical) subsets. The $CD3^{dim}$ cells were $CD7^-CD60^+$, whereas the converse was found for the $CD3^{bright}$ cells. Three other patients had two lymphoid populations based on cell size (similar to three of our patients). The larger cells were $CD7^-CD60^+$ and the smaller cells $CD7^+CD60^-$. These observations show that T cells in the blood of Sézary patients can be quite heterogeneous in terms of both CD7 expression and activation markers.

In our series, we observed somewhat lower levels of activation markers in Sézary patients with low CD7 expression (**Table III**). In fact, a statistically significant positive correlation rather than a negative correlation was actually observed between the calculated level of CD7 expression on $CD4^+$ cells and CD38 and CD57. Furthermore, the possibility that malignant T cells may aberrantly express low levels of natural killer cell markers CD16 (and CD57 as well) was raised by the observed positive association between CD16 and the calculated level of CD7 expression on $CD4^+$ cells, which was derived by subtracting CD8 and CD16 from CD7. Additional three-color flow studies that combine CD4, CD7, and CD16 are required to substantiate this observation.

Based on the above observations, it seems plausible that the level of CD7 expression on malignant T cells (and perhaps the cerebriform nucleus that characterizes Sézary cells) could be the consequence of sustained immunologic stimulation *in vivo* that converts a proportion of malignant T cells from $CD7^+$ to $CD7^-$. In contrast to other peripheral T cell lymphomas and leukemias that retain CD7, the loss of CD7 on the malignant T cells of CTCL could be the consequence of "memory" cells programmed to home into the skin where chronic immunologic stimulation may occur. Consistent with this hypothesis is the observation that most (nine of 17) patients in our series whose tumor cells expressed CD7 on more than 50% of $CD4^+$ cells eventually became $CD7^-$ later in the course of disease. Conversely, none of the patients with a predominance of $CD4^+CD7^-$ cells on initial studies subsequently developed a $CD4^+CD7^+$ cell phenotype. The discrepancy between the $CD4^+CD7^+$ and $CD4^+CD7^-$ phenotype of small and large tumor cells, respectively, in the blood of patient 29 discussed above might also be explained by this mechanism. On the other hand, if chronic antigenic stimulation results in the progressive loss of CD7 on malignant T cells, then a significant negative correlation might be expected between duration of disease and the level of CD7 expression on $CD4^+$ cells, but this was not observed in our patients.

With regard to the immunobiologic implications of CD7 expression by malignant T cells, most studies indicate that the usual cytokine secretion profile in SS consists of low Th1 cytokines (IL-2 and IFN-γ), and increased Th2 cytokines (IL-4, IL-5, and IL-10). Exceptions, however, occur in almost all series (Bigler *et al*, 1994; Saed *et al*, 1994; Tendler *et al*, 1994; Dummer *et al*, 1996). This indicates that malignant $CD4^+$ cells in SS are not functionally homogeneous in terms of cytokine production. Although CD7 expression correlated with the Th1 cytokine profile in the case reported by Yagi *et al* (1996), no consistent difference in cytokine mRNA expression was found between $CD7^+$ and $CD7^-$ subsets in five cases of SS studied by Dummer *et al* (1999).

Our observations did not reveal a significant correlation between the level of CD7 expression on malignant T cells and either eosinophil counts or serum IgE levels. In addition, two other studies found no correlation between IL-4 and serum IgE levels (Vowels *et al*, 1992; Tendler *et al*, 1994). The reason for this lack of association is unclear. In order for B cells to undergo an isotopic switch from IgM to IgE, two signals are required: stimulation of the IL-4 receptor by IL-4 followed by binding of CD40 to its ligand (Bacharier *et al*, 1998). Bigler *et al* (1994) observed that CD40L is not expressed constitutively by circulating Sézary cells, but when stimulated by phorbol myristate acetate-ionomycin but not phytohemagglutinin alone, CD40L is expressed by cells from eight of 10 cases. Moreover, CD40L expression on stimulated Sézary cells of some patients persisted, suggesting a defect in downregulation of CD40L. Patients whose cells could be stimulated to express CD40L were more likely to have elevated serum IgE levels. Finally, Sézary cells could be induced to secrete IL-4 when stimulated with phorbol myristate acetate-ionomycin, but not phytohemagglutinin. These observations suggest that activation of malignant T cells in tissues such as the skin may provide the necessary costimulus of CD40L expression in the presence of increased IL-4 production by Th0/Th2 malignant cells.

In conclusion, our hypothesis is that persistent antigen stimulation *in vivo* leads to loss of CD7 expression on some malignant T cells analogous to what has been proposed to occur normally with aging. In this regard, loss of CD7 is in effect a normal differentiation phenomenon occurring with malignant T cells. Similar mechanisms may account for the observed association between lack of CD7 expression and the number of cells with cerebriform nucleus that characterizes SS.

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