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PROGRAMMED DEATH-1 EXPRESSION IN CUTANEOUS B-CELL LYMPHOMA

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ABSTRACT

Background: Numbers of programmed death-1 (PD-1) positive T cells have prognostic significance in some types of nodal B-cell lymphomas, but data on PD-1 expression in cutaneous B-cell lymphoma (CBCL) are few. In this study we determined the expression and distribution of PD-1 on neoplastic B cells and reactive T cells in skin sections from primary CBCLs.

Methods: By means of immunohistochemical staining, PD-1 expression was investigated in skin biopsies from 10 patients with primary cutaneous marginal zone lymphoma (PCMZL), 18 patients with primary cutaneous follicle center lymphoma (PCFCL) and 12 patients with primary cutaneous diffuse large B-cell lymphoma–leg type (PCLBCL–LT).

Results: Neoplastic B cells were negative for PD-1 in all cases, except for two cases of PCLBCL–LT. The frequency of PD-1+ T cells was significantly higher in PCFCL than in PCMZL and PCLBCL–LT, accounting for 20%, 10% and 3% of the total number of infiltrating cells, and 60%, 20% and 15% of the total number of CD3+ T cells, respectively.

Conclusions: PD-1 is rarely expressed by the neoplastic B cells in CBCL. High percentages of PD-1+ T cells, particularly if found outside germinal centers, support a diagnosis of PCFCL.
INTRODUCTION
Programmed death-1 (PD-1; CD279) belongs to the CD28/CTLA-4 receptor family and is expressed by CD4+ and CD8+ T cells after activation. Binding of PD-1 to its ligands PD-L1 (CD274) or PD-L2 (CD273) inhibits T-cell functions, such as proliferation, cytokine production and cytotoxic activity.1,2 Follicular helper T (TFH) cells are a special subset of CD4+ T cells, which have a constitutive high expression of PD-1. TFH are important for the formation of germinal centers and regulate the differentiation of germinal-center B cells into plasma cells and memory B cells.3,4 Interaction between PD-1 on the TFH cells and PD-L1 on the germinal-center B cells appears to enhance, rather than inhibit the germinal center reaction, however, the exact mechanism is not known.3,4

Several studies investigated PD-1 expression in nodal B-cell non-Hodgkin lymphomas (B-NHLs). Except for small lymphocytic lymphoma and rare cases of nodal follicular B-cell lymphoma and diffuse large B-cell lymphoma, PD-1 is generally not present on neoplastic cells in B-NHLs.5-7 However, PD-1 is detectable on variable amounts of tumor infiltrating lymphocytes, in particular in follicular lymphoma.7,11 Several studies reported an association in these nodal follicular lymphomas between increased numbers of PD-1+ T cells and a significantly improved survival, while in one study an association with a poorer outcome was found.7-10 Information on the expression of PD-1 in primary cutaneous B-cell lymphomas (CBCLs) is limited. Fanoni et al. reported positive immunohistochemical staining for PD-1 in primary cutaneous follicle center lymphoma (PCFCL), but not in primary cutaneous marginal zone lymphoma (PCMZL) and primary cutaneous diffuse large B-cell lymphoma–leg type (PCLBCL–LT).12 However, the authors did not mention whether neoplastic B cells or reactive T cells were stained. In a very recent study, PD-1 was found to stain tumor-infiltrating T cells, but no neoplastic B cells in these three types of CBCL.13

In this study, we investigated the expression of PD-1 in skin sections from primary CBCLs. The aim of this study was to find out if PD-1 is expressed by the neoplastic B cells of these lymphomas. In addition, the number and distribution of PD-1+ T cells was investigated and correlated with clinical behavior.

METHODS
Patients
Paraffin-embedded skin biopsies from 10 patients with PCMZL, 18 patients with PCFCL and 12 patients with PCLBCL–LT were selected for this study. In addition, five cases of cutaneous lymphoid hyperplasia (pseudo-B-cell lymphoma) were included as controls. All skin samples were collected from the archives of the Department of Pathology, Leiden University Medical Center (LUMC) according the "Dutch Code..."
Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>PCMZL</th>
<th>PCFCL</th>
<th>PCDLBCL–LT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>10</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>Male / Female</td>
<td>6/4</td>
<td>12/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td></td>
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</tr>
<tr>
<td>Median</td>
<td>48</td>
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<td>78</td>
</tr>
<tr>
<td>Range</td>
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<td>53–91</td>
</tr>
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<td>Localization</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>6</td>
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<tr>
<td>Trunk</td>
<td>9</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Leg(s)</td>
<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>Arm</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Multifocal</td>
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<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Follow-up (months)</td>
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</tr>
<tr>
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<td>76</td>
<td>39</td>
</tr>
<tr>
<td>Range</td>
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<td>2–160</td>
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<td>10</td>
<td>4</td>
</tr>
<tr>
<td>A^-</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>D^0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>D^-</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
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</table>

PCMZL, primary cutaneous marginal zone lymphoma; PCFCL, primary cutaneous follicle center lymphoma; PCDLBCL–LT, primary cutaneous diffuse large B-cell lymphoma–leg type; A^+, indicates alive with disease; A^0, alive without disease; D^+, died of lymphoma; D^0, died of unrelated cause.

for Proper Secondary Use of Human Tissue”, which is approved by the Medical Ethics Committee of the LUMC. All CBCL 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 (WHO–EORTC) classification for primary cutaneous lymphomas. All patients were adequately staged; physical examination, blood count, computed tomography scan and bone marrow biopsy were performed and showed no signs of extracutaneous disease manifestation at the time of diagnosis. The main clinical characteristics of these three groups of CBCL are presented in Table 1.

Histology and Immunohistochemistry

Sections from all biopsies had routinely been stained with hematoxylin-eosin and immunostainings to detect B-cell–associated antigens (CD20, CD79a, cytoplasmic immunoglobulins), germinal center B-cell–associated antigens (BCL6 and CD10), T-cell–associated antigens (CD3, CD4, and CD8), BCL2 and IRF4/MUM-1. For the purpose of this study sections from all patients were stained for PD-1 and CXCL13. Staining procedures for PD-1 and CXCL13, using antibodies from R&D Systems
PD-1 in Cutaneous B-Cell Lymphoma

(Abingdon, UK), have previously been described.\textsuperscript{16,17} The percentages of CD3\textsuperscript{+} T-cells, PD-1\textsuperscript{+} cells and CXCL13\textsuperscript{+} cells were expressed as a percentage of the total number of skin-infiltrating cells (both reactive and neoplastic). Using serial sections percentages of these cells were estimated independently by three observers (FC, PJ, RW) to the nearest 5\% for the whole section. In the few cases in which there was disagreement, sections were read jointly and consensus was reached.

**Statistical analysis**

Statistical significance was determined with one-way analysis of variance (ANOVA) and Student’s t-test using SPSS software, with $p< 0.05$ considered as significant.

Figure 1. Histopathologic features of a representative primary cutaneous marginal zone lymphoma (PCMZL) patient. (A) Patient presenting with a tumor in the right popliteal area. (B) Hematoxylin-eosin staining of the lesion showing nodular to diffuse infiltrates containing (C) CD79a\textsuperscript{+} neoplastic B cells and reactive follicles, and (D) high numbers of admixed T cells. (E) A small proportion of the T cells showed programmed death-1 (PD-1) positivity; these cells were typically found within reactive germinal centers. (F) A higher magnification of the marked area in (E) showing a germinal center containing PD-1\textsuperscript{+} T cells. (B–E, original magnification x25; F, original magnification x100).
RESULTS

PD-1 expression in PCMZL

PCMZL showed nodular to diffuse infiltrates containing variable numbers of marginal zone B cells, lymphoplasmacytoid cells and plasma cells and considerable numbers of admixed T cells (Figure 1). Reactive follicles were observed in 8 of 10 cases. Plasma cells and/or lymphoplasmacytoid cells were characteristically found at the periphery of the infiltrates or at the subepidermal border showing either kappa or lambda monotypic immunoglobulin light chain expression. The neoplastic B cells, including marginal zone B cells and plasma cells, were PD-1 negative in all cases (Table 2).

Admixed CD3+ T cells were numerous, in all cases comprising 50–75% (median, 65%) of the total infiltrate (Figure 1). The proportion of PD-1+ T cells varied between 10% and 20% (median, 10%; mean, 13%) of the total infiltrate, and made up 15–30% (median, 20%) of the CD3+ T cells (Table 2). Examination of serial sections showed that CXCL13 generally stained 25–50% of the PD-1+ cells. These PD-1+ T cells were particularly found within reactive germinal centers and less frequently outside, and were spatially not associated with plasma cells (Figure 1). A very similar pattern was found in five cases of cutaneous pseudo-B-cell lymphoma. In these reactive lymphoid follicles PD-1+ T cells showed the same distribution as in normal reactive lymph nodes or tonsils, routinely included on the same glass slide as positive control. Clusters of four to six PD-1+ T cells rosetting around large B cells were regularly observed.

Table 2. Frequency of admixed CD3+ T cells and PD-1+ T cells

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Number</th>
<th>CD3+ * median (range)</th>
<th>PD-1+ † median (range)</th>
<th>PD-1+/CD3+ ‡ median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCMZL</td>
<td>10</td>
<td>65% (50–75%)</td>
<td>10% (10-20%)</td>
<td>20% (15-30%)</td>
</tr>
<tr>
<td>PCFCL</td>
<td>18</td>
<td>35% (10–50%)</td>
<td>20% (5–40%)</td>
<td>60% (25–90%)</td>
</tr>
<tr>
<td>&gt; follicular / follicular and diffuse</td>
<td>9</td>
<td>40% (15–50%)</td>
<td>25% (15–40%)</td>
<td>65% (30–90%)</td>
</tr>
<tr>
<td>&gt; diffuse</td>
<td>9</td>
<td>25% (10–50%)</td>
<td>10% (5–30%)</td>
<td>50% (25–75%)</td>
</tr>
<tr>
<td>PCLBC–LT</td>
<td>12</td>
<td>13% (5–25%)</td>
<td>3% (1–15%)</td>
<td>15% (5–50%)</td>
</tr>
</tbody>
</table>

PCMZL, primary cutaneous marginal zone lymphoma; PCFCL, primary cutaneous follicle center lymphoma; PCLBC–LT, primary cutaneous diffuse large B-cell lymphoma–leg type; PD-1, programmed death-1.

* proportion of CD3+ reactive T cells expressed as percentage of total number of infiltrating cells.
† proportion of PD-1+ reactive T cells expressed as percentage of total number of infiltrating cells.
‡ proportion of PD-1+ cells expressed as percentage of reactive CD3+ T lymphocytes.
Figure 2. Representative histopathologic features of a primary cutaneous follicle center lymphoma (PCFCL) patient with a mainly follicular growth pattern. (A) Hematoxylin-eosin staining of the lesion showing nodular infiltrates containing neoplastic follicles with irregular mantles. (B) Neoplastic cells are CD79a positive. (C) BCL6 positive neoplastic germinal centers. (D and E) The neoplastic B cells are admixed with considerable numbers of small CD3+ T cells, of which (F and G) many were programmed death-1 (PD-1) positive. The insets in (E) and (G) are showing rosettes of CD3+ T cells and PD-1+ cells around large B cells, respectively. (A–D and F, original magnification x25; E and G, original magnification x100; inserts, original magnification x400).
Figure 3. Representative histopathologic features of a primary cutaneous follicle center lymphoma (PCFCL) patient with a diffuse growth pattern. (A) Patient presenting with confluent tumors on the back. (B) Hematoxylin-eosin staining of the lesion showing diffuse infiltrates containing (C) CD79a+ neoplastic B cells. (D and E) Neoplastic B cells are admixed with considerable numbers of small CD3+ T cells, of which (F and G) many were programmed death-1 (PD-1) positive. The inset in (G) is showing the absence of PD-1 on neoplastic B cells (black arrowhead) and strong PD-1 expression on T cells (white arrowhead). (B–D and F, original magnification x25; E and G, original magnification x100; insert, original magnification x400).
PD-1 expression in PCFCL

PCFCL showed nodular to diffuse infiltrates containing medium-sized to large CD20+/CD79a+ cleaved cells (large centrocytes), variable numbers of centroblasts and considerable numbers of admixed small T cells (Figures 2 and 3). On the basis of routine histology and immunostains recognizing (remnants of) follicular dendritic cell networks (CD35 and CD21) these PCFCL showed either a purely follicular (n = 1), a follicular and diffuse (n = 8) or a diffuse growth pattern (n = 9). Characteristically, the neoplastic B cells expressed BCL6 (18/18 cases), but were negative for BCL2 (17/18 cases) and MUM-1 (18/18 cases), while CD10 was (partly) expressed in 6 of 16 cases investigated. Neoplastic B cells were PD-1 negative in all 18 cases (Table 2).

The proportion of admixed CD3+ T cells varied between 10% and 50% (median, 35%) of the total infiltrate, and was significantly lower than in PCMZL (p < 0.01). PD-1+ cells made up 5–40% of the total infiltrate (median, 20%) and were more frequent in cases with a (partly) follicular growth pattern than in cases with a diffuse growth pattern (Table 2). CXCL13 stained 25–50% of the PD-1+ cells. Examination of serial sections showed that PD-1 was expressed by more than 50% of the CD3+ T cells in 14 of 18 cases (Figures 2 and 3). The median percentage of 65% (range, 25–90%) was significantly higher than observed in PCMZL (p < 0.01) (Table 2). These PD-1+ T cells were particularly located within germinal centers or in areas with remnants of follicular dendritic cell networks. As in PCMZL, clusters of PD-1+ T cells rosetting around large B cells were regularly observed (Figure 2).

PD-1 expression in PCLBCL–LT

PCLBCL–LT biopsies showed diffuse infiltrates of centroblasts and immunoblasts, characteristically expressing BCL2 (11/11 cases) and MUM1 (10/12 cases), while BCL6 was expressed by 8 of 12 cases. Staining for CD10 was consistently negative. In 2 of 12 cases (17%) the neoplastic B cells showed weak PD-1 positivity (30% and 60% of the tumor cells, respectively; Table 1; Figure 4).

There were few reactive CD3+ T cells (median, 13%; range, less than 5–25%) and significantly less than in PCMZL (p < 0.01) and PCFCL (p = 0.01; Table 2; Figure 5). In 10 of 12 cases scattered PD-1+ cells made up less than 5% of the total infiltrate, while CXCL13+ cells were observed only occasionally. Serial sections showed that PD-1 was expressed by 5% to 25% of CD3+ T cells in 11 of 12 cases. In one patient, a 53-year-old man presenting with tumor on the trunk, CD3+ T cells made up 25% of the total infiltrate and 50% of these CD3+ T cells stained for PD-1. Compared to the nine PCFCL with a diffuse growth pattern, which also showed a diffuse population of large neoplastic B cells, these twelve PCLBCL–LT showed significantly less admixed T cells expressing PD-1 (50% versus 15%, respectively; p = 0.01)
**DISCUSSION**

In this study we investigated PD-1 expression in three types of primary CBCL: PCMZL, PCFCL and PCLBCL–LT. PD-1 was not expressed by the neoplastic B cells in these CBCL, except for two cases of PCLBCL–LT showing weak PD-1 staining in 30% and 60% of the neoplastic B cells, respectively. This observation is consistent with studies in nodal B-cell NHL, which showed that PD-1 is not or rarely expressed by the neoplastic B cells in follicular lymphomas and diffuse large B-cell lymphomas, neither in extranodal marginal zone lymphomas at non-cutaneous sites.\(^5\)\(^-\)\(^7\),\(^18\) In contrast to B-NHL, PD-1 is expressed by the neoplastic cells of several types of T-NHL, most notably angioimmunoblastic T-cell lymphoma (AITL), which is currently considered as the prototype of neoplasms derived from TFH cells.\(^19\),\(^20\) Regarding cutaneous T-cell lymphomas, PD-1, CXCL13 and BCL6, but –unlike AITL– not CD10, are consistently expressed by CD4\(^+\) small to medium-sized pleomorphic CTCL, but not or rarely by other types of primary cutaneous peripheral T-cell lymphoma, not otherwise specified, and not by primary cutaneous CD30-positive lymphoproliferative disorders.\(^16\),\(^21\) In recent studies of our group, PD-1 was almost without exception strongly expressed by the neoplastic T cells in Sézary syndrome, but uncommonly by the tumor cells in skin lesions of mycosis fungoides.\(^17\)

In this study we also looked for the number and distribution of reactive PD-1\(^+\) T cells in CBCL and found that PD-1\(^+\) T cells were significantly more numerous in PCFCL than in PCMZL and PCLBCL–LT, which is consistent with the results of previous studies.\(^12\),\(^13\) In accordance, studies in nodal B-NHL showed significantly higher numbers of PD-1\(^+\) lymphocytes in follicular lymphomas than in diffuse large B-cell lymphomas.\(^7\),\(^8\) In PCFCL these PD-1\(^+\) T cells were not only located within germinal centers, but also outside germinal centers. Examination of serial sections
stained with follicular dendritic cell markers (CD21 and CD35) revealed that these areas with many PD-1+ T cells outside germinal centers still contained remnants of follicular dendritic cell networks, that were easily overlooked in routine hematoxylin-eosin stainings. A very similar distribution has been described in nodal follicular lymphomas.\textsuperscript{7-11} In contrast, in PCMZL as well as the pseudo-B-cell lymphomas included as controls, PD-1+ T cells were preferentially localized within the germinal centers of reactive lymphoid follicles, and rarely present outside these follicles, which is in line with previous studies.\textsuperscript{12,13} The presence of many PD-1+ cells outside germinal centers may therefore be considered as an adjunct to differentiate PCFCL from pseudo B-cell lymphomas. Both in PCFCL and in PCMZL clusters of PD-1+ T cells, part of which were also CXCL13+, formed rosettes around large B cells, similar to TFH cells in reactive lymph nodes, as also reported in previous studies.\textsuperscript{12,13}

Several independent studies indicate that higher frequencies of intratumoral PD-1+ lymphocytes are associated with improved overall survival in nodal follicular lymphomas.
lymphomas, although in one study a relationship with poorer outcome was reported. In addition, the PD-1+ T cell abundance correlates with histological grade, as grade III lesions tend to contain fewer PD-1+ cells than grade I and II follicular lymphomas. In line with this, we found that PCFCL with a follicular or a follicular and diffuse growth pattern contained higher frequencies of PD-1+ T cells than PCFCL with a diffuse growth pattern. In contrast, in the study of Mitteldorf et al. no relation between PD-1+ cells and growth pattern was found, which may relate to the small patient groups studied. However, PD-1 expression has no prognostic significance in this group, as PCFCL has an excellent prognosis, irrespective of the growth pattern and the proportion of admixed (PD-1+) T cells.

For many years there has been discussion about the distinction between PCFCL with a diffuse growth pattern and PCLBCL–LT. In the WHO 2001 classification both were still classified as diffuse large B-cell lymphoma. However, studies in the last decade have firmly established that these represent distinct entities with a different clinical presentation, clinical behavior and prognosis, which are therefore classified separately in recent consensus classifications. In this study PCFCL with a diffuse growth pattern contained significantly more admixed PD-1+ T cells than PCLBCL–LT (50% versus 15%, respectively), suggesting that staining for PD-1 may serve as an adjunct diagnostic tool in difficult cases.

In conclusion, our results show that PD-1 is rarely expressed by the neoplastic B cells in CBCL. Moreover, apart from germinal centers of reactive lymphoid follicles in PCMZL, high numbers of PD-1+ T cells are particularly found in PCFCL. In particular, the presence of many PD-1+ T cells outside germinal centers may support a diagnosis of PCFCL.

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REFERENCE LIST


