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Dysregulated Synthesis of Intracellular Type 1 and Type 2 Cytokines by T Cells of Patients with Cutaneous T-Cell Lymphoma

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Mycosis fungoides (MF) and Sezary syndrome (SS) are the two main clinical entities of cutaneous T-cell lymphoma (CTCL). As the disease progresses from MF to SS, a switch from a type 1 (interleukin [IL]-2 and gamma interferon [IFN-γ]) to a type 2 (IL-4) cytokine production profile occurs. Although roles for type 1 and type 2 cytokines in the pathogenesis of CTCL have been proposed, the cellular origins of these cytokines are unclear. Using flow cytometry to identify individual T-cell subsets, we studied cytokine synthesis by the T cells of 13 patients with SS and 12 with MF and 9 hematologically healthy donors. Upon activation with phorbol 12-myristate 13-acetate (PMA), the numbers of T cells synthesizing IL-2 were similar for all study groups. Whereas the predominant T-cell producing IL-2 in healthy donors and in those with MF was $CD7^+$, in patients with SS, it was $CD7^-$. Although the number of IL-4^+ $CD4^+$ T cells was low for all study groups, there was a significantly higher number of IL-4^+ $CD8^+$ T cells in patients with MF than in those with SS or healthy donors. There was a decline in the number of IFN-γ-producing T cells in CTCL donors compared to that in healthy donors. More importantly, there was a significant decrease in the number of IFN-γ-producing T cells with disease progression from MF to SS. The inability of these T cells to synthesize IFN-γ may have prognostic value in CTCL, since it may be responsible for the progression of the disease from MF to SS.

The cutaneous T-cell lymphomas (CTCL), mycosis fungoides (MF) and the Sezary syndrome (SS), are the most frequent lymphomas involving the skin (8). Patients with CTCL may have an extremely long natural history of disease, with a median 6-year duration from the onset of skin symptoms to diagnosis of MF and finally to the leukemic phase of SS (16, 46). The development of SS as a consequence of the persistent stimulation of T cells by a variety of bacterial antigens has been hypothesized (12, 13, 17, 41, 42). Additionally, persistent colonization with toxigenic bacteria can result in the expansion of epidermotropic T cells (35). Activated T cells produce cytokines and/or chemokines that perpetuate the inflammatory reaction and recruit additional T cells to the skin (36, 37).

Leukemia T cells or Sezary cells are frequently identified in the peripheral blood of patients with either MF or SS (25). Sezary cells represent an expansion of circulating memory $CD4^+$ T cells that lack CD7 antigen expression (15). The defective expression of CD7 has been described on memory T cells from healthy donors (19), from patients infected with human immunodeficiency virus (2), and from patients with SS (15). Compared with $CD7^+$ T cells, $CD7^-$ T cells are less responsive to activation with anti-CD3 (2, 14) due to a lower-level expression of the T-cell receptor (TCR) and the suboptimal triggering of the TCR-CD3 complex (44). Defective triggering of the TCR-CD3 complex on memory T cells may also account for the reduction in the production of type 1 cytokines in CTCL (14, 26, 32).

Type 1 and type 2 cytokines play a vital role in immunity (30, 40). Patients with MF and SS differ in their production of (26, 32, 45) and in their response to (10, 32) cytokines. Whereas a type 1 cytokine production profile consisting of interleukin-2 (IL-2) and gamma interferon (IFN-γ) is common in MF, a type 2 cytokine production profile, consisting of IL-4, is more likely to occur in SS (26, 32, 33, 45). Although previous studies demonstrated the cytokine production in peripheral blood mononuclear cell cultures of patients with CTCL (26, 32, 45), none of these studies was capable of identifying the phenotype of the cells producing cytokines. In the present study, T cells were activated with phorbol 12-myristate 13-acetate (PMA), which directly stimulated calcium and phospholipid-dependent protein kinase C activity (43). Furthermore, we took advantage of a flow cytometric technique (31) that is capable of identifying the phenotype of the cytokine-producing cells at the single-cell level. We believe that such an approach provides opportunities to investigate the role of these soluble factors in exacerbating tumor pathogenesis in CTCL.

MATERIALS AND METHODS

Patient population and staging. A complete medical history of each patient was taken to assess previous treatments for CTCL. Each patient was assigned to a disease stage based on a physical examination to assess performance status, concurrent nonmalignant disease, and skin involvement as previously described (7, 20). The breakdown of the 25 patients by stage of disease is shown in Table 1 and consisted of four patients with stage I, four with stage II, nine with stage III, and eight with stage IV. The laboratory examination included the following: a complete blood count and a differential count of a Wright-Giemsa-stained smear, a platelet count, and T-cell immunophenotyping. The percentage of Sezary cells in the peripheral blood was determined by the presence of malignant lymphoid cells with hyperchromatic, indented (cerebriform) nuclei identifiable under light microscopy according to previously defined criteria (38).

Peripheral blood was obtained from patients with CTCL (13 with SS and 12 with MF) and 9 control subjects for the purpose of measuring the synthesis of type 1 and type 2 cytokines by $CD7^+$ and $CD7^-$ T-cell subsets. In a subset of the patients, seven with MF and five with SS, the synthesis of cytokines by $CD4^+$ and $CD8^+$ T-cell subsets was studied. A total of 15 ml of peripheral blood was drawn from each subject: 10 ml was drawn in heparin for the cytokine studies and another 5 ml was collected in EDTA for the determination of surface phenotypes. Informed consent was obtained from all patients and control subjects, and approval for the study was obtained from the Human Experimentation Committee of The University of Texas M. D. Anderson Cancer Center.

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TABLE 1. Disease stage and hematologic characteristics of patients with CTCL

Patient	Type CTCL	Stage[a]	WBC/μl[b]	Sezary cells in blood	
				%	Cells/μl
1	MF	Ia	4,800	0	0
2	MF	Ia	4,200	0	0
3	MF	Ib	8,300	0	0
4	MF	Ib	5,100	5	255
5	MF	IIa	7,200	28	2,016
6	MF	IIb	3,500	0	0
7	MF	IIb	3,500	0	0
8	MF	IIb	4,300	5	215
9	MF	III	5,000	16	800
10	MF	III	4,800	0	0
11	MF	IVa	4,000	0	0
12	MF	IVa	2,600	20	520
13	SS	III	4,400	28	1,232
14	SS	III	5,600	17	952
15	SS	III	10,700	9	963
16	SS	III	5,500	28	1,540
17	SS	III	7,900	20	1,580
18	SS	III	4,800	35	1,680
19	SS	III	2,500	14	350
20	SS	IVa	5,300	24	1,272
21	SS	IVa	6,800	11	748
22	SS	IVa	11,300	2	226
23	SS	IVa	10,000	11	1,100
24	SS	IVa	9,900	16	1,584
25	SS	IVa	6,300	32	2,016

[a] All patients had a complete physical examination and staging workup, including a blood smear for Sezary cells and skin biopsy for histology.
[b] WBC, leukocytes.

Phenotype of T cells in CTCL. Aliquots of whole blood (100 μl) were mixed with pretitrated amounts of monoclonal antibodies, each conjugated with one of the following fluorochromes: fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin chlorophyll protein (PerCP), all purchased from Becton-Dickinson Immunocytometry Systems Inc., San Jose, Calif. Each sample was incubated in the dark at ambient temperature for 15 min with a combination of monoclonal antibody reagents to detect the common leukocyte antigen ($CD45^+$), monocytes ($CD14^+$), total T cells ($CD3^+$), and $CD3^+$ T-cell subsets coexpressing CD7, CD4 (helper and inducer), or CD8 (cytotoxic and suppressor) surface antigens. At the end of this incubation period, the erythrocytes in each tube were disrupted. The samples were washed three times with phosphate-buffered saline and concentrated by centrifugation. The cell pellets were fixed with 200 μl of a 1% paraformaldehyde solution, stored at 4°C, and analyzed within 4 h with a FACSCalibur flow cytometer (Becton-Dickinson). Isotype-matched negative controls conjugated to each of the fluorochromes were used to determine nonspecific binding of human leukocytes to mouse monoclonal antibodies; lymphocyte purity was assessed by setting a gate around those cells that were positive for CD45 and negative for CD14.

Determination of cytokine synthesis by T cells. Preparation for the synthesis of cytokines and for the detection of cytokines in the cytoplasm of T cells was done as previously described (28, 31). Briefly, the cells were prepared in four sequential steps: (i) the cells were activated by PMA (25 ng/ml) for 4 h at 37°C in the presence of 1 μg of ionomycin/ml and 10 μg of brefeldin A (BFA; a nontoxic but potent inhibitor of intracellular transport)/ml; (ii) the activated cells were divided between two sets of tubes and reacted with either anti-CD3–PerCP and anti-CD7–FITC or with anti-CD3–PerCP and anti-CD8–FITC to determine the surface immunophenotype of the T cells; (iii) the cells were treated with a permeabilization solution (Becton-Dickinson) for 1 h at 37°C to allow entry of the monoclonal antibody; and (iv) the cells were stained with a cytokine-specific monoclonal antibody conjugated with PE to detect IL-2, IL-4, or IFN-γ. All cell preparations were fixed in a solution of 1% paraformaldehyde and stored at 4°C until they were analyzed with the FACSCalibur flow cytometer. In parallel experiments, the blood from each patient and control subject was incubated with BFA alone and served as a resting or unstimulated preparation.

The analysis was conducted by gating on $CD3^+$ cells among the 8,000 total events acquired. List-mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescence parameters) were analyzed with the CellQuest software program (Becton-Dickinson). Isotype controls were used to verify the staining specificity of experimental conditions and as a guide for setting markers to delineate positive and negative populations. In one set of studies, cytokine synthesis by $CD7^+$ and $CD7^-$ T-cell subsets was identified on the basis of the reactivities of the cells with anti-CD7. In another set of studies, the reaction of lymphocytes with anti-CD8 defined two additional T-cell subsets, $CD8^+$ and $CD8^-$ (or $CD4^+$), and permitted the evaluation of type 1 and type 2 cytokine synthesis in these T-cell subsets. The addition of anti-CD69–PE to the panel identified subsets of activated $CD3^+$ T-cells bearing these phenotypes. Likewise, the addition of an anti-cytokine specific monoclonal reagent to the above combinations of reagents permitted the identification of subsets of $CD3^+$ T-cells that were capable of cytokine synthesis.

Data analysis. The percentages of cytokine-producing T cells were calculated for total T cells and subsets of T cells for each patient and control subject. The data are presented as both the mean number of cytokine-producing T cells per microliter of peripheral blood and the mean percentage of cytokine-producing cells per specific T-cell subset. Statistical differences in the mean percentages and numbers of cytokine-producing T cells between study groups were determined by the unpaired *t* test.

RESULTS

Detection of Sezary cells in peripheral blood smears of patients with CTCL. Table 1 lists the clinical characteristics and hematology parameters of patients with CTCL. Sezary cells were detected in the peripheral blood of patients with CTCL at every stage of the disease, with an increasing percentage of Sezary cells associated with disease progression. We were able to identify Sezary cells in 18 of the 25 patients with CTCL, 14 of whom had >10% Sezary cells in their peripheral blood (Table 1). An increase in the percentage of Sezary cells in patients with more severe CTCL was also supported by a recent study with PCR-based analysis (24).

Immunophenotype of peripheral blood lymphocytes. Three-color flow-cytometric analysis was performed on all blood samples to determine the percentages of total T cells ($CD3^+$) and of the T-cell subsets in circulation (Table 2). The immunophe-

TABLE 2. Phenotypes of peripheral blood T cells in patients with CTCL

Phenotype[a]	T cells in circulation					
	Control subjects		MF		SS	
	%	Count/μl	%	Count/μl	%[b]	Count/μl[c]
Total $CD3^+$	74.9 (3.3)	1,440 (165)	71.3 (2.6)	873 (138)	88.5 (4.8)	2,193 (440)
$CD3^+$ $CD7^+$	67.3 (2.9)	1,306 (142)[d]	63.0 (2.0)	777 (122)	15.4 (5.6)	375 (91)
$CD3^+$ $CD7^-$	7.7 (1.6)	134 (25)[d]	8.3 (1.0)	96 (21)	73.0 (8.2)	1,818 (447)
$CD3^+$ $CD4^+$	44.6 (4.0)	802 (101)[d]	43.4 (3.7)	511 (84)	82.8 (5.5)	2,075 (443)
$CD3^+$ $CD8^+$	23.8 (3.6)	509 (89)	26.9 (2.6)	358 (81)	5.3 (2.2)	102 (19)
$CD4^+$ $CD45RO^+$	34.7 (6.2)	625 (78)	40.5 (3.7)	464 (79)	68.0 (7.9)	1,438 (222)

[a] Phenotyping of T-cell subsets was done as stated in Materials and Methods, and results are presented as means ± standard errors of the mean (in parentheses).
[b] Statistical differences in the mean percentages of all the immunophenotypes between patients with SS and both patients with MF and control subjects.
[c] Statistical differences in counts per microliter of all the T-cell subsets between patients with SS and both patients with MF and control subjects.
[d] Significant differences in counts per microliter between control subjects and patients with MF.

TABLE 3. Expression of CD69 by T-cell subsets

Phenotype	CD69 expression[a]					
	Control subjects		MF		SS	
	%	Count/μl	%	Count/μl	%	Count/μl
Total $CD3^+$	96.6 (0.8)[a]	1,394 (155)	92.5 (1.7)	811 (125)[b]	82.6 (4.4)	1,472 (228)
$CD3^+$ $CD7^+$[c]	96.4 (0.5)[d]	1,263 (131)	93.8 (1.5)[d]	727 (112)	92.3 (2.4)	344 (87)
$CD3^+$ $CD7^-$	91.4 (2.3)	123 (24)	86.1 (2.6)	82 (17)	82.9 (4.4)	1,116 (215)[e]
$CD3^+$ $CD4^+$	97.9 (0.4)[f]	786 (114)	88.2 (4.2)	437 (97)	86.9 (2.4)	1,953 (747)[g]
$CD3^+$ $CD8^+$	95.6 (1.0)[f]	500 (81)	92.9 (4.2)	656 (108)	83.9 (8.1)	91 (19)[g]

[a] Figures represent means ± standard errors of the mean (in parentheses).
[b] Patients with MF had significantly lower numbers of $CD69^+$ T cells than patients with SS and control subjects.
[c] There were significant differences in the numbers of $CD69^+$ $CD7^+$ T cells between study groups.
[d] $CD7^+$ T cells of patients with MF and control subjects had significantly higher percentages of CD69 expression than their autologous $CD7^-$ T cells.
[e] Patients with SS had significantly higher numbers of $CD69^+$ $CD7^-$ T cells than the other two groups.
[f] Control subjects had significantly higher percentages of $CD69^+$ $CD4^+$ and $CD69^+$ $CD8^+$ T cells than patients with MF and SS.
[g] Patients with SS had significantly higher numbers of $CD69^+$ $CD4^+$ T cells and significantly lower numbers $CD69^+$ $CD8^+$ T cells than patients with MF.

notypes of total T cells and subsets of T cells were similar for patients with MF and controls. The immunophenotype of patients with SS was significantly different from those of patients with MF and control subjects. In particular, patients with SS had significantly higher percentages of $CD3^+$, $CD7^-$, $CD4^+$, and $CD4^+$ $CD45RO^+$ T cells and significantly lower percentages of $CD7^+$ and $CD8^+$ T cells than both patients with MF and control subjects (Table 2).

Patients with MF had significantly lower numbers of $CD3^+$ (873 ± 138 versus 1,440 ± 165), $CD3^+$ $CD7^+$ (96 ± 21 versus 134 ± 25), and $CD3^+$ $CD4^+$ (511 ± 84 versus 802 ± 101) T cells per microliter than controls. On the other hand, patients with MF had significantly higher numbers of $CD3^+$ $CD7^+$ (777 ± 122 versus 375 ± 91) and $CD3^+$ $CD8^+$ (358 ± 81 versus 102 ± 19) T cells per microliter but significantly lower numbers of $CD3^+$ $CD7^-$ (96 ± 21 versus 1,818 ± 447), $CD3^+$ $CD4^+$ (511 ± 84 versus 2,075 ± 443), and $CD4^+$ $CD45RO^+$ (464 ± 79 versus 1,438 ± 222) T cells per microliter than patients with SS. Compared with control subjects, patients with SS had significantly higher numbers of $CD3^+$ $CD7^-$ (1,818 ± 447 versus 134 ± 25), $CD3^+$ $CD4^+$ (2,075 ± 443 versus 802 ± 101), and $CD4^+$ $CD45RO^+$ (1,438 ± 222 versus 625 ± 78) T cells per microliter and significantly fewer $CD3^+$ $CD7^+$ (375 ± 91 versus 1,306 ± 142) and $CD3^+$ $CD8^+$ (102 ± 19 versus 509 ± 89) T cells per microliter. Whereas the majority of $CD4^+$ $CD45RO^+$ memory T cells in patients with SS were $CD3^+$ $CD7^-$, the converse was true for patients with MF and control subjects.

In vitro activation of peripheral blood T lymphocytes of patients with CTCL. When the peripheral blood lymphocytes were cultured in vitro in the presence of BFA and without PMA, only negligible numbers of T cells from all groups synthesized cytokine (data not shown). The numbers of $CD69^+$ $CD3^+$ T-cell subsets per microliter in patients with MF (811 ± 125) was lower than those in patients with SS (1,472 ± 228) and controls (1,394 ± 155) (Table 3). In addition, in the patients with MF and the control donors, CD69 expression was significantly higher in the $CD7^+$ T cells than in their autologous $CD7^-$ T cells. Compared to control donors, significantly lower percentages of $CD4^+$ and $CD8^+$ T cells of patients with MF and SS expressed CD69 (Table 3).

Synthesis of IL-2 by PMA-activated T-cell subsets. The synthesis of IL-2 by T-cell subsets following stimulation with PMA is shown in Table 4. The mean percentages and counts per microliter of $CD3^+$ T cells synthesizing IL-2 were similar for all groups. Within the $CD3^+$ T cells of patients with MF and controls, the number of IL-2^+ $CD7^+$ T cells was significantly higher than the autologous IL-2^+ $CD7^-$ T-cell subset (P = 0.01). On the other hand, patients with SS had significantly fewer IL-2^+ $CD7^+$ and significantly more IL-2^+ $CD7^-$ T cells per microliter than both patients with MF and control subjects. Patients with SS also had significantly higher numbers of IL-2^+ $CD4^+$ T cells than patients with MF and significantly lower numbers of IL-2^+ $CD8^+$ T cells than both patients with MF and controls.

TABLE 4. Synthesis of IL-2 by T-cell subsets

Phenotype	T cells synthesizing IL-2[a]					
	Control subjects		MF		SS	
	%	Count/μl	%	Count/μl	%	Count/μl
Total $CD3^+$	35.0 (5.5)	466 (58)	40.2 (4.4)	343 (57)	35.0 (6.9)	493 (108)
$CD3^+$ $CD7^+$	35.2 (4.6)	414 (54)	38.7 (4.3)	303 (52)	32.0 (4.3)	134 (51)[b]
$CD3^+$ $CD7^-$	42.5 (5.7)	49 (8)	41.9 (4.6)	39 (7)	42.2 (7.2)	426 (121)[b]
$CD3^+$ $CD4^+$	49.9 (5.4)	383 (58)	24.0 (4.7)	236 (49)	28.6 (6.3)	561 (148)[c]
$CD3^+$ $CD8^+$	13.3 (2.3)	53 (8)	37.6 (6.5)[d]	192 (49)[d]	17.9 (2.9)	22 (7)[c]

[a] Figures represent means ± standard errors of the mean (in parentheses).
[b] There were significantly lower numbers of $IL2^+$ $CD7^+$ T cells and significantly higher numbers of $IL2^+$ $CD7^-$ T cells in patients with SS than in patients with MF and controls.
[c] There were significantly higher numbers of $IL2^+$ $CD4^+$ T cells and significantly lower numbers of $IL2^+$ $CD8^+$ T cells in patients with SS than in patients with MF.
[d] Mean percentages and numbers of $IL2^+$ $CD8^+$ T cells of patients with MF were significantly higher than those of controls.

TABLE 5. Synthesis of IFN-γ by T-cell subsets

Phenotype	T cells synthesizing IFN-γ[a]					
	Control subjects		MF		SS	
	%	Count/μl	%	Count/μl	%	Count/μl
Total $CD3^{+}$[b]	36.4 (3.7)	519 (67)	28.6 (3.0)	238 (35)	8.4 (2.3)[c]	102 (21)
$CD3^{+}$ $CD7^{+}$[b]	34.9 (3.4)	430 (56)	25.7 (2.7)	194 (31)	21.8 (4.1)	76 (25)
$CD3^{+}$ $CD7^{-}$	60.5 (2.2)	79 (15)	49.6 (5.1)	42 (7)	9.9 (4.1)[c]	52 (11)
$CD3^{+}$ $CD4^{+}$	25.9 (7.1)	201 (30)[d]	24.0 (4.7)	110 (22)	4.6 (0.8)[e]	108 (45)
$CD3^{+}$ $CD8^{+}$	46.6 (5.0)	216 (35)	37.6 (6.5)	243 (33)	52.3 (11.2)	46 (11)[f]

[a] Figures represent means ± standard errors of the mean (in parentheses).
[b] There were significant differences between the three study groups with respect to the number of IFN-γ^{+} $CD3^{+}$ T cells and IFN-γ^{+} $CD7^{+}$ T-cell subsets.
[c] Patients with SS had significantly lower percentages of IFN-γ^{+} $CD3^{+}$ T cells and of IFN-γ^{+} $CD7^{-}$ T cells than patients with MF and controls.
[d] Control subjects had significantly higher numbers of IFN-γ^{+} $CD4^{+}$ T cells than patients with MF or SS.
[e] Patients with SS had significantly lower percentages of IFN-γ^{+} $CD4^{+}$ T cells than patients with MF and controls.
[f] Patients with SS had significantly lower numbers of IFN-γ^{+} $CD8^{+}$ T cells than patients with MF and controls.

Synthesis of IFN-γ by PMA-activated T-cell subsets. The highest percentages and numbers microliter of IFN-γ-producing T cells were observed in controls, followed by those of patients with MF and finally by those of patients with SS (Table 5). There were significant decreases in the percentages and counts of IFN-γ^{+} $CD3^{+}$ T cells associated with disease progression in CTCL. The majority of IFN-γ^{+} $CD3^{+}$ T cells of patients with MF belonged to the $CD7^{+}$ T-cell subset. In contrast, less than 10% of $CD7^{-}$ T cells of patients with SS produced IFN-γ, indicating that the majority of $CD7^{-}$ T cells of these patients were not capable of producing IFN-γ. In addition, patients with SS had significantly lower percentages of IFN-γ^{+} $CD4^{+}$ T cells than patients with MF and controls. Whereas patients with MF had more IFN-γ^{+} $CD8^{+}$ (243 ± 33 per μl) than IFN-γ^{+} $CD4^{+}$ (110 ± 22 per μl) T cells, patients with SS had more IFN-γ^{+} $CD4^{+}$ (100 ± 45 per μl) than IFN-γ^{+} $CD8^{+}$ (46 ± 11 per μl) T cells.

Synthesis of IL-4 by PMA-activated T-cell subsets. The synthesis of IL-4 by T-cell subsets following stimulation with PMA is shown in Table 6. In patients with SS, a significantly higher percentage of $CD7^{+}$ T cells than $CD7^{-}$ T cells synthesized IL-4. Conversely, fewer $CD7^{+}$ T cells from control donors synthesized IL-4 than their autologous $CD7^{-}$ T cells. Equivalent percentages of $CD7^{+}$ and $CD7^{-}$ T cells of patients with MF synthesized IL-4. More importantly, the total numbers of $CD3^{+}$ T cells synthesizing IL-4 were similar for all three groups. Whereas the percentage of IL-4^{+} $CD4^{+}$ T cells in patients with SS was significantly lower than that in patients with MF ($P = 0.01$) and controls ($P = 0.01$), the percentage of IL-4^{+} $CD8^{+}$ T cells in control donors was significantly lower than that in patients with MF ($P = 0.01$) and SS ($P = 0.01$). In addition, patients with MF had a significantly higher number of IL-4^{+} $CD8^{+}$ T cells per microliter than patients with SS and controls.

DISCUSSION

Although CD7 is expressed on major subsets of peripheral blood T cells (15), its function is unknown and no ligand has been identified. The loss of CD7 antigen expression is found on memory T cells in healthy individuals (19), in patients infected with human immunodeficiency virus (2, 22), and in the synovial fluids of patients with rheumatoid arthritis (21). In patients with SS, the lack of CD7 antigen expression on $CD4^{+}$ T cells is a classical feature of malignant Sezary cells (19). Consistent with previous studies (3, 18), we found a significant increase in the percentage and absolute number of memory-helper T cells that lacked CD7 antigen expression in patients with SS (Table 2).

It is commonly recognized that CTCL patients are defective in triggering the TCR-CD3 complex (14, 26, 32). In the present study, we stimulated T cells with PMA via a pathway that is independent of that triggering the CD3-TCR complex (43). Compared with $CD7^{+}$ T cells, a lower percentage of autologous $CD7^{-}$ T cells of patients with CTCL coexpressed the activation marker CD69 (Table 2), thereby indicating that the defect is not restricted to activation of TCR (9) but extends to nonspecific activation mechanisms. Since the malignant cells in patients with SS are predominantly $CD7^{-}$ T cells, their reduced responsiveness to activation (Table 3) accompanied by their reduced capacity to proliferate (14) may be a consequence of their abnormal phenotype. Because T lymphocytes in patients with SS are likely to be clonal (24) and to express activation antigens (1), further stimulation of these T cells may lead to suboptimal activation to avoid programmed cell death (9).

By measuring cytokine proteins in individual cells rather than in culture supernatants as others have done (27, 45), we found that similar percentages and counts of total T cells from all study groups synthesized IL-2 (Table 4). Since patients with

TABLE 6. Synthesis of IL-4 by T-cell subsets

Phenotype	T cells synthesizing IL-4[a]					
	Control subjects		MF		SS	
	%	Count/μl	%	Count/μl	%	Count/μl
Total $CD3^{+}$	3.6 (0.4)	52 (9)	5.6 (1.0)	48 (9)	3.1 (0.7)	48 (12)
$CD3^{+}$ $CD7^{+}$	3.1 (0.3)[b]	40 (6)	5.5 (0.9)	41 (8)	6.0 (1.2)	22 (6)
$CD3^{+}$ $CD7^{-}$	9.3 (1.8)	11 (2)	6.8 (1.0)	7 (1)	2.1 (0.6)[c]	24 (8)[d]
$CD3^{+}$ $CD4^{+}$	5.2 (0.6)	42 (9)	7.3 (1.2)	34 (7)	1.6 (0.4)[e]	31 (8)
$CD3^{+}$ $CD8^{+}$	2.6 (0.5)[f]	12 (3)	7.0 (1.7)	45 (10)[g]	11.5 (2.7)	15 (4)

[a] Figures represent means ± standard errors of the mean (in parentheses).
[b] Mean percentage of IL-4^{+} $CD7^{+}$ T cells of control subjects was significantly lower than those of patients with MF or SS.
[c] Mean percentage of IL-4^{+} $CD7^{-}$ T cells of patients with SS was significantly lower than those of patients with MF and control subjects.
[d] Patients with SS had significantly higher numbers of IL-4^{+} $CD7^{-}$ T cells than patients with MF.
[e] Patients with SS had significantly lower percentages of IL-4^{+} $CD4^{+}$ T cells than the other two groups.
[f] Control subjects had significantly lower percentages of IL-4^{+} $CD8^{+}$ T cells than patients with MF and SS.
[g] Count per microliter of IL-4^{+} $CD8^{+}$ T cells of patients with MF was significantly higher than those of patients with SS and control subjects.

MF and healthy donors had significantly more $CD7^+$ than $CD7^-$ T cells in circulation, it was expected that their $IL\text{-}2^+$ $CD7^+$ T cells would outnumber their $IL\text{-}2^+$ $CD7^-$ T cells (Table 4). In contrast, patients with SS had a higher number of $IL\text{-}2^+$ $CD7^-$ T cells per microliter and the bulk of the IL-2 was more likely to be made by $CD7^-$ than by $CD7^+$ T cells, as others have suggested (45).

By blocking cytokine transport with BFA, we found no difference in the percentages of $IL\text{-}2^+$ $CD7^+$ ($32.0\% \pm 4.3\%$) and $IL\text{-}2^+$ $CD7^-$ ($42.2\% \pm 7.2\%$) T-cell subsets in patients with SS (Table 4). Since there were similar numbers of $IL\text{-}2^+$ $CD3^+$ T cells in patients with SS and control donors (Table 4), we cannot confirm a deficiency in IL-2 production in patients with SS as previously described (27, 32, 45). Furthermore, the difference in results may be explained by a defect in the post-translational processes or by an enhancement in the degradation of intracellular protein in malignant cells (11). Alternatively, it can be explained by the reabsorption of IL-2, acting as an autocrine growth factor for $CD7^-$ T cells (10), through membrane-bound IL-2 receptors (6, 23) and/or by the formation of complexes with soluble IL-2 receptors (5, 6).

As expected, there was a significant decline in the number of $IFN\text{-}\gamma^+$ $CD3^+$ T cells in patients with CTCL compared to that in healthy donors (Table 5). Between MF and SS, there was a further significant decrease in the percentage and number of $IFN\text{-}\gamma^+$ $CD3^+$ T cells, indicating an inverse relationship between the number of $IFN\text{-}\gamma^+$ $CD3^+$ T cells and disease stage. Our data support earlier observations that T cells in SS produced less IFN-γ following stimulation with mitogen (32, 45). Furthermore, the deficiency in IFN-γ synthesis in MF is more attributable to the $CD4^+$ T cells than to the $CD8^+$ T cells (Table 5). There were fewer IFN-γ-producing $CD8^+$ T cells in patients with SS than in other groups, and this may account for the suppressed cytotoxic-T-lymphocyte activity in these patients (39).

In healthy individuals, whereas stimulation with antigen resulted in a higher production of IFN-γ by $CD7^-$ T cells (34), IL-4 was preferentially produced by $CD7^+$ T cells following stimulation with lectin (4). Previous reports based on a variety of methodologies have generally shown patients with SS to have a type 2 cytokine response profile (45). Accordingly, we expected to find the majority of IL-4 producers to be $CD7^-$ T cells in patients with SS. Instead, we found the total number of $IL\text{-}4^+$ T cells to be similar for all three study groups (Table 6). There were few $IL\text{-}4^+$ $CD7^-$ T cells in patients with SS even through the majority of T cells of patients with SS were of $CD7^-$ phenotype (Table 2). The synthesis of IL-4 by T-cell lines is known to be transient, and not all Th2 cells are primed to produce this cytokine (29). Hence, our observation of a few $IL\text{-}4^+$ T cells in all study groups may be due to the low frequency of IL-4-synthesizing Th2 cells in peripheral circulation coupled with the transient synthesis of this cytokine upon PMA stimulation. Furthermore, our method of evaluating IL-4 synthesis is a snapshot of cytokine production in cells and is unlikely to reflect the amount of IL-4 accumulated over an extended period in cultures.

Our observations did not favor the previously reported Th0-Th2 profile of $CD7^-$ cells in patients with SS and raised the possibility that $CD7^-$ T cells, presumably leukemic in patients with SS (15), could be distinct from Th0-Th2-like T cells of healthy donors. Moreover, there were more $IL\text{-}4^+$ $CD7^+$ than $IL\text{-}4^+$ $CD7^-$ T cells in controls, indicating that $CD7^+$ T cells could have a greater contribution to the overall production of IL-4. In conclusion, we found no defect in the ability of $CD7^-$ T cells in patients with SS to synthesize IL-2, even though a defect in the production of IFN-γ in these patients was found in this and other studies. Our data expand on the observations of others by showing that both $CD7^+$ and $CD7^-$ T-cell subsets are less capable of synthesizing IFN-γ than those of patients with MF and control subjects. These data support the hypothesis that disease progression from MF to SS in patients with CTCL may be associated with the defective IFN-γ production accompanied by a decrease in cytotoxic T lymphocyte activity (39). Finally, our experimental approach can be used to address the association of cytokine induction by bacterial superantigens and pathogenesis in CTCL.

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