Abstract

Intravascular large B-cell lymphoma (IVLBCL) is a distinct disease, but the neoplastic PD-L1 expression on tumor cells may vary among cases. We evaluated 10 IVLBCL autopsy cases for neoplastic PD-L1 expression, and had positive results in two cases. In one case, neoplastic PD-L1 expression (SP142, 28-8, and E1J2J clones) was dependent on the organ and anatomical site (capillaries vs. vessels) of the tumor tissue. Neoplastic PD-L1 expression was found in tumor cells located in capillaries in the central nervous system, pituitary gland, kidneys, lung, and gastrointestinal tract; sinuses/sinusoids of the spleen, liver, bone marrow, and lymph nodes; and an extravascular location. However, this expression was not detected in tumor cells located in the adrenal gland, thyroid gland, pancreas, ovaries, uterus, pleura, and small or larger-sized vessels of the lung. The other case showed constant neoplastic PD-L1 expression on the tumor cells, and in addition to the affected organs, capillaries, and vessels with two anti-PD-L1 antibodies (28-8 and E1J2J, but not SP142). The divergence and heterogeneity of neoplastic PD-L1 expression were clearly demonstrated in our cases. To the best of our knowledge, this is the first description of divergent neoplastic PD-L1 expression among the affected organs and anatomical sites in IVLBCL.

Key Words: autopsy, intravascular large B-cell lymphoma, neoplastic PD-L1 expression divergence or heterogeneity

Introduction

Programmed Death Ligand 1 (PD-L1) is an inhibitory immune checkpoint regulator that suppresses T cell activity and plays a vital role in the maintenance of peripheral tolerance by protecting bystander tissues from immune-mediated damage. However, this pathway also promotes tumor development by inhibiting the immune activity of tumor specific CD8+ T cells, allowing tumor cells to escape T-cell-mediated tumor-specific and pathogen-specific immunity.[1, 2] There is much interest in tumor cell neoplastic PD-L1 expression in lymphoid malignancies, primarily classic Hodgkin lymphoma (CHL). However, our understanding of neoplastic PD-L1 expression remains limited, because it is typically assessed in biopsy samples and is rarely comprehensively examined in multiple affected sites in autopsy cases.

Intravascular large B-cell lymphoma (IVLBCL) is a rare variant of extranodal diffuse large B-cell lymphoma (DLBCL), characterized by the proliferation of large tumor cells within the lumina of small or intermediate-sized blood vessels and capillaries.[3, 4] This disease is systemic, can potentially involve any organ, and is often widely disseminated.[3, 5, 6] Recent advances in molecular analyses indicated a high prevalence of the L265P activating mutation in the *MYD88* gene and mutations of Y196 in the *CD79B* gene,[7, 8] resulting in constitutive activation of NFκβ-signaling. These mutations were found in both patients with skin-limited and systemic disease, despite the heterogeneity of their clinical characteristics.[8] There is

currently no explanation for the vast heterogeneity of clinical behaviors found in patients with IVLBCL.

We recently reported an autopsy case of IVLBCL with neoplastic PD-L1 expression using an anti-PD-L1 antibody (SP142).[3] This further promoted us to comprehensively screen for neoplastic PD-L1 expression in tumor cells from the affected organs and tissues using formalin-fixed paraffin-embedded (FFPE) blocks in a series of autopsy cases with IVLBCL. We found a heterogeneous pattern of PD-L1 expression with and without immunohistochemical divergence that was dependent on the anatomical location (capillaries vs. vessels) of the tumor cells.

We initially surveyed 10 autopsy cases with IVLBCL (male: female=5:5, a median onset age of 61 years [51-99 years]) by immunostaining the FFPE sections with three anti-PD-L1 antibodies (SP142, 1:50, Spring Bioscience; E1J2J, 1:50, Cell Signaling Technology; and 28-8, 1:50, Abcam), but only two previously reported cases presented with successful antigen retrieval.[3,9] The eight remaining cases showed no positivity in the neoplastic cells or cells in the tumor microenvironment probably due to the prolonged formalin fixation.

Clinical Summary

The clinicopathologic findings and prognoses of our patients have been already documented in details as separate case reports[3,9] and are briefly summarized in Table 1.

Pathological Findings

Cases #1 and 2 exhibited typical histopathologic features of IVLBCL with CD5 and CD10 positivity on their tumor cells, respectively. [3,9]

In Case #1, the immunohistochemical staining results with anti-PD-L1 antibodies are summarized in Table 2. In the central nervous system (CNS), a small number of scattered neoplastic PD-L1-positive (nPD-L1+) tumor cells were distributed in the capillaries and small vessels (Figure 1A and 1B). The pituitary gland showed a marked proliferation of nPD-L1+ tumor cells in the intricate capillaries, as previously reported.[3] In the liver, the nPD-L1+ tumor cells were largely distributed in the sinusoids. Tumor cells were also diffusely distributed in portal areas (Figure 1C). In the spleen, nPD-L1+ cells were predominantly distributed in the perifollicular zone (Figure 1D) and were infrequently scattered in the red pulp. In the bone marrow (BM), nPD-L1+ tumor cells showed diffuse extravascular invasion (Figure 1E) with a small number of scattered tumor cells in the sinuses. In the lymph nodes (LN), nPD-L1+ tumor cells were distributed in the sinuses without any parenchymal invasion (Figure 1F).

In the lungs, tumor cells were widely distributed in the capillaries and vessels, highlighted by CD20 immunostaining (Figure 2A). Neoplastic PD-L1 expression was largely restricted to tumor cells in the lung capillaries (Figure 2B-D) but not in the vessels, with one small exception. In the kidney, tumor cells were predominantly distributed in the glomerular capillaries,

and easily detected with CD20 immunostaining (Figure 2E). As seen in the lung, neoplastic PD-L1 expression was mainly found in tumor cells in the capillaries and rarely in the small vessels (Figure 2F). In the gastrointestinal tract, a small number of nPD-L1+ tumor cells and/or small foci were found in the capillaries and small vessels of the esophagus (Figure 1G), jejunum, ileum, cecum, and rectum, but not the transverse colon. The adrenal gland, thyroid gland, pancreas, ovaries, uterus, and pleura had tumor cells in the vessels, and no detectable neoplastic PD-L1 expression (Figure 1H and 1I). Only one exception was an eventually detected focus of nPD-L1+ tumor cells with minimal extravascular invasion in the ovary (Figure 1J). Notably, these differences in neoplastic PD-L1 expression were well conserved for all of the anti-PD-L1 antibodies tested, all of which resulted in the same or similar immunostaining pattern. The PD-L1 expression was considered positive or negative when there was >50% and <1% of positive tumor cells, respectively.

In Case #2, the tumor cells were universally positive for PD-L1 with the 28-8 and E1J2J clones (but not SP142) in the affected vessels and capillaries, as well as in all the organs examined, including the lung, liver, heart, kidney, gastrointestinal tract, pancreas, adrenal gland, and prostate (Figure 3). No exceptional findings were noted.

Discussion

Here, we document the divergence and heterogeneity of neoplastic PD-L1 expression in IVLBCL tumor cells by comprehensively screening

affected organs in two autopsy cases. We found immunohistochemically-detectable positive neoplastic PD-L1 expression in our on-going survey of a larger IVLBCL series using the SP142 clone (data not shown), consistent with a previous report by Kiyasu et al;[10] although they showed positive neoplastic PD-L1 expression in approximately half of the examined cases. However, the diagnostic sites varied between cases, and this required us to comprehensively examine the organs affected by IVLBCL to determine whether neoplastic PD-L1 expression is conserved beyond these anatomical sites. This supplementary study revealed the heterogeneity of neoplastic PD-L1 expression patterns among cases, suggesting that IVLBCL constitutes a distinct disease entity, but may have a broader spectrum of biological properties. The tumor cells of our two cases showed different immunophenotypes (CD10+ vs. CD5+) and neoplastic PD-L1 expression patterns with and without immunohistochemical divergence, depending on the anatomical sites of the tumor, despite that they shared similar clinicopathological findings.

Interestingly, Case #1 showed that neoplastic PD-L1 expression is highly restricted in the tumor cells found in the capillaries of the CNS, pituitary gland, and lung, and the sinuses/sinusoids of the spleen, liver, BM, and LN. These sites are the primary diagnostic sites for IVLBCL.[11] Tumor cells with diffuse extravascular invasion in the liver and BM were also positive for PD-L1. In sharp contrast, there was an absence of neoplastic PD-L1 expression in tumor cells distributed in the vessels of the adrenal

gland, thyroid gland, pancreas, ovaries, uterus, pleura, and lung. Notably, immunostaining with the three commercially available anti-PD-L1 antibodies yielded the same or a similar pattern of neoplastic PD-L1 expression, indicating that this divergence is not related to the sensitivity of the applied antibodies. On the other hand, the neoplastic PD-L1 expression was universally detected with the 28-8 and E1J2J clones (but not SP142) in Case #2 beyond the organs and anatomical sites of the tumor tissue. The molecular pathogenesis of PD-L1 expression was variable among cancer types, and the different binding characteristics were found for these three PD-L1 antibody clones in a linear epitope mapping, i.e., the 28.8 clone binding to predominantly intracellular epitopes, but the SP142 clone binding to both intracellularly and extracellularly located epitopes.[12,13] Kataoka and colleagues also demonstrated that PD-L1 expression induced by 3'-untranslated region disruption with truncated protein was detected only with an antibody (E1J2J) directed against the N-terminal, but not the C-terminal domain.[14] Regrettably, we could not genetically analyze *PD-L1* gene alterations in our case on the FFPE sections, and this issue should be clarified in a future study.

Suehara et al.[7] and Schrader et al.[8] recently reported a high prevalence of the L265P activating mutation in the *MYD88* gene and mutations of Y196 in the *CD79B* gene in 44-49% and 26-67% of their patients, respectively. Schrader et al. also reported that these mutations were detected in patients with both skin-limited and systemic disease.[8] These data

suggest that the genetic alterations in IVLBCL may be relatively homogeneous despite the vast heterogeneity of clinical manifestations between Western and Asian patients. The hypothesis that IVLBCL tumor cells share the same or at least similar genetic alterations includes the speculation that the suppression of neoplastic PD-L1 expression is mediated by the interaction between tumor cells and non-malignant cells, especially vascular endothelial cell (VEC), in the microenvironment. Indeed, we found the unusual focus of the nPD-L1+ tumor cells with minimal extravascular invasion in the ovary, suggesting that the loss or disruption of the intravascular compartmentalization of the tumor cells may lead them to have the PD-L1 positivity. PD-L1 interact with PD-1 and CD80 (B7.1).[15] The expression of PD-L1 and its receptor CD80 on VEC was shown to have an inhibitory function on endothelial cell proliferation and angiogenesis.[16] The presence or not of CD80 on VEC of the organs affected by the tumor cells might be speculated to induce the divergent neoplastic PD-L1 expression divergence among the anatomical sites. This phenomenon also provided us an alarm that the immunohistochemical PD-L1 negativity does not necessarily mean the absence of its neoplastic expression among the patients with the systemic extranodal lymphomas.

Notably, PD-L1 immunostaining highlighted the diffuse extravascular invasion of IVLBCL in the affected organs, begging the question whether neoplastic PD-L1 expression is associated with either additional genetic alterations or the loss of the intravascular microenvironment. We recently

reported a short series of immune escape-related extranodal large B-cell lymphoma cases, featuring exclusively extranodal sites, neoplastic PD-L1 expression, and often a partially intravascular pattern.[17] These data were obtained from biopsy specimens and not autopsy material. The results from the cases presented here indicated that a negative finding for PD-L1 expression may not mean it is completely absent, especially in diffuse large B-cell lymphomas characterized by exclusive extranodal site involvement, such as the primary DLBCL of CNS. This issue should also be further investigated in the future. To the best of our knowledge, this is the first and only description of neoplastic PD-L1 expression divergence among the affected organs and anatomical sites in IVLBCL.

Disclosure Statement

None declared.

Author Contributions

A. Sakakibara and K. K. reviewed the slides and wrote the paper. Y. I. and E. Imaoka provided the clinical information. Y. Sakai and M. I. made the original diagnosis. E. Ishikawa, S. S., Y. Suzuki and A. Satou reviewed the slides. K. S. and S. N. supervised the project.

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Table 1. Clinical summary of our patients

Case	Age/sex	Presentation	Diagnosis	Immunophenotype		Treatment	Outcome
				Positive	Negative		
#1	51/F†	Cognitive deficits and truncal ataxia	IVLBCL	CD10, CD20, BCL2, BCL6, MUM1	CD3, CD5	No therapy	Died 5 months after onset
#2	57/M‡	Spiking fevers and left epigastralgia	IVLBCL	CD5, CD20, CD45, CD79a	CD3, CD10, CD23, CD30, CD45RO§	DeVIC, CHOP	Died 11months after onset

Abbreviations: IVLBCL, intravascular large B-cell lymphoma; DeVIC, Dexamethasone, etoposide, ifosfamide, and carboplatin; and CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisolone.

† Refer to 3.

‡ Refer to 9.

§ Over expression of cyclin D1 or SOX11 protein was not detected.

Table 2. Neoplastic PD-L1 expression divergence among the affected organs and anatomical site in Case #1

Affected organ	nPD-L1	Tumor cell orientation
CNS	+	Capillaries
Pituitary gland	+	Intricate capillaries
Liver	+	Sinusoids and portal areas with diffuse extravascular invasion
Pancreas	-	Vessels
Spleen	+	Primarily in the perifollicular zones
BM	+	Sinuses and diffuse extravascular invasion
LN	+	Sinuses
Lung	+ -	Lung capillaries Vessels with an exception of only a small part
Pleura	-	Vessels
Kidney	+	Glomerular capillaries
Adrenal gland	-	Subcapsular vascular plexus
Thyroid gland	-	Vessels
GI	+ -	A small number of tumor cells in the capillaries of the esophagus, jejunum, ileum, cecum, and rectum Capillaries of the transverse colon
Ovaries	-	Vessels
Uterus	-	Vessels

Abbreviations: nPD-L1, neoplastic PD-L1; CNS, central nervous system; BM, bone marrow; LN, lymph node; and GI, gastrointestinal tract.

Figure Legends

Figure 1. Case #1. Intravascular large B-cell lymphoma with neoplastic PD-L1 expression (SP142 clone). **A**. Neoplastic PD-L1 (nPD-L1)+ tumor cells are seen in the capillaries of the central nervous system (CNS), and are often deformed in shape. **B**. Only a small number of nPD-L1+ cells are found in the small vessels of the CNS. **C**. nPD-L1+ tumor cells in the liver are distributed in both the sinusoid (right) and portal area with diffuse invasion (left). **D**. nPD-L1+ tumor cells in the spleen are seen predominantly in the perifollicular zone. **E**. The PD-L1 immunostaining highlights the diffuse extravascular invasion of the tumor cells in the bone marrow. **F**. nPD-L1+ tumor cells are seen in the sinuses of the affected lymph node. **G**. Neoplastic PD-L1 expression is found in only a small part of the tumor within the vessels of the esophagus. **H-I**. PD-L1 is not detected in tumor cells in the vessels of the pleura (H) and the ovary (I). **J**. The focus of nPD-L1+ tumor cells with minimal extravascular invasion in the ovary.

Figure 2. Case #1. Intravascular large B-cell lymphoma with neoplastic PD-L1 expression (Sp142 clone). **A**. CD20 immunostaining highlights the wide distribution of tumor cells in both the vessels (left) and capillaries (right) of the lung. **B**. Neoplastic PD-L1 expression is largely restricted in the tumor cells of lung capillaries (right), but not vessels (left). The immunostain for CD20 (A) and PD-L1 (B) on the semi-serial sections. Notable, the PD-L1 expression is undetectable in the CD20+ large tumor cells of the bronchiolar

vessels, but highlights those scattered in distribution and deformed in the alveolar capillaries. **C**. Higher magnification shows the deformed and scattered PD-L1+ tumor cells contrasted not matching the pattern of alveolar endothelial cells. **D**. Immunohistochemical double staining of PD-L1 (blue) and CD20 (brown). Note the merging of their positive images on the tumor cells distributing in the lung capillaries. **E-F**. CD20+ tumor cells are predominantly distributed in the glomerular capillaries of the kidney (E), and are characterized by neoplastic PD-L1 expression (F).

Figure 3. Case #2. Intravascular large B-cell lymphoma with neoplastic PD-L1 expression (Sp142 [**A** and **B**], 28-8 [**C** and **D**], and E1J2J clones [**E** and **F**]). **A**, **C**, and **E**. Tumor cells of the kidney are positive with the 28-8 and E1J2J clones, but not SP142. **B**, **D**, and **F**. Tumor cells of the prostate are positive with the 28-8 and E1J2J clones, but not SP142.