SHORT COMMUNICATION

Comparison of CD163^{+} Macrophages and CD206+ Cells in Lesional Skin of CD30^{+} Lymphoproliferative Disorders of Lymphomatoid Papulosis and Primary Cutaneous Anaplastic Large-cell Lymphoma

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CD30^{+} lymphoproliferative disorders represent a spectrum of diseases from lymphomatoid papulosis (LyP) to primary cutaneous anaplastic large-cell lymphoma (PCALCL) (1, 2). LyP is characterized by recurrent papulonodular lesions, which undergo spontaneous regression (1, 2). Survival is unaffected by LyP, but patients are at risk of a second cutaneous or lymphoid malignancy (1). PCALCL has a favourable prognosis with a 5-year survival rate between 76% and 96%. In this report, we used immunohistochemical (IHC) staining of LyP type A and PCALCL specimens to examine for macrophage markers as well as dendritic cell (DC)-specific markers and their functional markers.

MATERIALS AND METHODS

Reagents

Full details of reagents are given in Appendix S1[1]. We collected archival formalin-fixed, paraffin-embedded skin specimens from 4 patients with LyP and 4 patients with PCALCL who were treated in our department (Table SI[1]). All patients gave informed consent. This study was approved by the ethics committee of Tohoku University Graduate School of Medicine. Full details are available in Appendix S1[1].

Optical densitometric mean values were determined in IHC stained sections using KS400 image analysis system, and the staining intensity was presented reciprocally (3). For image cytometry, stained images were analysed using HistoQuest software(TissueGnostics, Vienna, Austria). Full details are given in Appendix S1[1].

Student's t-test was used for single comparison of 2 groups. The level of significance was set at 0.05.

RESULTS

First, we investigated the profiles of tumour-associated macrophages (TAMs). IHC staining of LyP (Fig. 1A, C) and PCALCL (Fig. 1B, D) showed infiltration of substantial numbers of CD163^{+} macrophages (Fig. 1A, B) and CD206^{+} cells (Fig. 1C, D) in lesional skin.

Next, to quantify the CD163^{+} cells and CD206^{+} cells in lesional skin, we performed TissueFAXS analysis (3). The percentages of CD163^{+} cells in LyP and PCALCL were $7.9 \pm 2.9\%$ and $14.6 \pm 4.2\%$ ($p = 0.24$), respectively, and the percentages of CD206^{+} cells were $15.2 \pm 3.4\%$ and $48.7 \pm 10.0\%$ ($p = 0.0195$), respectively (Fig. 1E).
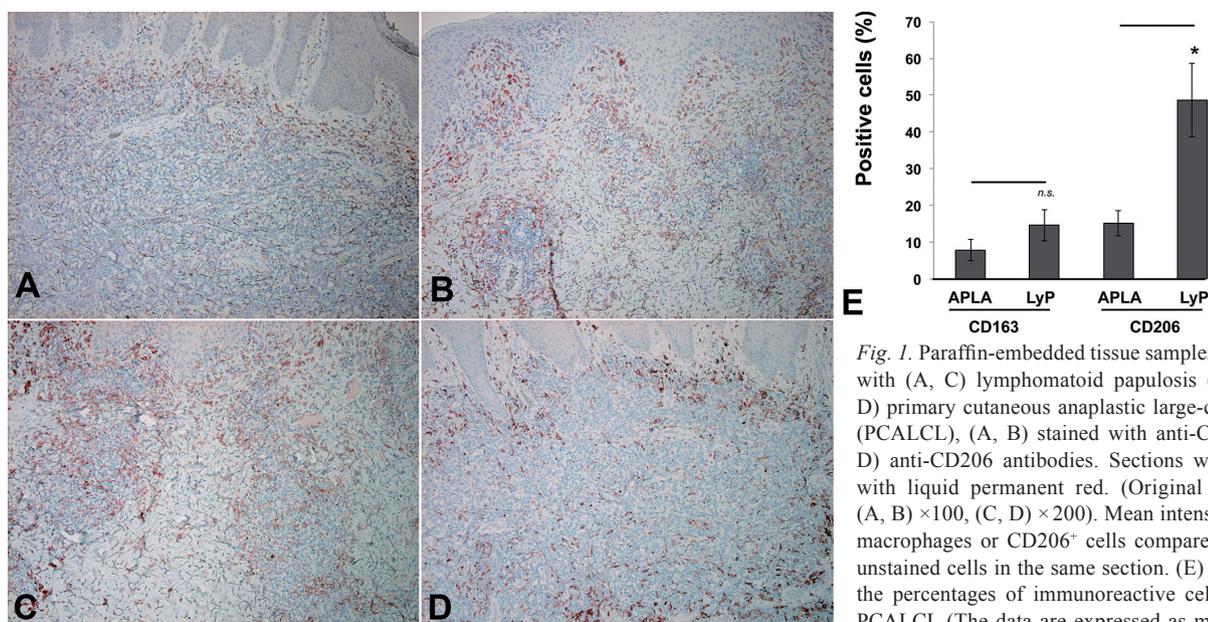
Fig. 1. Paraffin-embedded tissue samples from patients with (A, C) lymphomatoid papulosis (LyP) and (B, D) primary cutaneous anaplastic large-cell lymphoma (PCALCL), (A, B) stained with anti-CD163 and (C, D) anti-CD206 antibodies. Sections were developed with liquid permanent red. (Original magnification (A, B) ×100, (C, D) ×200). Mean intensity of CD163^{+} macrophages or CD206^{+} cells compared with that of unstained cells in the same section. (E) A summary of the percentages of immunoreactive cells in LyP and PCALCL (The data are expressed as mean ± standard error in each group. $p = 0.05$).

Because both macrophages and dendritic cells (DC) express CD206, also studied CD205 (specific for DC) to investigate the possibility of DC infiltration. IHC staining for CD205 revealed substantial numbers of CD205+ DC among the stromal cells of PCALCL (Fig. S1C, D[1]). In contrast, few CD205+ DC were detected among the stromal cells of LyP (Fig. S1A, B[1]). Other DC-specific markers, CD83 and CD208, were not detected in the lesional skin of LyP or PCALCL (data not shown).

To further investigate the functional phenotypes of TAMs and DC in LyP and PCALCL, we employed IHC staining to detect PD-L1 and CCL18, both of which are reported to be markers of TAMs and DC, and are associated with a poor prognosis in tumour-bearing hosts (4–6). Staining for PD-L1 revealed substantial numbers of PD-L1^high+ cells among the stromal cells of LyP (Fig. S2A[1]). In contrast, few PD-L1^high+ cells were detected among the stromal cells of PCALCL (Fig. S2B[1]). Together with PD-L1 staining, substantial numbers of CCL18-producing cells were detected in LyP (Fig. S2C[1]), but not in PCALCL (Fig. S1D[1]). The intensity of IHC staining was scored on a semi-quantitative scale (Table SI[1]).

DISCUSSION

In this report, we employed IHC staining, focusing on the phenotype of tumour-infiltrating histiocytes, specifically CD163, CD206, CD83, CD205 and CD208, as well as their functional markers, PD-L1 and CCL18. Interestingly, TissueFAXS analysis revealed a relatively higher percentage of CD206+ cells in LyP, suggesting that tumour-infiltrating histiocytes in the lesional skin of LyP and PCALCL have different profiles.

Other reports have suggested that the presence of TAMs correlates with therapy failure and poor prognosis in cancer patients (7, 8). It has also been reported that M2 macrophages have an important role in the production of an immunosuppressive cytokine, IL-10, and various chemokines, which leads to the induction of Tregs and Th2, and the consequent creation of an immunosuppressive tumour microenvironment (9–11). Since IL-10 is also known to increase the expression of co-inhibitory molecules, such as PD-L1 on TAMs and DCs (5, 11), we hypothesized that the expression of PD-L1 might be different between LyP and PCALCL. Indeed, the expression of PD-L1 was prominent in LyP, while there were few PD-L1^high+ cells in the lesional skin of PCALCL, which might correlate with the incidence of secondary cutaneous malignancies in LyP (1). In parallel with the expression of PD-L1, the expression of CCL18, which was previously reported to correlate with the prognosis of CTCL (6), was prominent in LyP.

CD205 is highly expressed in human DCs and can mediate antigen uptake, processing and proliferation (12, 13). *In vivo*, CD205 is almost exclusively expressed by DCs in lymphoid organs (14). Targeting anti-gens to CD205-expressing DCs in the steady state has been shown to induce tolerance (13). However, this can be overcome by co-administration of an adjuvant that activates DC, leading to induction of the immune response (14). Because the microenvironment of CTCL is proinflammatory (15), CD205-expressing DCs might work as immunogenic DCs in CTCL. In the present study, CD205-expressing DCs were more prominent in PCALCL than in LyP, suggesting that histiocytes in PCALCL are activated, immunogenic DCs.

Since the analysed number of cases was small, and IHC analysis examines only a single time-point within the life of a tumour, further cases are needed to gain additional insight into the pathomechanisms of LyP and PCALCL.

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