Expression of Follicular Helper T-cell Markers in Primary Cutaneous T-cell Lymphoma

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감사의 글

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2013 년 1 월

저자 씀

-ABSTRACT-

Expression of Follicular Helper T-cell Markers in Primary Cutaneous T-cell Lymphoma

Background and objectives: A distinct subset of T helper cells, named follicular helper T- (T_{FH}) cells, have been recently described. The T_{FH}-cells can be identified on the basis of the expression of several markers including: the chemokine CXCL13; the costimulatory molecules PD-1 and inducible costimulator (ICOS); and the transcription factor Bcl-6. The T_{FH}-cells appeared to be localized in several subsets of T-cell lymphomas. However, their localization in cutaneous T-cell lymphomas has been rarely described. In this study, the clinical features, histopathological morphology, and expression of T_{FH} markers in cutaneous T-cell lymphomas were investigated.

Materials and methods: Forty-nine Korean patients (24 men, 25 women) diagnosed with cutaneous T-cell lymphoma were examined: 25 patients with mycosis fungoides (MF), 9 with lymphomatoid papulosis, 8 with anaplastic large cell lymphoma, 4 with subcutaneous panniculitis-like T-cell lymphoma, 2 with peripheral T-cell lymphoma, and 1 with NK/T-cell lymphoma. Chart review was performed to evaluate the clinical features. Hematoxylin and eosin (H&E) staining was used to study the general histopathological changes. Additionally, immunohistochemical staining for CD10, Bcl-6, ICOS, CXCL13, and PD-1 were performed. The extent of staining was scored from 0 to 3 by 2 independent observers.

Results: PD-1 was frequently expressed in most of the MF cases, but was rarely expressed in other cutaneous T-cell lymphomas. Bcl-6, CXCL13, ICOS, and CD10 were scantly expressed in most T-cell lymphomas, including MF. Interestingly, the staining for PD-1 was negative in all the MF cases with large-cell transformation. No correlation was observed among clinical morphology, disease course, and PD-1 expression rate in the MF cases.

Conclusions: Most of the MF samples expressed T_{FH}-cell markers. This finding suggests that some MF tumor cells might consist of T_{FH}-cells. In addition, higher PD-1 expression rates in MF than in other cutaneous T-cell lymphomas implies that PD-1 can be an MF-sensitive marker, and it might be useful in differentiating MF from other cutaneous T-cell lymphoma. The loss of PD-1 expression in large-cell–transformed MF suggests that PD-1

downregulation may be related to the processes responsible for large-cell transformation.

Key words: Follicular helper T-cell, cutaneous T-cell lymphoma, mycosis fungoides, PD-1

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I. INTRODUCTION

Follicular helper T- (T_{FH}) cells are a specific subset of $CD4^+$ helper T-cells (T_H), which are normally found in the germinal centers of the B-cell follicles. The T_{FH}-cells help B-cells to differentiate into long-lived, antibody-secreting plasma cells or memory B-cells. The T_{FH}-cells can be distinguished from subtypes T_H1 and T_H2 by using several criteria, which include the expression of: chemokine CXCL13; the costimulatory molecules PD-1 and inducible costimulator (ICOS); and the transcription factor Bcl-6 (Laurent et al., 2010; Gaulard and de Leval, 2011).

Some T-cell lymphomas express T_{FH}-cell markers. The gene expression profiling studies of angioimmunoblastic T-cell lymphoma (AITL) have demonstrated that the neoplastic T-cells are derived from the T_{FH}-cells (de Leval et al., 2007). The neoplastic T-cells in AITL indeed express CD10, Bcl-6, ICOS, CXCL13, and PD-1 (Dupuis et al., 2006; Yu et al., 2009). Recently, follicular peripheral T-cell lymphoma (PTCL), a subset of nodal peripheral T-cell lymphoma with a follicular growth pattern, has been identified. These lymphomas have been shown to express T_{FH} markers, and may show overlapping features with AITL (Huang et al., 2009). In primary cutaneous $CD4^+$ small/medium-sized pleomorphic T-cell lymphoma, atypical large $CD4^+$ cells were positive for T_{FH} markers (Rodriguez Pinilla et al., 2009), suggesting that they were derived from T_{FH}-cells.

However, in cutaneous T-cell lymphoma, T_{FH}-cell expressions have been rarely described. Recently, Battistella et al reported an original series of 5 primary cutaneous T-cell lymphomas showing expression of T_{FH} markers, and proposed naming them primary cutaneous T_{FH}-cell lymphomas (Battistella et al., 2012). More recently, several mycosis fungoides (MF) cases positive for the T_{FH} markers were reported. Meyerson et al identified 8 cases diagnosed with MF or Sézary syndrome. The results of immunohistochemical studies revealed that some cases of lymphoma cells expressed CD10, Bcl-6, PD-1, and occasionally, CXCL-13 (Meyerson et al., 2012). In addition, intertriginous MF (Gammon and Guitart, 2012) and CD4/CD8 double negative MF with T_{FH} markers expression cases (Kempf et al., 2012) have been reported. However, identification of T_{FH}-cells in cutaneous T-cell lymphomas in difficult because the low number of cases.

In this study, the clinical and histopathological features and the expression of T_{FH}-cell

markers in cutaneous T-cell lymphoma was investigated. In addition, I sought to identify a specific marker for differentiating cutaneous T-cell lymphomas, and assessed the correlation between the expression rate of T_{FH}-cell markers and the prognosis of the disease.

II. MATERIALS AND METHODS

A. Subjects

Forty-nine cases of primary cutaneous T-cell lymphoma (24 men, 25 women) diagnosed at the Department of Dermatology, Ajou University Hospital, Suwon, Korea, between January 2000 and December 2011 were examined. The diagnosis of cutaneous T-cell lymphoma was made on the basis of the clinical features, and the results of histopathological analyses and immunohistochemical studies. Twenty-five patients with MF, 9 with lymphomatoid papulosis, 8 with anaplastic large cell lymphoma, 4 with subcutaneous panniculitis-like T-cell lymphoma, 2 with peripheral T-cell lymphoma, and 1 with NK/T-cell lymphoma were included in the study. The mean age of the patients was 44.8 years (range 14–91 years). This study was approved by the institutional review board of the Ajou University Hospital (IRB number: AJIRB-MED-KSP-12-201).

B. Methods

1. Chart review

The charts of 49 patients with clinicopathologically proven cutaneous T-cell lymphoma were reviewed. The clinical features were investigated with respect to age, sex, locations of the skin lesions, duration of symptoms at presentation, cutaneous manifestations, treatments, and disease courses.

2. Skin biopsies

The subjects were given local anesthesia using 1% lidocaine and 3-mm punch- or excisional-biopsies were taken from the skin lesions. After collection, the biopsy samples were fixed overnight using 10% formalin and evaluated using light microscopy.

3. Staining

Paraffin-embedded tissue sections of 3-μm thickness were processed for examination by light microscopy. Hematoxylin and eosin (H&E) staining was used to study the general histopathological changes in the skin lesions of the cutaneous T-cell lymphoma.

4. Immunohistochemical analysis

Five-micrometer thick, paraffin-embedded sections of skin lesions were mounted on glass slides coated with 0.1% poly-D-lysine (Polysine microscope slides, Menzel-Glaser, Germany). The sections were deparaffinized and rehydrated by sequential immersion in xylene, graded concentrations of ethanol, and distilled water. The tissue sections were immunohistochemically stained using a modification of the avidin-biotin-peroxidase complex (ABC) method. The tissue sections were incubated for 30 minutes at room temperature in 0.5% hydrogen peroxidase in methanol to quench the endogenous peroxidase activity, followed by 3 washes with Tris-buffered saline (TBS, 0.1 M; pH 7.4; Dako, Carpinteria, CA, USA). The sections were subsequently incubated in 0.05% pepsin in TBS for 20 minutes at 37℃. The slides were then washed 3 times in TBS and were immersed in protein blocking agent (PBA, Immunon, Pittsburgh, PA, USA) for 10 minutes at room temperature to saturate non-specific protein-binding sites. The excess PBA was drained, and the slides were immersed in the primary antibodies, i.e. antibodies against PD-1, Bcl-6, CXCL13, ICOS, and CD10, at specified dilutions (Table 1). The slides were then incubated for 1 hour at room temperature and then for 30 minutes at 37℃ in a humid chamber. Following 3 washes in TBS, the slides were submerged under biotinylated universal secondary antibody reagent (Immunon, Pittsburgh, PA, USA) for 30 minutes at room temperature. The slides were then washed in TBS, followed by incubation in streptavidin alkaline phosphatase reagent for 30 minutes. After washes in TBS, the sections were incubated in fast red chromogen for 10 minutes. The sections were counter-stained with hematoxylin modified solution (Merck, Darmstadt, Germany) and mounted in an aqueous mounting medium (Biomeda, Foster City, CA, USA).

5. Evaluation of immunohistochemical staining

Expression of the T_{FH} markers was graded on the basis of the extent of staining (by percentage of positive tumor cells graded on a scale of 0 to 3; 0 = 0–10%, 1 = 11–40%, 2 = 41–70%, 3 = 71–100%). Positive cases were defined if positive tumor cells were 11% or more.

Table 1. Primary antibodies used and their working dilutions.

Antigen	Antibody	Manufacturer	Dilution
PD-1	Mouse monoclonal	Cell Marque, USA	1:100
Bcl-6	Mouse monoclonal	Cell Marque, USA	1:100
CXCL13	Goat polyclonal	R&D Systems, USA	1:10
ICOS	Rabbit polyclonal	Spring Bioscience, USA	1:100
CD10	Mouse monoclonal	Novocastra, UK	1:100

6. Statistical analysis

A chi-square test was performed using the SPSS Statistics Desktop 20.0.0 software (IBM, Armonk, NY, USA) to evaluate sensitivities and specificities with 95% confidence intervals for MF and other cutaneous T-cell lymphomas with respect to PD-1. A P value <0.05 was considered statistically significant.

III. RESULTS

A. Clinical findings

The charts of 49 patients diagnosed with cutaneous T-cell lymphoma were reviewed. The skin sites of disease involvement were diverse: multiple sites (trunk, extremities, and/or head and neck area; 21/49), extremities (17/49), trunk (7/49), and head and neck area (4/49). The MF patients consisted of 14 men and 11 women with an average age of 44.8 years, ranging from 16 to 83 years. The skin sites of disease involvement showed more involvement in multiple sites than in other cutaneous T-cell lymphoma: multiple sites (16/25), trunk (5/25), extremities (3/25), and the head and neck area (1/25). The erythematous patches or plaques typical for MF were seen in 23 of the 25 patients (patches, 18; plaques, 9). Papules were observed in 2 of the 25 patients, and tumors or nodules were seen in 3 patients of the 25. Hypo-pigmented patches were observed in 2 patients from the 25. Generalized erythema was not observed. Clinical follow-up dates were reviewed as available. Mean follow-up period was 2.2 years with follow-up failure for 6 patients. While the condition of 5 patients improved, 12 patients showed limited disease progression or recurrence at last follow-up. Two patients died of the disease, 2 and 2.3 years after diagnosis (Table 2).

Table 2. Clinical features of MF patients.

Case No.	Sex/ age	Skin sites	Disease duration (years)	Clinical morphology	Treatment	Outcome	F/u period (years)
1	F/41	Trunk, arm, leg	NA	Patches	MTX, topical steroid, NBUVB	Recurred	2.0
2	F/16	Arm, leg	1.0	Patches	NBUVB	Improved	1.5
3	F/45	Back, arm, leg, preauricular area	1.0	Patches, plaques	NBUVB, RTx	Recurred	0.8

4	F/60	Whole body	0.1	Papules, patches	MTX, topical steroid, NBUVB	Recurred	1.0
5	F/33	Whole body	2.5	Patches	NBUVB, topical steroid	Recurred	2.0
6	M/40	Trunk, leg	NA	Patches	Topical steroid	F/u loss	0.1
7	F/83	Face	NA	Papules	PUVA, CTx	DOD	2.0
8	M/26	Whole body	NA	Patches, plaques	PUVA, NBUVB, topical steroid	F/u loss	6.3
9	F/62	Trunk, axilla	5.5	Plaques	PUVA, CTx	F/u loss	5.8
10	M/52	Whole body	NA	Plaques	No treatment	F/u loss	0.2
11	M/38	Arm, leg	7.0	Plaques, nodules	CTx, RTx	Progressive	6.0
12	M/38	Arm, leg	8.0	Patches, plaques, nodule	CTx, RTx	Recurred	6.5
13	M/52	Whole body	10	Patches	PUVA, NBUVB, topical steroid	Recurred	6.0
14	M/39	Trunk	0.5	Patches	Topical steroid	F/u loss	0.3
15	F/23	Trunk, leg	2.0	Patches	PUVA, NBUVB, topical steroid	Recurred, progressive	1.0
16	M/44	Trunk, arm, leg	2.5	Patches	NBUVB	F/u loss	1.5
17	M/33	Whole body	NA	Patches	MTX, NBUVB	Progressive	4.0
18	M/28	Abdomen	NA	Patches	NBUVB	Improved	2.0

19	M/42	Abdomen, back, ankle	NA	Patches	PUVA, NBUVB, topical steroid	Recurred	2.0
20	M/56	Whole body	7.0	Plaques, nodules, tumor	CTx	DOD	2.3
21	F/70	Scalp, trunk	3.0	Plaque	CTx, RTx	Progressive	0.2
22	F/69	Trunk	3.0	Plaques	CTx, RTx	Progressive	0.7
23	M/70	Trunk, arm, thigh	10	Patches	NBUVB, topical steroid	Improved	0.1
24	M/38	Trunk, thigh	NA	Patches	NBUVB	Improved	0.8
25	F/23	Back	1.0	Patches	NBUVB	Improved	0.5

NA, not assessed; MTX, methotrexate; PUVA, psoralen plus UVA treatment; NBUVB, narrow band UVB therapy; CTx, chemotherapy; RTx, radiotherapy; f/u, follow-up; DOD, died of disease.

B. Results of immunohistochemical staining

Five T_{FH} markers in 49 primary cutaneous T-cell lymphoma tissues were investigated. The results are summarized in Tables 3 and 4. Figures 1 and 2 show the representative immunohistochemical staining of T_{FH} markers. PD-1 was frequently expressed in most of the MF cases, but rarely expressed in other cutaneous T-cell lymphoma. On the other hand, Bcl-6, CXCL13, and ICOS were scantly expressed in most T-cell lymphomas, including MF.

PD-1 was detected in 21 (84.0%) of 25 MF patients and in 11 (45.8%) of 24 other cutaneous T-cell lymphoma cases. Cases positive for Bcl-6, CXCL13, and ICOS were 32.0% in MF patients and 25.0%, 37.5%, and 20.8% in other cutaneous T-cell lymphoma, respectively (Table 4). CD10 expression was not detected in any of the cases.

Three MF cases with large-cell transformation were enrolled in this study, and all were negative for PD-1 expression (case 12, 20, 21; Table 3).

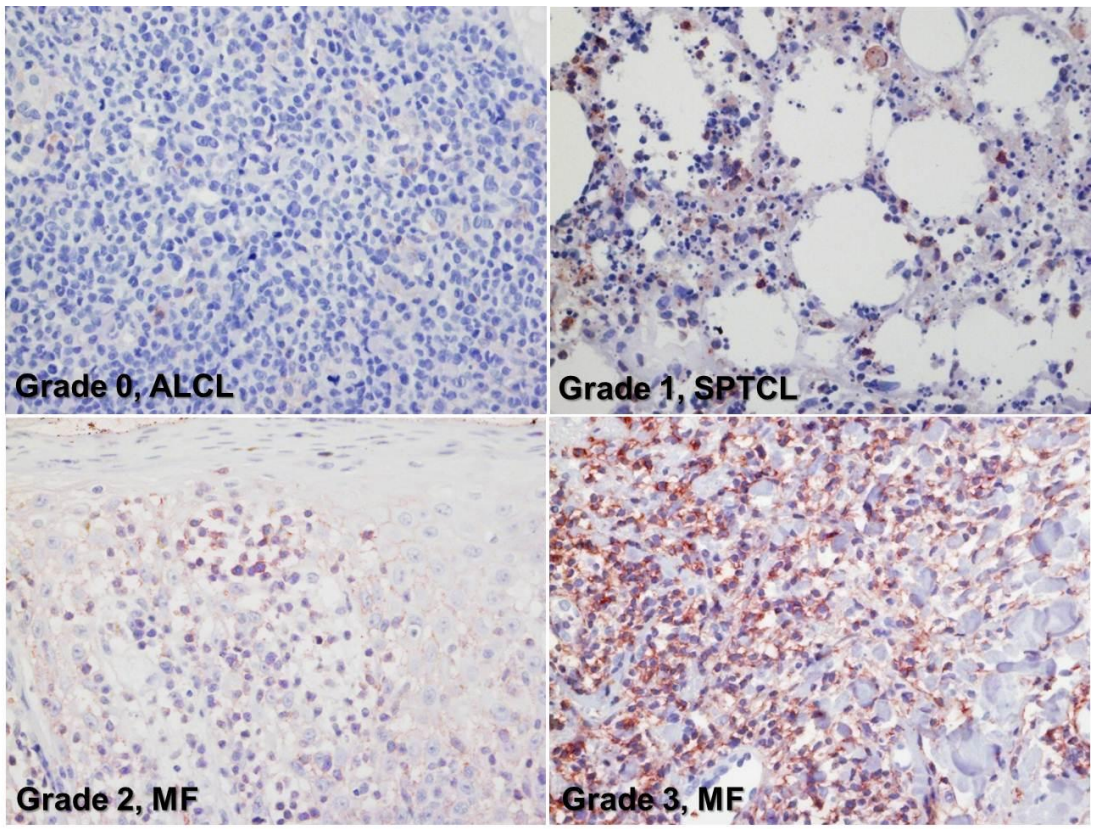
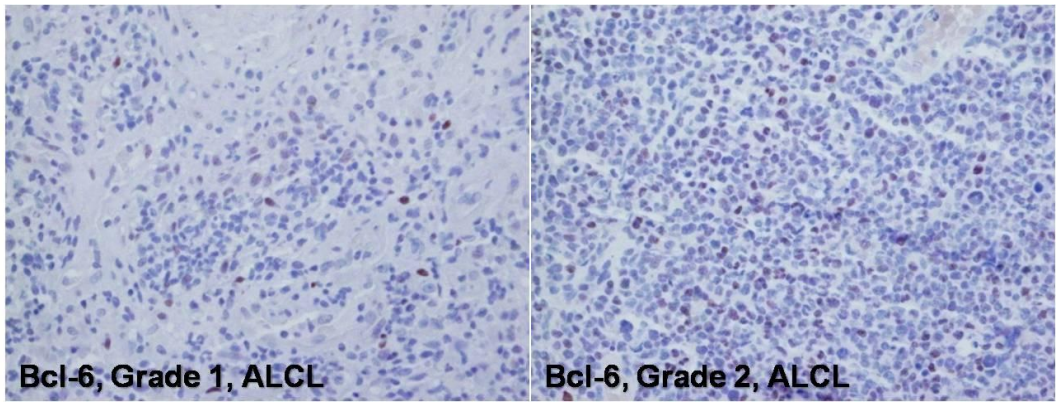
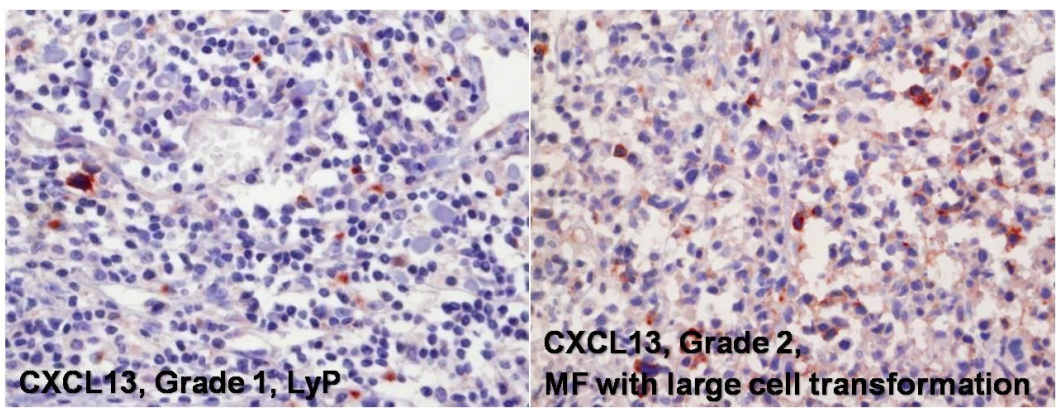


Fig. 1. Results of immunohistochemical staining for PD-1. The expression level was categorized as grade 0 to 3. Most MF specimens showed high PD-1 expression, whereas some cutaneous T-cell lymphomas showed low expression (original magnification ×200).

(A) Bcl-6



(B) CXCL13



(C) ICOS

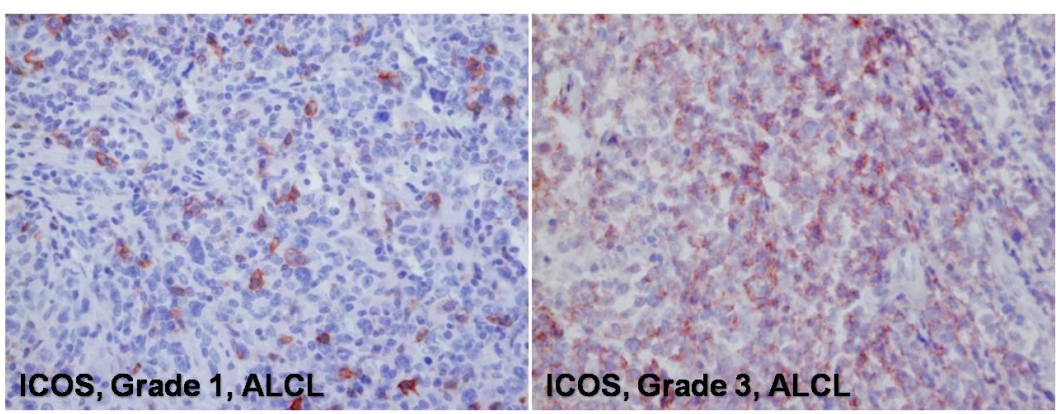


Fig. 2. Results of immunohistochemical staining for Bcl-6, CXCL13, and ICOS. In most cutaneous T-cell lymphomas, including MF, scant expression of Bcl-6, CXCL13, and ICOS was observed (original magnification ×200).

Table 3. Results of immunohistochemical staining for PD-1, Bcl-6, CXCL13, and ICOS.

Case No.	Diagnosis	PD-1	Bcl-6	CXCL13	ICOS
1	MF	3	0	0	1
2	MF	1	0	0	0
3	MF	1	0	0	0
4	MF	3	1	1	1
5	MF	3	0	1	0
6	MF	3	1	0	0
7	MF	1	1	1	0
8	MF	3	1	1	0
9	MF	1	0	1	1
10	MF	3	0	0	0
11	MF	1	1	1	0
12	MF*	0	1	0	1
13	MF	0	0	0	0
14	MF	3	0	0	0
15	MF	2	0	0	1
16	MF	3	0	0	0
17	MF	3	0	0	0
18	MF	3	0	0	0
19	MF	2	1	1	1
20	MF*	0	0	2	1
21	MF*	0	0	0	0
22	MF	1	0	0	1
23	MF	3	0	0	0
24	MF	1	1	0	0
25	MF	3	0	0	0
26	LyP	0	0	1	0
27	LyP	0	1	0	0
28	LyP	0	0	0	0
29	LyP	1	0	0	0

30	LyP	1	0	1	0
31	LyP	1	1	0	0
32	LyP	1	0	1	0
33	LyP	2	0	0	1
34	LyP	0	0	0	0
35	ALCL	0	0	0	0
36	ALCL	0	1	1	0
37	ALCL	0	2	0	0
38	ALCL	0	1	0	0
39	ALCL	0	0	0	1
40	ALCL	0	0	1	0
41	ALCL	1	0	0	0
42	ALCL	1	0	0	0
43	SPTCL	2	0	0	0
44	SPTCL	1	0	0	2
45	SPTCL	0	0	0	0
46	SPTCL	0	0	1	0
47	NK/T	0	0	1	0
48	PTCL	1	0	1	2
49	PTCL	1	1	1	1

MF, mycosis fungoides; LyP, lymphomatoid papulosis; ALCL, anaplastic large cell lymphoma; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; NK/T, NK/T-cell lymphoma; PTCL, peripheral T-cell lymphoma.

*MF with large cell transformation.

Table 4. The expression rates of PD-1, Bcl-6, CXCL13, and ICOS in MF and other cutaneous T-cell lymphomas.

Subtype	PD-1 (%)*	Bcl-6 (%)	CXCL13 (%)	ICOS (%)
MF	84.0	32.0	32.0	32.0
Other CTCL	45.8	25.0	37.5	20.8

The differences were evaluated by the $\chi 2$ test. *$P < 0.05$

CTCL, cutaneous T-cell lymphoma.

C. Statistical analysis

The cases were assorted into 2 groups: MF and other cutaneous T-cell lymphoma. The PD-1 expression was more frequent in MFs than other cutaneous T-cell lymphomas ($P < 0.05$). Specificities for PD-1, Bcl-6, CXCL13, and ICOS were 76.5%, 51.4%, 46.9%, and 52.8%, respectively, and sensitivities for PD-1, Bcl-6, CXCL13, and ICOS were 65.6%, 57.1%, 47.1%, and 61.5%, respectively (Table 5).

Table 5. Specificity and sensitivity of PD-1, Bcl-6, CXCL13, and ICOS for MF.

	PD-1 (%)	Bcl-6 (%)	CXCL13 (%)	ICOS (%)
Sensitivity	65.6	57.1	47.1	61.5
Specificity	76.5	51.4	46.9	52.8

D. Relation between PD-1 expression and clinical course

Excluding patients who were lost to follow-up, 2 of 4 PD-1–negative MF patients (50%) and 6 of 15 PD-1–positive MF patients (40%) showed progressive course in spite of treatment or died of disease. No correlation was observed between clinical morphology, disease course, and PD-1 expression rate among MF cases (Table 6).

Table 6. Relation between PD-1 expression and clinical course. The PD-1 expression rate and clinical course among MF cases showed no correlation.

PD-1 expression	Number of cases (except cases lost to f/u)	Number of cases with poor prognosis*	Percentage (%)
0	4	2	50.0
1	6	3	50.0
2	2	1	50.0
3	7	2	28.6

f/u, follow-up.

*Poor prognosis was defined as treatment unresponsiveness with disease progression or fatality.

IV. DISCUSSION

The expression of T_{FH} markers in primary cutaneous T-cell lymphoma was evaluated under the assumption that some tumor cells may be derived from $CD4^+$ T-cells and that T_{FH}-cells are a subset of $CD4^+$ helper T-cells.

The T_{FH}-cell functions are mediated through the production of cytokines and through the engagement of costimulatory molecules (such as ICOS, CD28, CD40L, and PD-1) and other receptors (SLAM-associated protein, CXCR5, etc) that favor strong interactions with B-cells and consequently B-cell responses. Some of these cytokines, costimulatory molecules, and receptors can be used as markers to identify reactive or neoplastic T_{FH}-cells by using flow cytometry or by performing immunohistochemical analyses on routinely fixed tissue samples. For diagnostic purposes, CXCL13, PD-1, ICOS, and Bcl-6 may represent the most useful immunohistochemical T_{FH} markers (Gaulard and de Leval, 2011).

CXCL13 is a chemokine, which is critical for B-cell recruitment into the germinal centers and for B-cell activation. The transcription factor Bcl-6 drives T_{FH} formation (Nurieva et al., 2009). Bcl-6 expression in T_{FH}-cells upregulates expression of CXCR5, ICOS, PD-1, IL-21R, and IL-6R. Furthermore, the ICOS molecule, a CD28 homolog implicated in T-cell activation and differentiation, interacts with counter receptors on B-cells (Gaulard and de Leval, 2011). In this study, these markers were rarely expressed in most T-cell lymphomas, including MF.

CD10 is a cell surface glycoprotein with endopeptidase activity that is expressed on a variety of hematopoietic and epithelial cells (McIntosh et al., 1999). CD10 is also expressed on a subset of $CD4^+$ T-cells referred to as T_{FH}-cells, which reside in the lymph node follicle germinal center. However, CD10 expression was not detected in all of the cases in this study. Moreover, identification of tumor cells in skin biopsies by using immunohistochemical analyses to detect CD10 expression on tumor cells is complicated because tumor-associated dermal fibroblasts have also been shown to express CD10 (Bilalovic et al., 2004; Takahara et al., 2009). As an alternative to immunohistochemical tests, CD10 on tumor cells can be detected by using flow cytometry (Oshtory et al., 2007).

PD-1 cell-surface protein, an inhibitory member of the CD28 costimulatory receptor family, is a negative regulator of T-cell activity that presumably regulates selection and

survival of germinal center B-cells (Nishimura et al., 1996). In this study, PD-1 expression was observed in higher number of MF cases than in other cutaneous T-cell lymphoma cases. Moreover, PD-1 expression showed a significant correlation with MF.

Several cases of cutaneous T-cell lymphoma with T_{FH}-cell phenotype have been reported. Battistella et al reported 5 such cases and suggested that these cutaneous T-cell lymphomas with expression of T_{FH}-cell markers comprised a new phenotype (Battistella et al., 2012). They also proposed provisional criteria for these lymphomas, which include: clinical symptoms such as development of papules, plaques, and nodules (no patch stage); no B-cell lymphoma systemic signs; and histopathological features such as diffused dermal infiltrate of pleomorphic medium to large $CD4^+$ T-cells, prominent B-cell component with some immunoblasts, T_{FH}-cell phenotype of neoplastic cells (i.e., $\geq$2 positive markers among CD10, Bcl6, PD-1, CXCL13, or ICOS). In this study, however, the majority of the T_{FH} marker (+) MF cases showed patch stage, and B-cell component was not histologically prominent. Thus, the study cases did not match the new phenotype defined by Battistella et al.

Most of the MF samples expressed T_{FH}-cell markers, and PD-1 expression rates were significantly higher in the MF samples than in the other cutaneous T-cell lymphoma samples. These findings suggest that some MF tumor cells might consist of T_{FH}-cells. The recently reported MF cases that are positive for the T_{FH} marker raise this possibility (Gammon and Guitart, 2012; Kempf et al., 2012; Meyerson et al., 2012). In addition, the result in this study suggests that PD-1 can be an MF-sensitive marker, and it might be useful in differentiating MF from other cutaneous T-cell lymphomas.

A high PD-1 expression rate in MF might suggest that some MF tumor cells were derived from T_{FH}-cells. However, no single marker, including PD-1, Bcl-6, CXCL13, ICOS, and CD10, was found to be specific for the T_{FH}-cell lineage. To prove the derivation from follicular helper cell, a combination of various T_{FH}-cell marker expression is required (Samimi et al., 2010). In addition, the expression of T_{FH} markers in several cases of subcutaneous panniculitis-like T-cell lymphomas, which are usually $CD4^-$ and $CD8^+$, could not be explained. Additional studies with larger number of patients are needed to confirm this finding.

Large-cell transformation in MF is defined as the presence of large cells at a number that exceeds 25% of the cell population (Salhany et al., 1988). Although large-cell transformation

has been documented to occur in all stages of cutaneous T-cell lymphoma, it is most frequently diagnosed in patients with more advanced disease, and it is often associated with a poor response to treatment and poor prognosis. Studies have shown that MF and large-cell transformation have a common clonal origin; however, the morphologic, phenotypic, and molecular risk factors that result in the development of large-cell transformation are largely unknown (Wood et al., 1994; Wolfe et al., 1995). The results of a previous study suggested that expression of CD25 (interleukin-2R) may identify a subset of patients at risk for large-cell transformation (Stefanato et al., 1998). In addition, some researchers have demonstrated correlations between large-cell transformation and an increase or decrease of regulatory T-cells (Gjerdrum et al., 2007; Hallermann et al., 2007), chromosomal tetraploidy (Prochazkova et al., 2005), or aneuploidy (Dmitrovsky et al., 1987). Recently, Kantekure et al presented results of the immunohistochemical staining of the PD-1 and PD-L1 expression in MF, which were based on the clinical and histological stages (Kantekure et al., 2012). In that report, PD-1 was expressed frequently in the early patch and plaques stages of MF, and it was expressed less frequently in the tumor stage of the lymphomas. Additionally, PD-1 expression was rarely detected in the large, transformed lymphocytes.

In this study, all the MF cases with large-cell transformation were negative for PD-1 expression. This result is consistent with the results of a previous study (Kantekure et al., 2012). In this study, skin specimens had been obtained before the large-cell transformation for 2 out of the 3 large-cell transformation cases. Additional PD-1 immunostaining was performed in those 2 specimens and both were positive for PD-1. The loss of PD-1 expression in large-cell–transformed cases is intriguing because it raises the possibility that PD-1 downregulation may be related to the biological process that ultimately results in large-cell transformation. Although additional studies with larger number of patients are needed to confirm this observation, our findings suggest that loss of PD-1 expression in MF may be a possible marker to predict large-cell transformation, with potential impact on treatment modalities and patient survival.

V. CONCLUSION

In this study, most MF samples expressed T_{FH}-cell markers. In addition, PD-1 expression rates were significantly higher in MF than in other cutaneous T-cell lymphomas. This implies that PD-1 can be an MF-sensitive marker, and it might be useful in differentiating MF from other cutaneous T-cell lymphomas. Interestingly, loss of PD-1 expression in large-cell transformation was identified. This finding suggests that PD-1 downregulation may be related the biological process that ultimately results in large-cell transformation.

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– 국문요약 –

원발성 피부 T-세포 림프종의 여포성 보조 T-세포 표지자 발현에 대한 연구

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연구 배경 및 목적: 최근 보조 T-세포의 아집단 중 하나인 여포성 보조 T-세포에 관한 연구들이 보고되고 있다. 여포성 보조 T-세포는 CXCL13, PD-1, ICOS, Bcl-6 를 포함한 몇 가지 보조 T-세포 표지자의 발현을 통해 확인될 수 있다. 현재까지 T-세포 림프종의 일부에서는 여포성 보조 T-세포 표지자의 발현이 확인되었으나, 피부 T-세포 림프종에서의 여포성 보조 T-세포 발현에 대해서는 보고된 바가 드물다. 따라서 본 연구에서는 피부 T-세포 림프종의 임상, 조직학적 특징과 함께 여포성 T-세포 표지자의 발현을 확인하고자 하였다.

재료 및 방법: 피부 T-세포 림프종으로 진단된 49 명 (균상식육종 25 예, 림프종양구진증 9 예, 역형성큰세포림프종 8 예, 피하지방층염유사 T 세포림프종 4 예, 말초 T 세포림프종 2 예, NK/T 세포림프종 1 예)의 환자를 대상으로 연구를 시행하였다. 의무기록 검토를 통해 임상 소견을 확인하였고, H&E 염색 하에서 대상 환자들의 조직학적 소견을 확인하였다. 추가적으로 CD10, Bcl-6, ICOS, CXCL13, PD-1 에 대한 면역화학적 염색을 시행하였으며, 각 항체의 발현 정도를 0 부터 3 까지 등급을 나누어 점수화하였다.

결과: PD-1 의 발현은 대부분의 균상식육종 조직에서 높게 나타났으나, 균상식육종 외 다른 피부 T-세포 림프종에서는 낮은 발현률을 보였다. Bcl-6, CXCL13, ICOS, CD10 은 균상식육종을 포함한 대부분의 T-세포 림프종에서

거의 발현되지 않거나 매우 낮은 발현률을 보였다. 흥미롭게도, 대세포 전환을 보인 균상식육종 (MF with large cell transformation) 모두에서 PD-1 의 발현이 나타나지 않았다. 균상식육종 환자들의 임상양상, 질병경과와 PD-1 발현률 사이에는 관련성이 없었다.

결론: 대부분의 균상식육종에서 여포성 보조 T-세포 표지자가 발현되어, 균상식육종의 일부 종양세포는 여포성 보조 T-세포로 구성되어 있을 가능성을 유추해 볼 수 있다. 균상식육종에서 다른 피부 T-세포 림프종에 비해 PD-1 이 높은 발현률을 보이는 것을 볼 때, PD-1 이 균상식육종의 민감한 표지자로서 균상식육종과 다른 피부 T-세포 림프종 간의 감별진단에 유용하게 사용될 수 있을 것으로 생각된다. 대세포 전환을 보인 균상식육종에서 PD-1 의 발현이 소실된 것은 PD-1 의 하향조절이 대세포전환을 일으키는 생물학적 과정과 관련되어 있을 가능성을 나타낸다.

핵심어: 여포성 보조 T-세포, 피부 T-세포 림프종, 균상식육종, PD-1