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Expression patterns of the immunosuppressive proteins PD-1/CD279 and PD-L1/CD274 at different stages of cutaneous T-cell lymphoma (CTCL)/mycosis fungoides (MF)

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To the Editors

Programmed death-1 (PD-1/CD279) cell-surface protein, an inhibitory member of the CD28 costimulatory receptor superfamily, is expressed mainly on a subset of activated T lymphocytes including helper T cells located in the follicle germinal centers.[1,2] PD-1 inhibits T-cell activity[3] by providing a second signal to T cells in conjunction with signaling through the T-cell receptor for antigen. Two cell-membrane proteins designated PD-L1 (B7-H1CD274) and PD-L2 (B7-DC/CD273) have been identified as ligands for PD-1[4,5]. Interactions between PD-1 and the ligands control the induction and maintenance of peripheral T-cell tolerance during normal immune responses. These interactions may play a role also in immune evasion in malignancy,[6–8] since PD-1 expression tends to be upregulated on tumor infiltrating lymphocytes[9] and cancer cells of various types aberrantly express PD-L1[6].

In this report, we present results of the immunohistochemical examination of the PD-1 and PD-L1 expression at the patch/plaque, tumor, and large cell transformation stages of CTCL. CTCL cases for the study were retrieved from the Departments of Pathology and Laboratory Medicine and Dermatopathology at the University of Pennsylvania. The diagnosis of different stages of CTCL was established based on the clinical presentation and histologic and immunohistochemical evaluation. To perform the PD-1 and PD-L1 stains, the formalin-fixed paraffin-embedded CTCL tissue specimen slides were heat-treated for antigen retrieval by boiling in 10 mM citrate buffer pH 6 for 20 min. The sections were blocked with the peroxidase blocking system (Dako, Carpinteria, CA) for 10 min and 2% normal horse serum for 20 min at room temperature (RT). To detect PD1 expression, the slides were incubated with the primary PD-1 mouse monoclonal antibody (Abcam, Cambridge, MA) at 1:200 dilution (overnight at 4°C); secondary biotinylated anti-mouse antibody at 1:250 dilution (30 min at RT); the streptavidin-biotinylated horseradish peroxidase complex reagent (Dako) (30 min at RT); and three 5 min washes in buffer after each incubations. To detect the PD-L1 expression, the slides were incubated at RT with the rabbit PD-L1 antibody (Lifespan Biosciences, Seattle, WA) at 1:200 dilution for 30 min and an anti-rabbit-HRP polymer for 30 min followed by washes. The PD-1- or PD-L1-antibody stained slides were exposed to the DAB plus chromagen (Dako) for 5 min at RT and counterstained with hematoxylin. The

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results of this evaluation are summarized in Table 1 and the representative images are presented in Fig. 1. The retrieved 26 specimens were classified as 9 patch/plaque, 6 tumor, and 9 large cell transformation stage lesions.

PD-1 was frequently expressed at the early, patch and plaque stages of CTCL (Fig. 1, upper panel in the middle column). PD-1 was detected in all nine patch and plaque cases, in seven of the nine cases more than 25% of the atypical lymphocytes expressed PD-1 including three with staining was present in at least 50% of the lymphocytes (Table 1, middle column, rows 3–6). By comparison, PD-1 was expressed less frequently at the tumor stage of the lymphoma (Fig. 1, middle panel/middle column and Table 1, middle column, rows 8–11). Accordingly, PD-1-positive cells exceeded 25% in three of the six cases examined and in one they comprised more than 50%. Of note, the PD-1 expression loss preferentially affected lymphocytes that have undergone large cell transformation. (Fig. 1, lower panel/ middle column and Table 1, middle columns, rows 13–16); while the small atypical cells were frequently PD-1 positive, in 9 of the 11 cases examined PD-1-expressing large cells were extremely rare in none of the cases they comprised more than 25% of the large cell population. In contrast to PD-1, all stages of CTCL cases showed staining for PD-L1 in the majority of the atypical lymphocytes. If anything, PD-L1 expression actually seemed to increase with the lymphoma progression with essentially all large transformed lymphocytes strongly expressing the protein on their surface (Fig. 1, right panel column and Table 1, right column).

While the progressive nature of immunosuppression in CTCL is well recognized, the mechanisms that underlie the immune impairment remain essentially unknown. Our recent study demonstrated expression by the atypical lymphocytes in CTCL of FoxP3, the transcription factor defining the key type of regulatory T cells[11]. However, similar to PD-1, the FOXP3-expressing tumor cells were common at the patch and plaque stages but their frequency was profoundly diminished at the tumor stage and after the large cell transformation. These combined findings strongly suggest that the PD-1 and FoxP3 dependent immunosuppression is important at the early but not later stages of CTCL. As mentioned, PD-1 downregulation seems to result mainly from the large cell transformation of the CTCL cells; the FoxP3 loss appears to occur primarily at transition to the tumor stage[11]. Of note, the studies on the angioimmunoblastic T-cell lymphoma and other types of the nodal based peripheral T-cell lymphomas suggested that PD-1 expression is highly characteristic for, and most likely related to the follicle germinal center T helper cell derivation of the lymphoma cells.[1,12] Although CTCL cells share also another marker with follicular T helper cells: CXCL13,[13] they clearly are distinct from these cells given the lack of CD10 and BCL-6 expression as well as lack of follicle formation contrasted by the presence of epidermotropism in the typical CTCL lesions. Therefore, our observation that CTCL cells express PD-1 as well as the one made by others[1] regarding the primary cutaneous CD4+ pleomorphic T-cell lymphoma indicate that non-follicular T-cell lymphoma cells are also capable of expressing the receptor. The mechanisms leading to PD-1 expression by the various types of malignant T cells remain to be explored. Studies in animal model showed that PD-L1 expressed by tumors inhibits T cell activation, protects tumor cells from lysis, and in some cases induces death of the tumor-specific T cells.[14,15] Wilcox et al documented the importance of PD-L1 in the suppression of host immunity in the subset human T-cell lymphoproliferative disorders including CTCL and peripheral T cell lymphoma PTCL by showing that PD-L1 inhibits immune T-cells and promotes the induction of $FoxP3^+$ regulatory T cells.[16] Our current findings also point to PD-L1 as the likely key immunosuppressive protein in the advanced CTCL. Of note, the large T-cell lymphoma expressing anaplastic lymphoma kinase (ALK) is also PD-L1 positive,[10,17] suggesting some common pathogenic aspects for these two types of lymphoma. It may be related to the persistent activation of the transcription factor STAT3 since PD-L1 gene is

induced by the factor in the ALK-positive T-cell lymphomas[10] and the activated, tyrosine-phosphorylated form of STAT3 is expressed at all stages of CTCL[18] with some evidence suggesting particularly strong expression in advanced stage of the disease like tumor stage than MF[19]. Finally, our findings may have potential therapeutic implications for CTCL. Thus, antibody-induced blockade of PD-1[6] may improve the anti-tumor immunity and, in principle, could be used as an adjuvant to standard therapy, in particular at the early stages of CTCL when expression of this immunosuppressive receptor is the most pronounced. Similarly antibody-mediated inhibition of PD-L1[6,20] may increase the number and effectiveness of the tumor-specific immune T cells. This may be particularly important at the more advanced stages of CTCL, when the PD-L1 expression seems the strongest, the immunosuppression the most pronounced, and the current therapies are the least effective in controlling the lymphoma.

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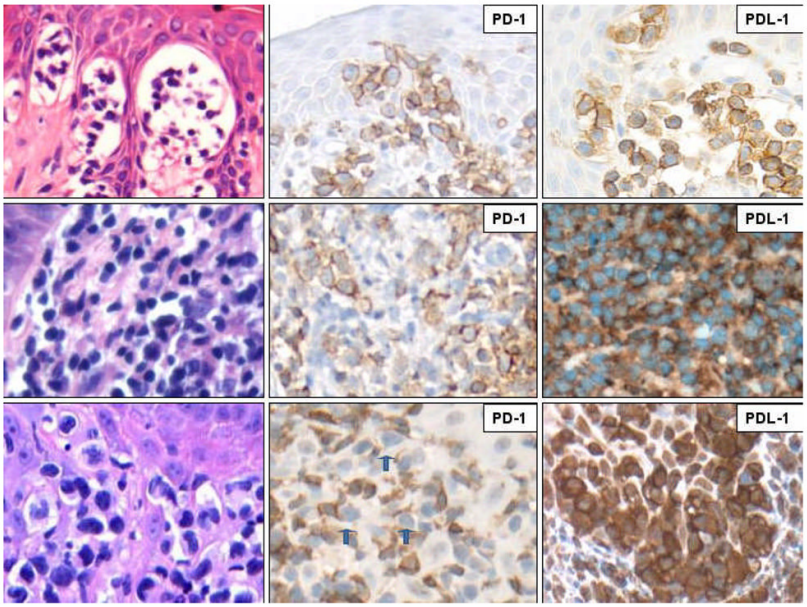

FIGURE 1.

PD-1 and PD-L1 expression at various stages of CTCL/MF: patch/plague (upper row), tumor (middle row), and large cell transformation (lower row). The formalin-fixed, paraffin-embedded skin biopsy tissue sections were stained with H&E (left column) or antibodies against PD-1 (middle column) or PD-L1 (right column). The depicted images are representative of the 26 CTCL cases examined and were captured at the 400X magnification. The blue arrows in the PD-1 stain of the large cell transformation case highlight the negative large lymphocytes.

Table 1

Expression of immunosuppressive proteins PD1 and PDL-1 at the depicted clinical-histological stages of CTCL/MF

	PD1		PDL-1	
Patch/plaque stage				
0–2 (% of atypical lymphocytes)	0 (number of cases)		0	
3–25%	2		1	
26–50%	4		1	
>50%	3		7	
Tumor stage				
0–2%	1		0	
3–25%	2		0	
26–50%	2		0	
>50%	1		6	
Large cell transformation	Small atypical lymphocytes	Large transformed lymphocytes	Small atypical lymphocytes	Large transformed lymphocytes
0–2%	1	9	0	0
3–25%	4	2	0	0
26–50%	3	0	0	0
>50%	3	0	11	11