Appendix S1.

MATERIALS AND METHODS

Reagents

The following antibodies (Abs) were used for immunohistochemical staining: mouse monoclonal Abs for human CD83 (Novocastra, Milton Keynes, UK), human CD163 (Novocastra), human CD205 (Novocastra), human CD206 (LifeSpan Bioscience, Seattle, WA), and rabbit polyclonal Abs for human PD-L1 (ProSci, Poway, CA, USA), CCL18 (Life Span Bioscience) and CD208 (LifeSpan Bioscience).

Tissue samples and immunohistochemical staining

Archival formalin-fixed, paraffin-embedded skin specimens were collected from 4 patients with lymphomatoid papulosis (LyP) and 4 patients with primary cutaneous anaplastic large-cell lymphoma (PCALCL) who were treated in the Department of Dermatology at Tohoku University Graduate School of Medicine (Table I). We diagnosed LyP based on typical histological features, such as a wedge-shaped infiltrate with scattered or clustered CD30$^+$ tumour cells, intermingled with numerous inflammatory cells including histiocytes, as described previously (1). We diagnosed PCALCL based on typical histological features, such as dense, nodular, dermal infiltrates composed of CD30$^+$ large pleomorphic, anaplastic or immunoblastic cells with large, irregularly shaped nuclei and abundant pale or eosinophilic cytoplasm (1). These samples were processed for single staining for CD83, CD163, CD205, CD206, CD208, CCL18 and PD-L1, and developed with liquid permanent red (DAKO, Tokyo, Japan). All patients gave informed consent. This study was approved by the ethics committee of Tohoku University Graduate School of Medicine, Sendai, Japan.

Quantitative analysis of immunohistochemical staining samples using image cytometry

Optical densitometric mean value of immunohistochemically stained sections were quantified with a KS400 image analysis system (Carl Zeiss Imaging Solutions), and the staining intensity was presented reciprocally (6). For image cytometry, immunohistochemical stained images were analysed using HistoQuest software (TissueGnostics, Vienna, Austria). Briefly, nucleated cells among the haematoxylin-positive cells were detected and determined by a dissection algorithm. Then, positive cells were detected by signals of liquid permanent red (LPR) in the nucleated cells. LPR intensity was plotted against the haematoxylin intensity to create scatter plots. The cut-off threshold was determined by the LPR signal intensity based on LPR-negative cells in the same section.