Differential Expression of Programmed Death-1 (PD-1) in Sézary Syndrome and Mycosis Fungoides

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Objective: To determine if there are differences in the expression of programmed death-1 (PD-1) between SS and MF, and in particular erythrodermic MF (E-MF). PD-1 is a marker of follicular helper T (T_fH) cells and is expressed by the neoplastic T cells of some types of malignant T-cell lymphoma, including mycosis fungoides (MF) and Sézary syndrome (SS). Reported results of PD-1 staining in MF and SS are, however, conflicting.

Design: Formalin-fixed, paraffin-embedded skin biopsy specimens were stained for PD-1. In addition, PD-1/H11001 cases were stained with antibodies against BCL6, CXCL13, and CD10 to find possible relationship with T_fH cells.

Setting: Tertiary referral center for cutaneous lymphomas.

Patients: Twenty-seven patients with SS and 60 patients with MF, including 8 patients with E-MF.

Results: In patients with SS, expression of PD-1 by more than 50% of the neoplastic T cells was observed in 24 of 27 cases (89%). In contrast, PD-1 expression by more than 50% of neoplastic T cells was found in only 8 of 60 patients with MF (13%), including only 1 of 8 patients with E-MF (12%). In PD-1/H11001 cases, serial skin sections showed that CXCL13 and BCL6 generally stained 25% to 50% of the PD-1/H11001 cells, while expression of CD10 was uncommon.

Conclusion: The results of the present study show differential expression of PD-1 between SS and MF/E-MF, which provides further support for the view that SS and MF are distinct entities.

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Recent studies described the expression of membrane molecule programmed death-1 (PD-1; CD279) by the neoplastic T cells of various types of malignant T-cell lymphomas, including Sézary syndrome (SS) and mycosis fungoides (MF).1-6 PD-1 belongs to the CD28/CTLA-4 receptor family and has a key role in regulating cellular immune responses.7,8 PD-1 has 2 identified ligands called PD-L1 (CD274), which has a broad expression profile, including nonimmune cells, and PD-L2 (CD273), which is found primarily on activated professional antigen-presenting cells.7,8 Engagement of PD-1 with its ligands has been shown to inhibit T-cell activation and proliferation.9 Since loss of PD-1 is associated with the development of autoimmune diseases, PD-1 is considered to play an important role in peripheral tolerance.7,8 Whereas activation is necessary to induce PD-1 expression by T cells, B cells, and dendritic cells, some cell types, such as follicular helper T (T_fH) cells, have a constitutive high expression of PD-1.10 T_fH cells, which also highly express chemokine (C-X-C motif) ligand 13 (CXCL13) and B-cell lymphoma 6 (BCL6), are localized in the lymph node follicles and provide essential support to germinal center formation and plasma cell development.

Recent studies showed that the neoplastic cells in angioimmunoblastic T-cell lymphoma (AITL) express PD-1, CXCL13, BCL6, and CD10.11,12 Because of this phenotypical resemblance to T_fH cells, AITL is now commonly considered to be a tumor of T_fH cells. More recently, we and others reported that the atypical medium- to large-size CD4 T cells in primary cutaneous CD4 small- to medium-size pleomorphic T-cell lymphoma (PCSM-TCL) also express PD-1. Since 25% to 75% of these PD-1+ cells also express CXCL13 and BCL6, it has been suggested that this type of cutaneous T-cell lymphoma (CTCL) may also originate from T_fH cells.11,12

Samimi et al found increased PD-1 expression in the circulating neoplastic T cells in patients with SS, but not in CD4+ peripheral blood T cells from patients with MF or healthy controls, and suggested that PD-1 may contribute to immunosuppression in SS. In other studies, PD-1 expression by the neoplastic T cells was found in 50% to 60% of skin biopsy samples of
patients with MF and 73% of skin biopsy samples of those with SS. 
In contrast, in our recent study on PCSTMTC, PD-1 expression was detected in only 2 of 21 MF biopsy samples, included as a control group. These discrepant results prompted us to investigate PD-1 expression in a large group of biopsy samples obtained from patients with SS and those with different stages of MF. In addition, we also investigated all PD-1 patients with SS and those with different stages of MF.

**PATIENTS**

Paraffin-embedded skin biopsy specimens from 27 patients with SS and 60 patients with MF were selected for this study. All skin samples were collected from the archives of the Department of Pathology, Leiden University Medical Center (LUMC) (Leiden, the Netherlands) according the “Dutch Code for Proper Secondary Use of Human Tissue,” which is approved by the medical ethics committee of the LUMC. In some patients, multiple skin biopsy specimens obtained from different stages of disease or during follow-up, and additional specimens from involved lymph nodes were studied. All cases had been reviewed by an expert panel of dermatologists and pathologists during one of the quarterly meetings of the Dutch Cutaneous Lymphoma group, and were classified according to the criteria of the World Health Organization–European Organization of Research and Treatment of Cancer classification (WHO-EORTC) for primary cutaneous lymphomas. Stage of disease was based on the revised International Society for Cutaneous Lymphoma/EORTC criteria. All patients with SS had erythroderma and peripheral blood involvement at first presentation (T4N0-3B2). Twenty-five of 27 patients with SS showed clonal T-cell receptor gene rearrangement in combination with a CD4/CD8 ratio of more than 10 (range, 12-232) in the peripheral blood. In the other 2 patients, peripheral blood involvement was confirmed by the presence of a T-cell clone in combination with either a CD4/CD8 ratio greater than 40 or the presence of more than 1000 Sézary cells/µL.

The MF group included 30 biopsy specimens from patients with patches or plaques (stage IA-IB), 22 biopsy specimens from skin tumors (19 from patients with stage IIB disease, 3 from patients with stage IV) and 8 biopsy specimens from patients with E-MF (3 patients with stage III disease; 5 patients with stage IV). Twenty-one of these 60 skin biopsy specimens were included in a previous study, where they served as a control group. With respect to the E-MF group, 5 patients had a history of classical (folliculotropic) MF and developed E-MF with (cases 2-4) or without (cases 1 and 5) (Table 1) peripheral blood involvement 24 to 240 months after initial diagnosis, while 3 presented with erythroderma with involvement cases 6-8) Table 1). Since the definition and terminology of cases with E-MF are subject to debate, the main features of these 8 cases have been summarized in Table 1.

**METHODS**

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**HISTOLOGIC AND IMMUNOHISTOCHEMICAL ANALYSIS**

In sections from all biopsy specimens, we routinely performed hematoxylin–eosin stainings and immunostainings to detect T-cell–associated antigens (CD2, CD3, CD4, CD5, CD8), B-cell–associated antigens (CD20 and/or CD79a), and histiocytic antigens (CD68) and CD30. Sections from all patients were stained for PD-1, and in addition, PD-1 cases for expression of BCL6, CXCL13, and CD10 to determine if these cells have a TFH-cell phenotype. Our results show differential expression of PD-1 in SS and MF, which provides further support to the view that SS and MF are distinct entities.

**STATISTIC ANALYSIS**

Statistical calculations were performed using SPSS statistical software (version 17.0; SPSS Inc). Comparison of PD-1 expres-
SÉZARY SYNDROME

Routine immunostaining revealed a CD3+, CD4+, CD8− T-cell phenotype in all SS cases. Marker loss of CD2, CD3, or CD5, defined as absent staining by more than 50% of the neoplastic T cells, was detected in only 1 of 27 SS cases (3.7%), in which the neoplastic cells lacked CD2. In 2 other cases there was partial loss (< 50% loss) of either CD3 or CD2. Staining for CD30 was performed in 24 of 27 cases. In 4 cases, CD30 expression was found in almost 100%, more than 50%, 30%, and 15% of the neoplastic T cells, respectively, while in the other 20 cases, no or few (<10%) CD30+ neoplastic T cells were found.

In 24 of the 27 SS cases, more than 50% of the neoplastic T cells in the skin expressed PD-1, in 22 cases even more than 75% (Figure 1 and Table 2). Six follow-up biopsy specimens of 3 of these 24 patients, obtained 24 to 48 months after diagnosis, also showed expression of PD-1 by more than 75% of the neoplastic T cells. PD-1 was expressed by small to large atypical T cells with cerebriform nuclei (Sézary cells) as well as by (CD30+) blast cells. In the 3rd of these cases, PD-1 was expressed by approximately 25% in 1 case, less than 10% in another case, while in the third case the neoplastic T cells were completely negative for PD-1. In the patient with less than 10% PD-1+ neoplastic T cells in the skin infiltrate, the lymph node biopsy specimen showed a diffuse population of PD-1+, CD3+, and CD4+ neoplastic T cells. A diffuse infiltration by PD-1+ tumor cells was also observed in the lymph node biopsy specimens from 3 other patients with SS.

Serial skin sections showed that CXCL13 and BCL6 generally stained 25% to 50% of the PD-1+ cells (Figure 1). In the 2 cases with less than 10% PD-1+ tumor cells, CXCL13 was expressed by 25% to 50% and BCL6 by approximately 25% of the neoplastic T cells. Staining for CD10 was performed in 25 cases, and results were negative in 21 cases. In the other 4 cases, all of them with more than 50% PD-1+ tumor cells, CD10 was expressed by 100% of the neoplastic T cells in 1 case and by 25% to 50% in the other 3.

MYCOSIS FUNGOIDES

Immunostaining showed a CD4+CD8− T-cell phenotype in 36 patients, a CD4+CD8+ T-cell phenotype in 9 patients, and a CD4+CD8− T-cell phenotype in 15 of 60 patients. Including the 15 CD4+CD8+ patients, marker loss was observed in 35 of 60 biopsy specimens (58%), including 16 of 30 patches or plaques (53%), 14 of 22 tumors (64%), and 5 of 8 cases of E-MF (63%). Expression of CD30 by more than 25% of the neoplastic T cells was observed in 11 biopsy specimens, including 5 plaques, 5 tumors, and 1 biopsy obtained from E-MF.

Expression of PD-1 by more than 50% of the neoplastic T cells was observed in 8 of 60 patients (13%), including 4 of 30 patches or plaques (13%), 3 of 22 tumors (14%), and, interestingly, only 1 of 8 biopsy specimens from E-MF (13%). PD-1 expression was significantly higher in the SS group compared with the total MF group (P < .001), as well compared with the E-MF group (P < .001). The results of PD-1 staining in a second biopsy, obtained from either a preceding plaque or a subsequent tumor in 8 of 60 patients, were identical to those of the first biopsy. The 8 PD-1+ cases included both patients with a CD4+CD8+ phenotype (4 cases), a CD4+CD8− phenotype (1 case) and a CD4+CD8− T-cell phenotype (3 cases). In these cases, PD-1 stained both atypical T cells with cerebriform nuclei and (CD30+) blast cells. In the 11 biopsy specimens containing more than 25% CD30+ tumor cells, PD-1 was expressed by 30% to more than 75% of the CD30+ blast cells in 3 cases, while these cells were negative for PD-1 in the other 8 cases. In addition to the 8 cases with more than 50% PD-1+ tumor cells, in 1 case 25% to 50% and in 3 cases 1% to 25% of the neoplastic T cells expressed PD-1 (Table 2). In the other 48 cases, no or sometimes few (but always < 10%) neoplastic T cells expressed PD-1 (Figure 2), while in all biopsy specimens, small numbers of reactive PD-1+ T cells were observed, serving as an internal control. Examination of serial sections showed that both CXCL13 and BCL6 generally stained 25% to 50% of the PD-1+ cells. CD10 was expressed by almost all neoplastic T cells in only 1 of 8 biopsy specimens with more than 50% PD-1+ tumor cells, and in none of the 10 biopsy specimens with 10% to 50% PD-1+ tumor cells.

ADDITIONAL STUDIES IN E-MF

After this initial analysis additional skin and lymph node biopsy specimens were collected from 6 of 8 patients with E-MF. These biopsy specimens were obtained from plaques 4 and 20 years before the development of erythroderma (cases 2 and 3) (Table 1), from erythrodermatous skin during follow-up 12 to 60 months after diagnosis (cases 3, 6, and 8) (Table 1), and from diffusely involved lymph nodes (N3 stage; cases 1, 5, 6, and 8) (Table 1). Four patients showed folliculotropic infiltration in the first biopsy specimens at the nonerythrodermic stage and in the follow-up biopsy specimens at the erythrodermic stage. In the single patient who was PD-1+ (Table 1) (case 2), PD-1 was expressed by more than 75% of the neoplastic T cells both in the erythrodermatous skin and in the plaque biopsied 20 years before. In the 5 other patients, PD-1 was consistently negative, both in preceding plaques, follow-up biopsy specimens and involved lymph nodes (Table 1).

In the present study we observed a differential expression of PD-1 in skin biopsy specimens from patients with SS and MF. In 24 of 27 patients with SS (89%), PD-1 was expressed by more than 50% of the malignant T cells, while in 1 of the 3 remaining cases the neoplastic cells...
in the involved lymph node were PD-1+. In contrast, in only 8 of 60 patients with MF (13%), PD-1 was expressed by more than 50% of the neoplastic cells.

The proportion of PD-1+ MF cases (13%) in our study contrasts with the results of 3 other studies. Wada et al6 reported a percentage of more than 50% PD-1+ tumor cells in 6 of 15 patches or plaques (40%), 9 of 15 tumors in patients with MF (60%), and in 8 of 11 biopsy specimens from patients with SS (63%). In the study of Kantekure et al,13 16 of 26 MF biopsy specimens (62%) contained more than 25% PD-1+ tumor cells, but if the cutoff point of 50% of the present study was used, only 7 of 26 biopsy specimens from patients with MF (26%) were PD-1+. Roncador et al14 observed PD-1 expression in 5 of 9 MF cases (56%) but did not provide a cutoff point for PD-1 positivity. The results of the present study

Figure 1. Histopathologic features of a representative patient with Sézary syndrome. The hematoxylin-eosin staining of the lesion showed a superficial bandlike dermal infiltrate and epidermal Pautrier microabscesses (A) with CD4+ (B) and CD3+ (C) neoplastic T cells. These neoplastic T cells showed strong PD-1 positivity (D), and serial skin sections showed that BCL6 (E) and CXCL13 (F) was expressed by 25% to 50% of the PD-1+ cells. A-D, Original magnification ×100. E and F, Original magnification ×400. Insets (in A and D), original magnification ×2.
are consistent with those of a previous study of our group. In that study, which was focused on PD-1 expression in PCSM-TCL and/or pseudo–T-cell lymphomas, 21 cases of MF with a CD4+/CD8– T-cell phenotype were included as a control group, and expression of PD-1 by more than 50% of the neoplastic T cells was found in only 2 of 21 cases. In the present study, we did not only include cases with a CD4+/CD8– T-cell phenotype, but—to cover the whole spectrum of MF—our database was actively searched for cases with a CD4+CD8+ or CD4+CD8– T-cell phenotype and in both CD30+ and CD30– cases. PD-1 expression was found not only in cases with a CD4+/CD8– T-cell phenotype, but also in cases with a CD4+CD8+ or CD4+CD8– T-cell phenotype and in both CD30+ and CD30– cases. The expression of PD-1 by CD30+ blast cells in some cases of MF and SS is of interest, since the neoplastic CD30+ T cells in cutaneous anaplastic large-cell lymphoma and

Table 2. Results of Programmed Death-1 (PD-1) Expression in Skin Biopsy Specimens of Patients With Sézary Syndrome (SS) and Mycosis Fungoides (MF)

<table>
<thead>
<tr>
<th>Type of Lymphoma</th>
<th>Cases, No.</th>
<th>PD-1+ Neoplastic T Cells, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&gt;50%</td>
</tr>
<tr>
<td>SS</td>
<td>27a,b</td>
<td>24 (89)</td>
</tr>
<tr>
<td>MF</td>
<td>60a</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Patch/plaque</td>
<td>30</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Tumor</td>
<td>22</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Erythrodermic MF</td>
<td>8b</td>
<td>1 (12)</td>
</tr>
</tbody>
</table>

a Patients with SS (n = 27) vs those with MF (n = 60): P < .001.

b Patients with SS (n = 27) vs those with erythrodermic MF (n = 8): P < .001.

Figure 2. Histopathologic features of a representative patient who was negative for programmed death-1 (PD-1) with mycosis fungoides. The hematoxylin-eosin staining (original magnification ×100) of the lesion showed an epidermal and superficial dermal infiltrate (A) with CD4+ (B) and CD3+ (C) neoplastic T cells. PD-1 expression was seen in less than 10% of the tumor cells (D).
lymphomatoid papulosis are consistently negative for PD-1.12

Remarkably, expression of PD-1 by more than 50% of the neoplastic T cells was observed in only 1 of the 8 patients with E-MF. In this patient, both erythroderma and the initial plaquelike lesion biopsied 20 years before were strongly positive for PD-1. In the other 7 patients, all skin and lymph node biopsy specimens were consistently negative for PD-1. It should be noted that, during follow-up, 4 patients in this group developed peripheral blood involvement, meeting the criteria normally used for the diagnosis of SS (B2 stage) (Table 1). There is controversy as to how such patients with MF developing peripheral blood involvement during follow-up should be designated. Some might prefer to simply use the term SS,17 others like SS preceded by MF18 or secondary SS,19 but we designate such cases E-MF with blood involvement.

These observations are important for the ongoing discussion regarding the relationship between SS and MF. Often, SS is designated as a leukemic phase or leukemic variant of MF. Indeed, there are morphologic (atypical cells with cerebriform nuclei) and phenotypical (CD3+CD4+CD8− T cells) similarities between both conditions, which resulted in the concept of CTCL.20 Moreover, patients with MF may sometimes present with erythroderma and/or develop peripheral blood involvement during follow-up, while rare patients with SS may develop skin tumors as in MF. However, apart from the differences in clinical presentation and clinical course, there are also histologic, phenotypic, and genetic differences between both conditions. Histologically, the neoplastic T cells in early-stage MF preferentially colonize the basal layers of the epidermis, while the neoplastic T cells in SS show a preferential perivascular distribution with or without prominent epidermal infiltration, reflecting the leukemic nature of the disease.19,20 Consistently, involved lymph nodes in SS are generally overrun by a monotonous infiltration of Sézary cells.21 In contrast, involved lymph nodes in MF show increased numbers of both atypical T cells with cerebriform nuclei and interdigitating reticulum cells, and in advanced stages blast cell transformation is much more pronounced than in SS lymph nodes.21 Phenotypically, SS consistently has a mature CD4+ T-cell phenotype. Also in MF, a CD4+ phenotype is most common, but patients may also present with a CD4+CD8+ or a CD4+CD8− T-cell phenotype.22 Moreover, both in the present study and previous studies on involved lymph nodes, loss of pan–T-cell antigens (CD2, CD3, CD5) is frequently observed in skin lesions and involved lymph nodes in MF, in particular in patients with advanced disease.23 In contrast, the neoplastic T cells in skin and lymph nodes of patients with SS generally retain their mature CD4+ T-cell phenotype, even in patients with advanced disease.23 In the present study, loss of pan–T-cell antigens was observed in only 1 of 27 patients with SS. More recent phenotypical studies showed that the neoplastic T cells obtained from the peripheral blood of patients with SS have a central memory T-cell phenotype, while T cells isolated from MF skin lesions had skin resident effector memory T-cell phenotype.24,25 The authors suggested that SS and MF should be considered as separate lymphomas arising from distinct functional T-cell subsets.24,25 Also, recent genetic studies showing major oncogenic differences between SS and tumor-stage MF suggested that these are distinct entities.20,27 The almost consistent expression of PD-1 and CXCL13 in SS28 and the uncommon expression in MF also support the view that SS and MF should be considered as distinct entities. This would imply that in future trials these conditions should be considered separately.

The consistent expression of PD-1, CXCL13, and BCL6 by the neoplastic cells in SS is similar to that of TFH cells in the germinal centers of lymph nodes, and similar to the neoplastic T cells inAITL, now commonly designated as a tumor of TFH cells.13 This raises the question of whether the neoplastic cells in SS are derived from TFH cells and/or have the functional characteristics of these TFH cells. TFH cells are located in lymph nodes providing essential support for B-cell maturation and plasma cell development. The chemokine CXCL13 selectively recruits CXCR5+B cells into the follicle and is involved in the activation of B cells.29 CXCL13 is expressed by the malignant T cells in lymph nodes inAITL and has been suggested to contribute to B-cell dysregulation.30 However, such a role seems unlikely in SS. Admixture of B cells is uncommon in skin biopsy specimens of SS, and also in our MF biopsy specimens no relationship between PD-1 expression and the number of admixed B cells or plasma cells was found (data not shown).

Two recent studies suggest another role for CXCL13 and PD-1 in SS. Picchio et al26 found high expression of CXCL13 in skin lesions, lymph nodes, and peripheral blood of patients with SS and noted that plasma levels of functional active CXCL13 expression increased significantly during disease progression. Interestingly, while the neoplastic T cells in most patients with SS were positive for CXCL13 (13 of 16 cases [81%]), in patients with MF, only 4 of 14 cases (28%) expressed CXCL13, which nicely parallels our observations on PD-1 expression in these conditions. Sokolowska-Wojdylo et al30 proposed that CXCL13 acts in synergy with CCR7 agonists CCL21 (present in both skin and lymph node of patients with SS) to promote the skin and lymph node homing of the Sézary cells that are known to have high expression of CCR7. In another study, Samimi et al31 found increased PD-1 expression in the circulating neoplastic T cells in patients with SS compared with patients with MF and healthy controls. They observed a strong decrease in PD-1 expression with improvement of disease. Moreover, an increase in interferon gamma production was found in cultures of PBMC from patients with SS if the PD-1/PD-L1 interaction was interfered by blocking anti–PD-1 antibodies. They suggested that the high PD-1 expression may contribute to immunosuppression in SS, but the mechanisms involved are as yet unknown. Given the high expression of PD-1 in Sézary cells, it is tempting to speculate that PD-1 on Sézary cells may serve as a potential target for immunotherapy. Interestingly, fully human blocking antibodies for PD-1 have recently been tested in a phase I clinical trial, showing promising results for different types of PD-L1+ solid tumors that are assumed to attenuate the PD-1+ tumor infiltrating T lymphocytes.32 Although in case of SS the situation is differ-
ent—SS is a T-cell tumor and the neoplastic cells are PD-1+ themselves—it may very well be that patients with SS may benefit from anti–PD-1 immunotherapy as well.

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Author Contributions: All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Willemze. Acquisition of data: Çetinözmân, Jansen, Vermeer, and Willemze. Analysis and interpretation of data: Çetinözmân, Jansen, and Willemze. Drafting of the manuscript: Çetinözmân and Willemze. Critical revision of the manuscript for important intellectual content: Çetinözmân, Jansen, Vermeer, and Willemze. Statistical analysis: Çetinözmân. Administrative, technical, and material support: Çetinözmân, Jansen, Vermeer, and Willemze. Study supervision: Willemze.

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REFERENCES


