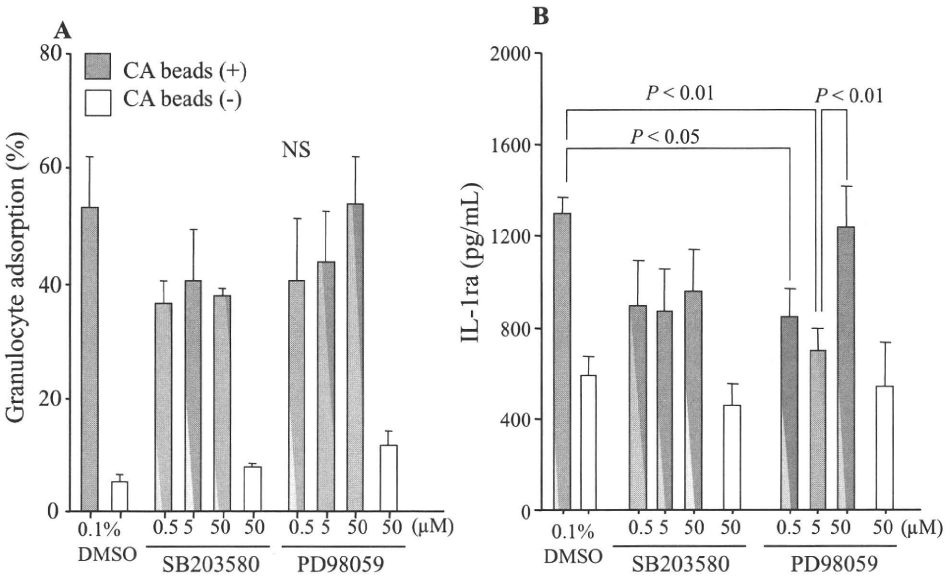

FIG. 3. Effects of signal transduction inhibitors on (A) the adsorption to cellulose acetate (CA) beads and (B) IL-1ra release. Blood cells were pre-incubated with various concentrations of SB203580 or PD98059 for 15 min at room temperature and then cultured with (■) or without (□) cellulose acetate beads for 1 h. For the control treatment, 0.1% dimethyl sulfoxide (DMSO) was used. Data are presented as mean ± standard error from three different blood donors. NS, not significant, by repeated-measures one-way ANOVA (post-hoc test with Bonferroni) in cellulose acetate beads (+).

inhibited using SB203580 and PD98059, respectively. These inhibitors did not show any cytotoxic effects on leukocyte viability after examination (flow cytometric assessment of viable cells using propidium iodide staining showed more than 95% viable cells), and the number of cells did not decrease after incubation. The down-regulation of L-selectin (CD62L) induced by fMLP (1 μmol/L) stimulation was clearly suppressed by treatment with SB203580 and PD98059 (data not shown). These inhibitors slightly, but not significantly, inhibited granulocyte adsorption, as with ulinastatin (Fig. 3A). SB203589 did not significantly inhibit IL-1ra release (Fig. 3A); however, IL-1ra release was significantly inhibited by the addition of a low dose of PD98059 (0.5 and 5 μmol/L), and a high dose of PD98059 (50 μmol/L) augmented IL-1ra release as compared to a low dose (Fig. 3B).

DISCUSSION

Ulinastatin has two functions: one is as a serine protease inhibitor, and the other is as a suppressor of pro-inflammatory cytokine production. In the present study we found that ulinastatin did not inhibit GM adsorption to cellulose acetate beads, and that 10 000 units/mL of ulinastatin augmented the release of IL-1ra induced by GM adsorption. Ulinastatin and cellulose acetate beads are separately used for patients with ulcerative colitis, and IL-1ra release is correlated with clinical efficacy (15,17); thus, the present study demonstrates the feasibility of combination therapy with ulinastatin and GMA.

Complement activation fragments produced by contact between cellulose acetate beads and blood play important roles in biological responses to GMA therapy. Our previous studies have demonstrated that IL-1ra release requires both granulocyte adhesion to cellulose acetate beads and C5a (anaphylatoxin) stimulation (8,18,19). The release of IL-1ra by cellulose acetate beads is similar to that of elastase, a serine protease. Granulocytes stimulated in suspension show little degranulation, but C5a induces the release of elastase from adherent cells in minutes (20). It is well known that granulocyte adhesion and C5a stimulation cause activation of ERK1/2 and p38 MAPK signaling pathways in granulocytes (21,22); thus, ERK1/2 and p38 MAPK signaling pathways should be involved in the release of IL-1ra.

Another serine protease inhibitor, gabexate mesilate, did not augment IL-1ra release (Fig. 2A), suggesting that IL-1ra release is unrelated to serine protease inhibition. Interestingly, gabexate mesilate also inhibits cytokine production, but this effect is mediated by the suppression of nuclear factor-κB (NF-κB) activation (11). On the other hand, ulinastatin inhibits phosphorylation of ERK1/2 and decreases expression of early growth response

factor-1 induced by lipopolysaccharide without affecting the activation of NF-κB and activator protein-1 (23). Since C5a/C5a receptor signaling requires the phosphorylation of ERK1/2 (21), it is reasonable to postulate that a low dose of ulinastatin and an ERK1/2-specific inhibitor (PD98059) inhibit the release of IL-1ra.

That high doses of these reagents augmented the release of IL-1ra is difficult to explain. Interestingly, PD98059 blocks neutrophil chemotaxis, but does not alter superoxide anion production and paradoxically enhances degranulation responses to stimuli (24). Indeed, the effect of PD98059 on IL-1ra release was similar to that of a previous report on degranulation (Fig. 3). The blocking of ERK signaling by ulinastatin may augment alternative signaling by a functional diversion of ERK signaling pathways, such as the activation and maintenance of cytosolic phospholipase A_2 activity (22).

Gabexate mesilate has an inhibitory effect on complement activation (9). We measured the production of C5a in blood samples from two volunteers after incubation with cellulose acetate beads using flow cytometry and found that the level of C5a in blood containing 800 μg/mL of gabexate mesilate was lower than in blood containing 10 000 units/mL of ulinastatin (Nishise, unpublished results). Since complement activation is required for granulocyte adsorption to cellulose acetate beads and the release of IL-1ra (8), the difference between ulinastatin and gabexate mesilate on GM adsorption should be attributed to the inhibitory effect on complement activation.

In patients with ulcerative colitis, ulinastatin has been used with steroids and is thought to be an effective therapy. It has been reported that rectal mucosal blood flow is decreased in active ulcerative colitis patients as compared with healthy controls, but it is improved after the intravenous injection of ulinastatin and prednisolone (15), and that intravenous injection of 200 000 units of ulinastatin weekly for three months in patients taking corticosteroids is effective in 64% of patients with moderate to severe ulcerative colitis who are resistant to steroid therapy (16). Combination therapy with ulinastatin and GMA has not been administered in patients with ulcerative colitis, and the biological effects of ulinastatin for GM adsorption to cellulose acetate beads are still unknown. Our study found that a high concentration of ulinastatin augmented the release of IL-1ra in GM adsorption to cellulose acetate beads. It has been reported that patients with ulcerative colitis who responded to GMA treatment show a significant increase in IL-1ra in the Adacolumn outflow (17), and that the mucosal tissue ratio of IL-1ra/IL-1β is significantly increased in ulcerative colitis patients with clinical remission after Adacolumn therapy (4). Therefore, ulinastatin administered during GMA may provide clinical efficacy in patients with ulcerative colitis through an increase in IL-1ra. There are some practical issues that remain to be resolved before ulinastatin can be applied with GMA in a clinical setting. First of all, it must be verified that a high dose of ulinastatin does not have adverse affects on the human body, because a larger dose of ulinastatin than that used in previous studies might be needed for ulcerative colitis patients during Adacolumn therapy.

CONCLUSION

The present study found that a high concentration of ulinastatin increased the release of IL-1ra without inhibiting granulocyte adsorption to cellulose acetate beads. Our results should help to clarify the anti-inflammatory effects of ulinastatin and potentially lead to new combination therapies for patients with ulcerative colitis.

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Differential immunophenotypic analysis of dendritic cell tumours

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ABSTRACT

Aims The phenotypic and biological characteristics of dendritic cell (DC) tumours have not been fully elucidated. The aim of this study was to compare the immunophenotypic characteristics of DC-related markers and cell-cycle-associated markers among DC tumours and finally to utilise them for differential diagnosis of DC tumours.

Methods Tissue sections from 28 patients with DC tumours were immunohistochemically examined using DC-related and cell-cycle-associated markers.

Results The Langerhans cell histiocytosis (LCH) and Langerhans cell sarcoma (LCS) samples were positive for S-100 protein, CD1a, Langerin, fascin, DEC-205 and DC-SIGN. Interdigitating dendritic cell sarcoma (IDCS) was positive for S-100 protein and fascin and negative for Langerin. In addition, two IDCS samples were positive for CD1a, DEC-205 and DC-SIGN. The labelling indices of Ki-67, cyclin A, cyclin B1 and acetylated histone H3 on the LCS and IDCS specimens were significantly higher than those on the LCH specimens. The expression of p53 was also significantly higher in the LCS specimens than in the LCH specimens. The numbers of infiltrating $CD123^+$ and $FOXP3^+$ cells were also significantly higher in the LCS samples than in the LCH and IDCS samples. Follicular dendritic cell sarcoma was distinguished from other DC tumours by the lack of DC-SIGN, Langerin and DCE-205.

Conclusions These results suggest that Langerin can be used to distinguish LCS from IDCS, and DC-SIGN and DEC-205 can be used to identify DC tumour cells. The frequency of cell-cycle-associated markers can be used for the differential diagnosis of malignant and benign DC tumours.

INTRODUCTION

Langerhans cell histiocytosis (LCH) is caused by the abnormal accumulation and/or proliferation of pathological LCs which are S-100 $protein^+$, $CD1a^+$ and $Birbeck\ granule^+$.[1] It displays a heterogeneous clinical feature that ranges from the involvement of a single organ system (SS) (primarily skin or bone) to the involvement of multiple organ systems (MS) complicated by organ dysfunction.[1 2] Only about 20 cases of Langerhans cell sarcoma (LCS) have been previously reported in English literature,[1 3 4] which can be considered as a higher-grade variant of LCH, and it can present de novo or progress from antecedent LCH. Interdigitating dendritic cell sarcoma (IDCS) is also a very rare disease, and fewer than 60 cases have been reported.[1 5–8] IDCS is a neoplastic proliferation with phenotypes similar to those of IDCs. The immunophenotype of LCS and IDCS has not been classified because of their rarity. Furthermore, it is not easy to morphologically distinguish LCS or IDCS tumours from lesions of the MS subtype of LCH. Follicular dendritic cell sarcoma (FDCS) is categorised as a neoplastic proliferation of spindle to ovoid cells showing morphological and immunophenotypic features of FDCs.[1]

The expression of the tumour suppressor gene p53 occurs at low levels in normal cells due to a very short half life; however, p53 is often abnormal in malignant tumours. There have been only a few reports addressing the levels of p53 expression in dendritic cell (DC) tumours.[9–11] $CD4^+CD25^+$ regulatory T cells (Tregs) express forkhead box protein 3 (FOXP3).[12 13] Recent studies have reported that high numbers of Tregs are present in cancer tissue and suppress the anti-tumour immune response; however, there has been no reported correlation between Tregs and DC tumours.[14]

The objective of this study was to evaluate the immunophenotype of DC-related markers and the expression of cell-cycle markers, acetylated histone H3 and p53 in the four types of DC tumours in order to determine their distinguishing characteristics. We also examined the degree of the infiltration of non-neoplastic plasmacytoid DC (pDC) and Treg in DC tumours to clarify the difference of the resistance of DC tumours to antitumour immune response mediated via the impact of pDCs and Tregs especially between benign (LCH) and malignant (LCS) LC tumours. The present study evaluated the identity of markers that would enable the differentiation of benign DC tumours from malignant DC tumours.

MATERIALS AND METHODS

Patients and specimens

Tissue samples were obtained from 26 patients who were diagnosed as having LCH, LCS, IDCS or FDCS in our hospital between 1980 and 2008. Twenty-two patients were male, and four were female, and the mean age was 28 years (6–71). Seventeen patients were diagnosed as having LCH (14 males and three females with a mean age of 11 years (6–35)), including 13 cases of SS and four cases of MS LCH. Four patients had LCS (four males with a mean age of 57 years (40–64)), and five patients were diagnosed as having IDCS based on lymph node (four males and one female with a mean age of 59 years (52–71)). Two patients were also treated with FDCS (two males with a mean age of 63 years (60 and 66)). Resected specimens were fixed in 10% formalin. These specimens were embedded in paraffin to use for Haematoxylin & Eosin staining and

Table 1 Primary antibodies used in this study

Antibody (clone)	Ig subclass	Source
S-100 protein	Rabbit, heterologous	Nichirei, Tokyo, Japan
CD1a (O10)	Mouse IgG1κ	Immunotech, Marseille, France
Langerin (CD207; 12D6)	Mouse IgG2b	YLEM, Rome, Italy
Fascin (55K-2)	Mouse IgG1κ	DAKO, Glostrup, Denmark
CD83 (1H4b)	Mouse IgG1κ	Novocastra, Newcastle upon Tyne, UK
DC-SIGN (CD209)	Rabbit IgG	Santa Cruz, Delaware Avenue, California
DEC-205 (CD205; 11A10)	Mouse IgG1	Novocastra
CD123 (S-12)	Mouse IgG1	Santa Cruz
FOXP3 (263A/E7)	Mouse IgG1	Abcam, Cambridge, UK
Ki-67 (MIB-1)	Mouse IgG1	Immunotech
Cyclin A (6E6)	Mouse IgG1κ	Novocastra
Cyclin B1 (7A9)	Mouse IgG1κ	Novocastra
p53 (DO-7)	Mouse IgG2b	Novocastra
Acetylated histone H3	Rabbit, heterologous	Upstate, Lake Placid, New York
CD163 (10D6)	Mouse IgG1	Novocastra

immunohistochemical study. All the patients provided informed consent, and the study was approved by the Yamagata University School of Medicine Ethics Committee.

Immunohistochemistry

Four-micrometre-thick sections were prepared and mounted on glass slides. The primary antibodies used in this study are listed in table 1. The labelled streptavidin-biotin-peroxidase method (Ultratech HRP Streptavidin-biotin Universal Detection System, Immunotech, Marseille, France) or the alkaline phosphatase method (streptavidin/AP; DAKO, Glostrup, Denmark) was used. A positive reaction was detected as a brown colour with 3,3′-diaminobenzidine (Dojin Chemicals, Kumamoto, Japan) or as a red colour with new fuchsin (DAKO). In the case of the former, the sections were counterstained with haematoxylin. The double immunostaining was done to evaluate whether S-100 protein$^+$ DC tumour cells simultaneously express DC-SIGN. The alkaline phosphatase method was applied for DC-SIGN (new fuchsin, red) in the first-step staining. After sections were subjected to heat-induced antigen retrieval for 20 min, the labelled streptavidin-biotin-peroxidase method was carried out for S-100 protein (TrueBlue, blue; Kirkegaard & Perry Laboratories, Gaithersburg, Maryland) in the second-step staining.

For the positive controls, we similarly immunostained tissue sections of lymph nodes obtained from three patients with reactive lymphadenitis. In addition, tissue sections were incubated with non-immune mouse or rabbit immunoglobulin (DAKO), respectively, instead of the primary antibody as negative control.

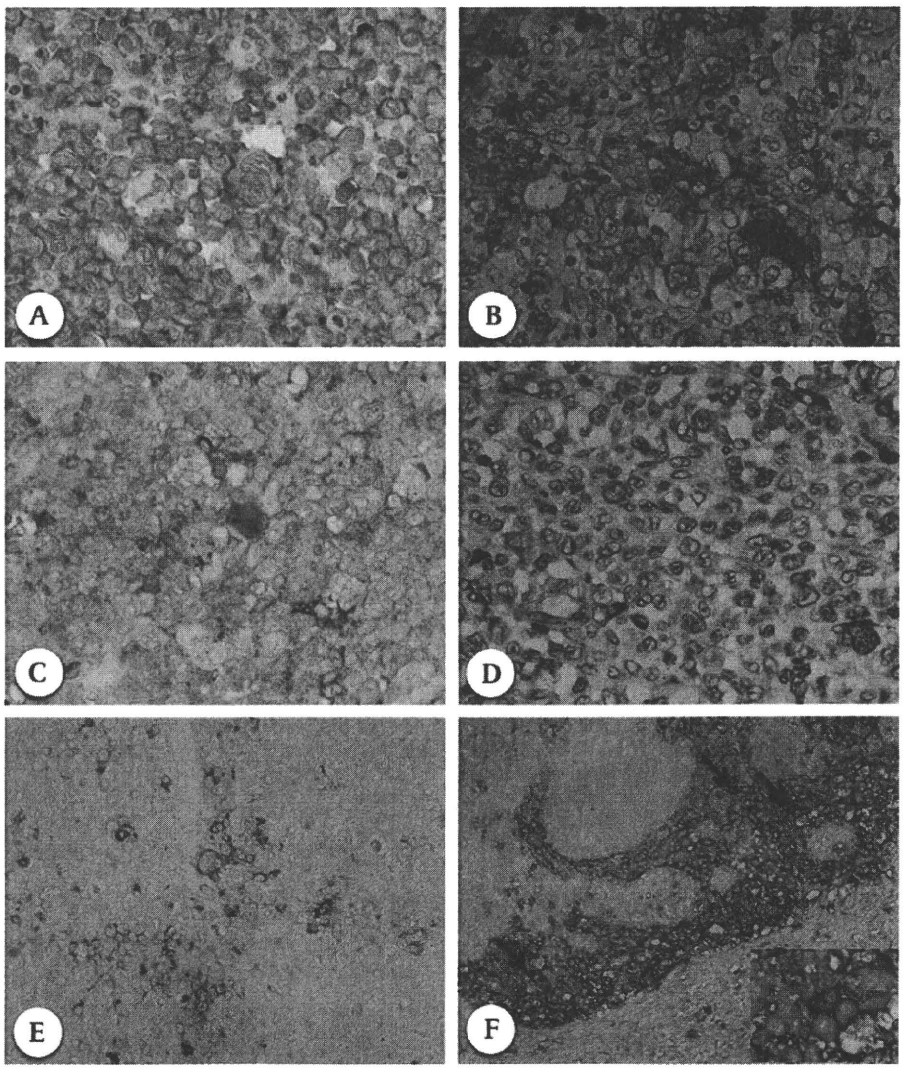

Figure 1 Immunophenotype of dendritic cell tumours. (A) Almost all Langerhans cell sarcoma cells express CD1a. (B) A majority of tumour cells in the case of interdigitating dendritic cell sarcoma express CD1a. (C) Almost all Langerhans cell sarcoma cells express DEC-205. (D) A majority of tumour cells in this case of Langerhans cell sarcoma also express DC-SIGN. (E) A part of follicular dendritic cell sarcoma cells express fascin. (F) Double immunostaining for DC-SIGN (red) and S-100 protein (blue) in Langerhans cell sarcoma showing a small number of double positive tumour cells (arrows). (Inset) High-power view of a double positive cell. (A–D) Counterstained with haematoxylin, original magnification ×400; (E–F) not counterstained, original magnification ×300 (inset ×800).

The tissue sections were immersed in 10 mM citrate buffer, pH 6.0 (Mitsubishi Kagaku Iatron, Tokyo, Japan), or in 50 mM Tris-HCl buffer, pH 9.0, containing 1 mM ethylenediaminetetraacetic acid for the CD123 immunostain. The sections were subjected to autoclave treatment for 20 min at 120°C to determine the antigenicity.

Counting of immunostained cells

Photographs of 10 high-power fields at ×400 magnification under a microscope with CCD camera were taken and stored as Photoshop files (Photoshop 5.0 Limited Edition, Adobe, San Jose, California). The ratios of tumour cells positive for S-100 protein, CD1a, Langerin, fascin, CD83, DC-SIGN, DEC-205, Ki-67, cyclin A, cyclin B1, acetylated histone H3 (H3Ac) and p53 per total tumour cells (%) were counted by two observers independently and expressed as the mean±SD. The number of cells positive for CD123 and FOXP3 was counted in 10 high-power fields, and the mean number±SD of positive cells per a microscopic field (×400) was calculated.

Statistical analysis

Statistical analysis was carried out using StatView 4.5 software package (Abacus Concepts). The Mann–Whitney U test was used for immunohistochemical data. P values <0.05 were considered to be significant.

RESULTS

Immunophenotype of DC-related markers in DC tumour cells

The majority of tumour cells in the 26 cases of LCH, LCS and IDCS tumours expressed S-100 protein and fascin. However, none of the tumour cells expressed CD83. In most cases of LCH and LCS, at least 75% of the tumour cells expressed CD1a (figure 1A), Langerin and DEC-205 (figure 1C). Most of the cases of LCH and LCS expressed DC-SIGN in variable frequencies (figure 1D). The double immunostaining demonstrated the presence of DC-SIGN$^+$ S-100 protein$^+$ tumour cells (figure 1F). Langerin was not expressed in IDCS cells, and the other DC markers, CD1a, DEC-205 and DC-SIGN, were positive in only two cases (figure 1B). CD163$^+$ cells were scattered throughout the DC tumours in various degrees. Both cases of FDCS were often positive for S-100 protein and fascin but negative for CD1a, DC-SIGN, Langerin and DEC-205.

The frequency of tumour cells positive for DC markers (S-100 protein, CD1a, Langerin, fascin and DEC-205) was compared statistically among LCH, LCS and IDCS (table 2). Although there was a significant difference in the frequency of Langerin$^+$ tumour cells among the IDCS and LCH or LCS cells ($p<0.05$), there was no significant difference detected in the frequencies of any other DC markers.

Table 2 Frequency of S-100 protein, CD1a, Langerin, fascin, DC-SIGN, and DEC-205 in Langerhans cell histiocytosis (LCH), Langerhans cell sarcoma (LCS), interdigitating cell sarcoma (IDCS) and follicular dendritic cell sarcoma (FDCS)

		The frequency of immunopositive tumor cells (%)(mean ± standard deviation)		
Histology	Number of cases	S-100 protein	CD1a	Langerin
LCH (total)	17	87.44 ± 11.57	89.02 ± 13.13	78.00 ± 19.27
LCH (SS)	13	86.31 ± 12.86	92.05 ± 9.07	80.28 ± 17.00
LCH (MS)	4	91.13 ± 5.29	79.18 ± 20.51	70.59 ± 27.98
LCS	4	95.78 ± 2.24	96.43 ± 2.14	80.96 ± 13.83
IDCS	5	79.97 ± 18.02	43.99 ± 51.41	0.00 ± 0.00
FDCS	2	15.12 ± 1.28	0	0

Histology	Number of cases	Fascin	DC-SIGN	DEC-205
LCH (total)	17	82.97 ± 16.92	33.80 ± 24.13	87.85 ± 19.81
LCH (SS)	13	79.98 ± 18.00	29.36 ± 22.73	91.01 ± 17.29
LCH (MS)	4	92.68 ± 8.18	48.22 ± 26.01	77.59 ± 26.72
LCS	4	93.93 ± 3.96	35.38 ± 22.80	98.27 ± 1.70
IDCS	5	93.74 ± 5.37	34.45 ± 46.25	49.40 ± 55.87
FDCS	2	29.03 ± 5.97	0	0

SS; single system, MS; multisystem, [a]: $p < 0.01$ and [b]; $p < 0.05$ by Mann-Whitney U-test
FDCS was excluded from statistical analysis because of a lower number of cases.

The expression of cell-cycle markers, acetylated histone H3 (H3Ac) and p53 in DC tumour cells

The frequency of tumour cells positive for Ki-67, cyclin A, and cyclin B1 on LCS and IDCS was significantly higher than that on the LCH tumour cells ($p<0.01$)(table 3; figure 2A–C). Furthermore, there was a significant difference in the frequencies of Ki-67 and cyclin A expression between the LCS or IDCS specimens and the samples from the LCH subtypes (SS and MS). There was a significant difference in the frequency of cyclin B1 expression between the LCH MS samples and the IDCS samples. There were no significant differences in the frequencies of Ki-67, cyclin A and cyclin B1 between the SM and MM subtypes of LCH and between the LCS and IDCS tumour cells.

The frequency of H3Ac nuclear expression in LCS and IDCS tumour cells was significantly higher than that in the total LCH or the SS subtype of LCH tumour cells ($p<0.05$; figure 2D). There was also a significant difference in the frequency of nuclear p53 expression between the LCS tumour cells and of the total LCH or SS type of LCH tumour cells ($p<0.05$; figure 2E); however, there was no significant difference in the p53 frequency between the IDCS and LCH specimens.

Infiltration of CD123$^+$ and FOXP3$^+$ cells in DC tumours

The number of infiltrating CD123$^+$ cells (figure 2F) and FOXP3$^+$ cells (figure 2G) was significantly higher in the LCS specimens than in the LCH or the IDCS specimens (table 4).

DISCUSSION

The present study did not detect any remarkable immunophenotypic differences between LCH and LCS cells. The cells in both types of tumours expressed S-100 protein, CD1a, Langerin, fascin and DEC-205. Neither of the tumour types expressed CD83. The immunoreactivity of S-100 protein, CD1a, Langerin, fascin and CD83 on LCH cells was consistent with the observations in previous reports.[1 9] The immunoreactivity of DC-SIGN and DEC-205 in DC tumours has not been fully examined, but this study is the first to demonstrate the usefulness of these markers to pick up LCH, LCS and IDCS cells on routine formalin-fixed and paraffin-embedded tissue sections, although these markers are not available to make a differential diagnosis among LCH, LCS and IDCS. Generally, IDCS cells lack CD1a,[1 9] but some reports are controversial.[15 16] Many recent reports defined LCS as CD1a$^+$ and Langerin$^+$ in the Birbeck granules, which is identical to LCs. Some reports also define IDCS as Langerin$^-$ independent of CD1a immunoreactivity.[9] The present study showed similar results to these earlier reports.

The Ki-67 labelling index in DC tumours has been evaluated in some previous reports, but a wide variation among cases has been reported.[17] This study demonstrated that the frequency of expression of Ki-67 (non-cycle-specific), cyclin A (S-G2 phase) and cyclin B1 (G2-M phase) was significantly higher in LCS cells and IDCS cells in comparison with the frequency in LCH cells, indicating that LCS may have a higher malignant potential than

Table 3 Frequency of Ki-67, cyclin A, cyclin B1, acetylated histone H3, and p53 in Langerhans cell histiocytosis (LCH), Langerhans cell sarcoma (LCS), interdigitating cell sarcoma (IDCS) and follicular dendritic cell sarcoma (FDCS)

		The ratio of immunopositive tumor cells per total tumor cells (%)(mean ± standard deviation)					
Histology	Number of cases	Ki-67		Cyclin A			
LCH (total)	17	30.85 ± 11.23	a a	7.10 ± 3.71	a a		
LCH (SS)	13	28.11 ± 10.52	b a	6.01 ± 2.81	a a		
LCH (MS)	4	31.88 ± 9.02	b b	10.64 ± 4.49	b b		
LCS	4	53.77 ± 10.21		22.59 ± 2.18			
IDCS	5	62.15 ± 19.82		26.29 ± 8.41			
FDCS	2	25.20 ± 2.08		11.60 ± 0.80			
Histology	Number of cases	Cyclin B1		Acetylated histone H3		p53	
LCH (total)	17	1.81 ± 1.68	a a	83.16 ± 8.99	b b	43.75 ± 22.25	b
LCH (SS)	13	1.25 ± 0.99	a a	82.47 ± 8.42	b b	39.35 ± 20.59	a
LCH (MS)	4	3.63 ± 2.31	b	85.42 ± 11.78		58.06 ± 24.22	
LCS	4	11.29 ± 3.11		94.07 ± 2.28		72.90 ± 7.22	
IDCS	5	7.15 ± 2.67		93.76 ± 3.84		62.24 ± 24.60	
FDCS	2	1.13 ± 0.08		79.52 ± 2.48		24.35 ± 3.87	

SS; single system, MS; multisystem, [a]: $p < 0.01$ and [b]; $p < 0.05$ by Mann-Whitney U-test
FDCS was excluded from statistical analysis because of a lower number of cases.

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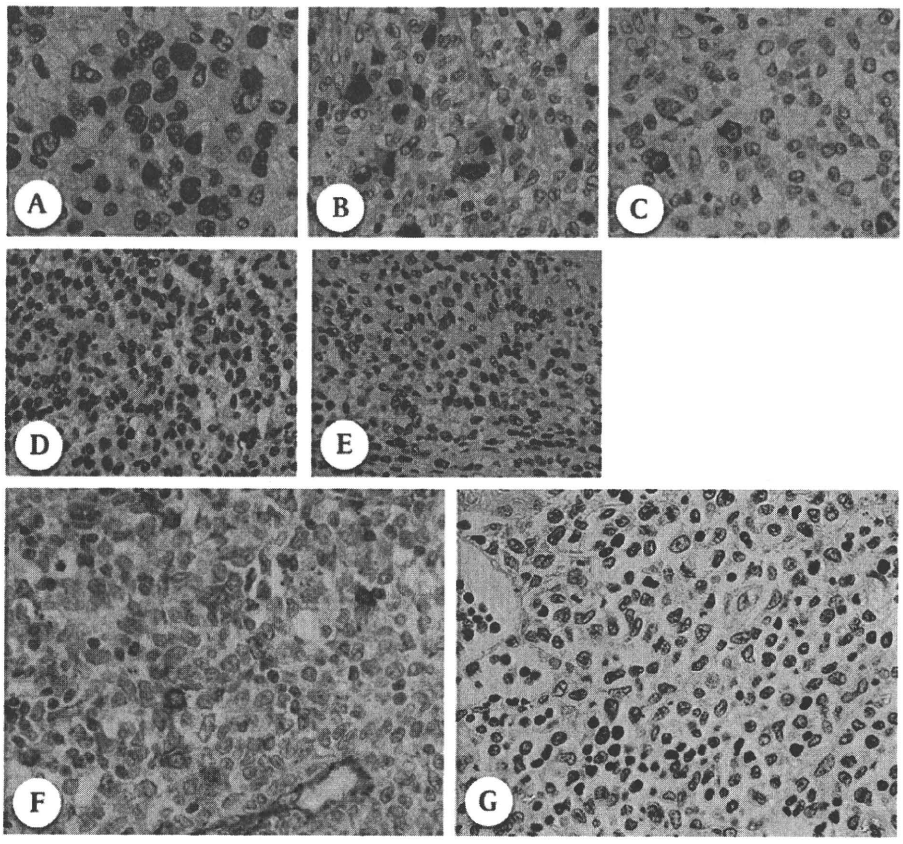

Figure 2 Immunostaining of cell cycle markers, acetylated histone H3, p53, CD123 and FOXP3 in Langerhans cell sarcoma. (A) The nuclei of a part of tumour cells are labelled with Ki-67. (B) Some tumour cells are labelled with cyclin A. (C) Occasional tumour cells express cyclin B1. (D) Almost all tumour cells are labelled with acetylated histone H3. (E) A majority of tumour cells are labelled with p53. (F) Some $CD123^+$ cells are scattering in the tumour. (G) Many $FOXP3^+$ cells are infiltrating in the tumour. Counterstained with haematoxylin, original magnification: ×400

LCH. The superiority of cyclin A rather than Ki-67 and cyclin D1 as an indicator of poor prognosis has previously been demonstrated.[18] A lower p value was found in the case of cyclin A ($p<0.0001$) in comparison with the p values for other cell-cycle markers used in this study. This observation strongly suggested that cyclin A may be a potent indicator for distinguishing LCH from LCS.

The inhibition of oncogene p53 expression during DNA damage stops cell proliferation and induces apoptotic cell death. The detection of mutant p53 or wild type p53 expression in IDCS and LCH tumour cells is controversial.[19 20] In this study, a significant difference in the frequency of p53 expression was found only between the LCH and LCS specimens; however, in combination with the cell-cycle-marker analysis, p53 may be an effective prognostic indicator in DC tumours.

Recent papers have demonstrated that the acetylation and deacetylation of histones regulate the cell cycle and the activation and suppression of transcription.[20 21] The present analysis demonstrated a higher frequency of H3Ac expression in LCS and IDCS cells in comparison with LCH cells. This result indicated

Table 4 Frequency of infiltrating $CD123^+$ and $FOXP3^+$ cells in Langerhans cell histiocytosis (LCH), Langerhans cell sarcoma (LCS), interdigitating cell sarcoma (IDCS) and follicular dendritic cell sarcoma (FDCS)

		Number of infiltrating cells/High-power field (mean ± standard deviation)			
Histology	Number of cases	CD123		FOXP3	
LCH (total)	17	0.18 ± 0.27	a	5.44 ± 3.25	a
LCH (SS)	13	0.15 ± 0.29	a	5.15 ± 3.55	a
LCH (MS)	4	0.28 ± 0.21	b	6.38 ± 2.11	b
LCS	4	6.30 ± 4.85	b	23.85 ± 8.05	b
IDCS	5	0.05 ± 0.10		4.13 ± 4.92	
FDCS	2	0.16 ± 0.03		2.86 ± 0.32	

SS; single system, MS; multisystem, [a]: $p < 0.01$ and [b]; $p < 0.05$ by Mann-Whitney U-test
FDCS was excluded from statistical analysis because of a lower number of cases.

the presence of active transcription and accelerated cell proliferation in LCS and IDCS.

The infiltration of both CD123$^+$ pDCs and FOXP3$^+$ Tregs was frequently detected in the LCS tumour samples in this study. The antitumour immune response in some patients with cancer is suppressed by Tregs.[22] A relationship between CD123$^+$ cells and FOXP3$^+$ cells has been demonstrated.[23] Tregs express chemokine receptors such as CCR4 and CCR6,[24] and LCH cells secrete CCL22 (a ligand for CCR4) and CCL20 (a ligand for CCR6).[25 26] LCS tumour cells may become resistant to antitumour immunity by producing these chemokines and inducing Treg infiltration, whereas LCH tumour cells occasionally experience spontaneous regression during the clinical course of the disease. The issue of the impact of infiltrating Tregs on tumour progression is a complex one, as the efficacy of effector T cell suppression by Tregs can be affected by a plethora of cellular and molecular players populating the tumour microenvironment. Therefore, data on the activation status of Tregs, on the ratio between Tregs and T-effectors as well as on the composition of the inflammatory cytokine microenvironment could provide useful elements to support the hypothesis of a differently effective antitumour response in LCH and LCS.

In conclusion, the present study confirmed that Langerin is a useful marker to distinguish LCS from IDCS, and DC-SIGN and DEC-205 can also be used to pick up DC tumours. In addition, we demonstrated the increased frequency of cell-cycle markers, H3Ac, and p53 in LCS and IDCS cells. It should be noted that these frequencies could be used to develop a differential diagnosis of malignant or benign DC tumours (figure 3).

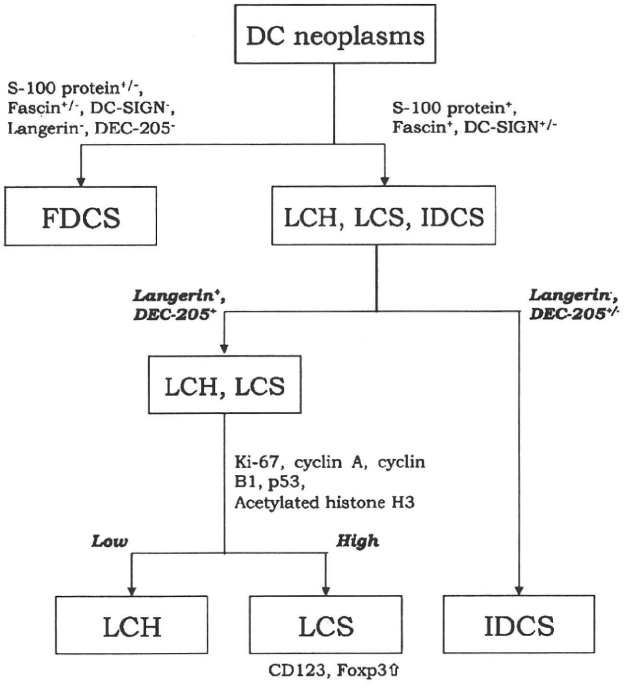

Figure 3 Diagnostic flow chart of dendritic cell neoplasms. Follicular dendritic cell sarcoma is negative for DC-SIGN, Langerin and DEC-205. Langerhans cell tumours and interdigitating cell sarcoma (IDCS) are recognised by S-100 protein$^+$, fascin$^+$ and DC-SIGN$^{+/-}$. Langerhans cell histiocytosis (LCH) and Langerhans cell sarcoma (LCS) are distinguished from IDCS by the immunostaining of Langerin and DEC-205. Expression of cell-cycle markers, acetylated histone H3 and p53 is low in LCH, whereas it is high in LCS.

Take-home messages

- **It is not easy to morphologically distinguish LCS or IDCS tumours from lesions of the multiple organ systems subtype of LCH.**
- **The immunostain of DC-SIGN and DEC-205 on routine formalin-fixed and paraffin-embedded tissue sections is useful to pick up LCH, LCS and IDCS cells.**
- **Cyclin A may be a potent indicator for distinguishing LCH from LCS.**

This study also demonstrated that the frequent infiltration of pDCs and Tregs in LCS tumours facilitates a resistance to the antitumour immune response.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the Yamagata University School of Medicine Ethics Committee.

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