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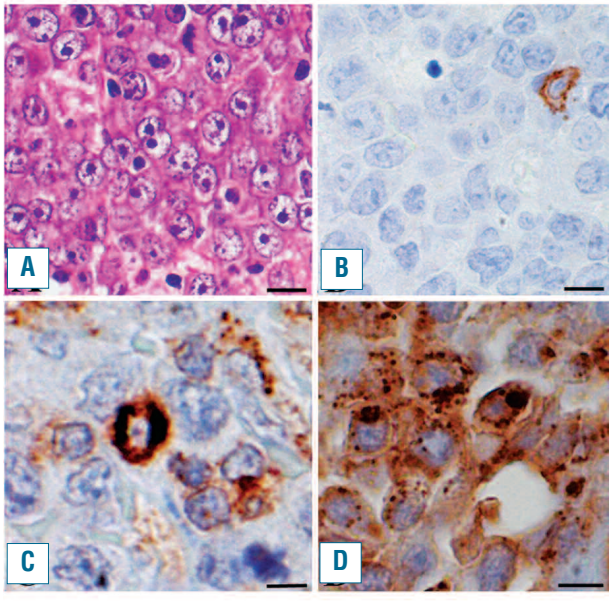
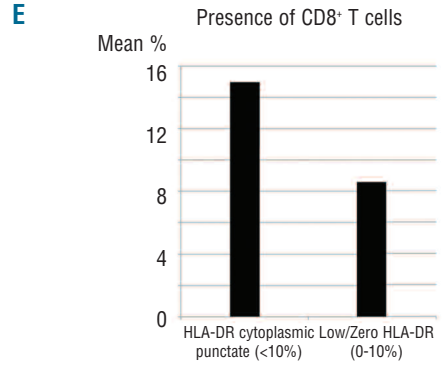
Lack and/or aberrant localization of major histocompatibility class II (MHCII) protein in plasmablastic lymphoma

Plasmablastic lymphoma (PBL) was recently reclassified as a distinct entity of mature B-cell neoplasm.[1,2] However, the diagnostic distinction of PBL from diffuse large B-cell lymphoma (DLBCL) is still a common problem due to the lack of biomarkers for PBL.[3] Recently, Montes-Moreno *et al.* published a study in Hematologica describing several PBL phenotypes that help to differentiate PBL from DLBCL.[4] A major characteristic of these PBL cases (full and variant plasmablastic phenotypes) which distinguished them from conventional DLBCL was PRDM1/Blimp1 positivity.[4] The authors further demonstrated that the rare acquisition of a partial, Blimp1 positive, plasmablastic phenotype in a minority of DLBCL cases was associated with poorer patient outcome.[4] Blimp1 and MHC class II protein expression are inversely related as normal B cells enter the terminal differentiation





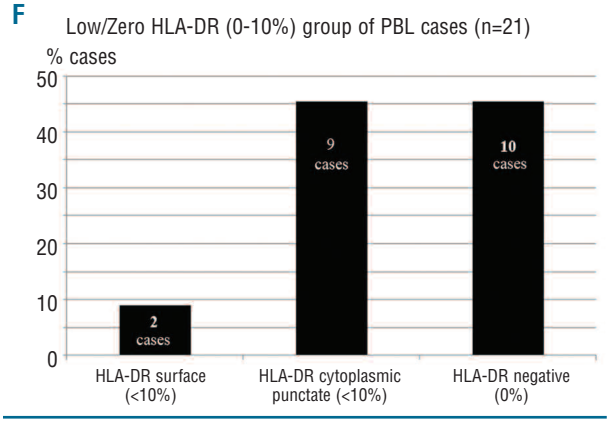


Figure 1. Hematoxylin and eosin (H&E) staining shows (A) the typical morphology of PBL. (B) CD8 staining reveals the presence of one T cell in a tumor area. (C and D) Immunohistochemical staining of PBL cases with anti-HLA-DR demonstrates the typical membrane staining in one cell (B) and the aberrant cytoplasmic punctate pattern in the absence of membrane staining (D). Bars 20 μm. (E) The "Low/Zero HLA-DR" PBL cases showed a median presence of 8.6% (±5 SD) $CD8^+$ T cells. In the one PBL case with 60% of cells strongly expressing the aberrant cytoplasmic punctate pattern in the absence of membrane staining, there were 15% $CD8^+$ T cells. (F) Within the "Low/Zero HLA-DR" group, 10 cases were completely negative. In 2 cases, a few cells (2% ±3 SD) expressed HLA-DR on the cell membrane. In 9 cases, 4% (±3 SD) of cells showed an aberrant cytoplasmic punctate pattern in the absence of membrane staining.

Table 1. HLA-DR expression and presence of CD8+ T cells in PBL cases (n=22). In the one case with more than 10% cells being positive, HLA-DR was exclusively localized in the cytoplasm with no surface expression.

HLA-DR expression	N. cases	Mean % HLA-DR+ cells (± SD)	Mean HLA-DR intensity (± SD)	Mean % CD 8 cells (± SD)	Range CD8 T cells (%)
Low/Zero (<10% + negative)	21 (95%)	1.7 (± 2.8)	1.8 (± 0.6)	8.6 (±5)	1.6-17
>10%	1 (5%)	60	3+	15	

Table 2. HLA-DR expression and presence of CD8+ T cells in the "Low/Zero" PBL group (n=21).

HLA-DR expression	N. cases	Mean % HLA-DR+ cells (± SD)	Mean HLA-DR intensity (± SD)	Mean % CD 8 cells (± SD)	Range CD8 T cells (%)
Negative	10	0		7 (± 6)	1.6-17
Cell membrane positive	2	2 (± 3)	2+ (± 1)	9 (± 4)	6-12
Cytoplasmic (only) positive	9	4 (± 3)	2+ (± 1)	9.7 (±5)	3-17

program towards plasma cells. Given the expression of PRDM1/Blimp1 and poor outcome of PBL, Montes-Moreno *et al.* hypothesized that PBL cases would lack MHC class II expression.[4] In DLBCL, Rimsza *et al.* previously showed that downregulation of the MHC class II mRNA and protein expression correlated with low numbers of tumor infiltrating CD8+ T cells and poor patient outcome, which may be likely due to a loss of immunosurveillance.[5-7] In the current study, in a collaborative effort, we extended the work carried out by Montes-Moreno *et al.* by analyzing MHC class II (HLA-DR) and CD8 expression using immunohistochemistry in 22 of the 35 PBL cases from the previously published case series.[4] Due to tissue limitations, 13 cases were not analyzed.

The area of tumor was identified by hematoxylin and eosin (H&E) staining (Figure 1A) and a tumor area with the lowest frequency of CD8+ cells was chosen for counting (Figure 1B).[5] Staining was quantified by counting the number of HLA-DR+ and CD8+ cells in the total number of malignant cells or lymphoid-appearing cells, respectively (3 consecutive 60X fields/950 cells each-per case; obvious stromal and histiocytic cells excluded).[5] HLA-DR staining intensity was semi-quantitatively scored: 0 = no staining; 1+ = faint partial staining; 2+ = complete or partial moderate staining; 3+ = complete strong staining. In addition, the HLA-DR staining pattern was qualitatively characterized as cell membrane (Figure 1C) or aberrant cytoplasmic (Figure 1D) as described previously,[8] or as negative.

PBL cases with less than 10% of cells showing HLA-DR expression (cell membrane and aberrant cytoplasmic) were grouped together with the negative cases ("Low/Zero HLA-DR"). The 21 "Low/Zero HLA-DR" cases showed a median presence of 8.6% (±5 SD) CD8+ T cells (range 1.6-17%) (Figure 1E, Table 1). In one case, 60% of cells strongly expressed the aberrant cytoplasmic punctate pattern in the absence of membrane staining with an intensity of 3+. In this case, the CD8+ T-cell percentage was 15% (Figure 1E, Table 1).

Within the "Low/Zero HLA-DR" group of PBL cases (Figure 1F, Table 2), 10 cases were HLA-DR negative with a median presence of 7% (±6 SD) CD8+ T cells (ranging 1-17%). In a further 2 cases of the "Low/Zero HLA-DR" group, a few cells (2% ±3 SD) expressed HLA-DR on the cell membrane with a median intensity of 2+ and showed a median presence of 9% (±4 SD) CD8+ T-cells (range 6-12%). In the remaining 9 cases of the "Low/Zero HLA-DR" group, 4% (±3 SD) of cells showed an aberrant cytoplasmic pattern in the absence of membrane staining with a median intensity of 2+ (±1 SD). The CD8+ T-cell percentages in the latter cases ranged from 3% to 17% with a median of 9.7% (±5 SD).

Both the negative and aberrant cytoplasmic HLA-DR expression patterns were observed equally in all the plasmablastic phenotypes described previously.[4] The PBL case series included 4 cases negative for Blimp1 which we also analyzed for HLA-DR expression:[4] 2 cases were negative for HLA-DR, and 2 expressed low levels of aberrant cytoplasmic HLA-DR ("Low/Zero HLA-DR"). The one PBL case with 60% of cells exclusively expressing cytoplasmic MHC class II showed the variant plasmablastic phenotype, being positive for Blimp1, MUM1 and CD38, weakly positive for CD20 and PAX5, and negative for XBP1s, CD138 and EBER.

In summary, this study demonstrates the lack of MHC II protein expression on the surface membrane of most PBL cases, which is associated with only a modest decrease in CD8+ tumor infiltrating T cells. In contrast, our previous studies of this kind in DLBCL cases showed a much more pronounced decrease in CD8+ T-cell numbers in MHC II negative cases in which there was an average of 11% CD8+ T cells in the presence of MHC II cell membrane expression, but only 2.8% CD8+ T cells in the absence of MHC II protein cell membrane expression.[5] The difference may be due to the low power of this study. The significance of the cytoplasmic localization of MHC class II protein is not yet clear, but may represent a stage of partial expression which is associated with an intermediate percentage of infiltrating T cells. The absence of MHC II protein expression may explain the poor outcome in PBL patients, and may serve as an additional diagnostic tool and as a biomarker for immunosurveillance.

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