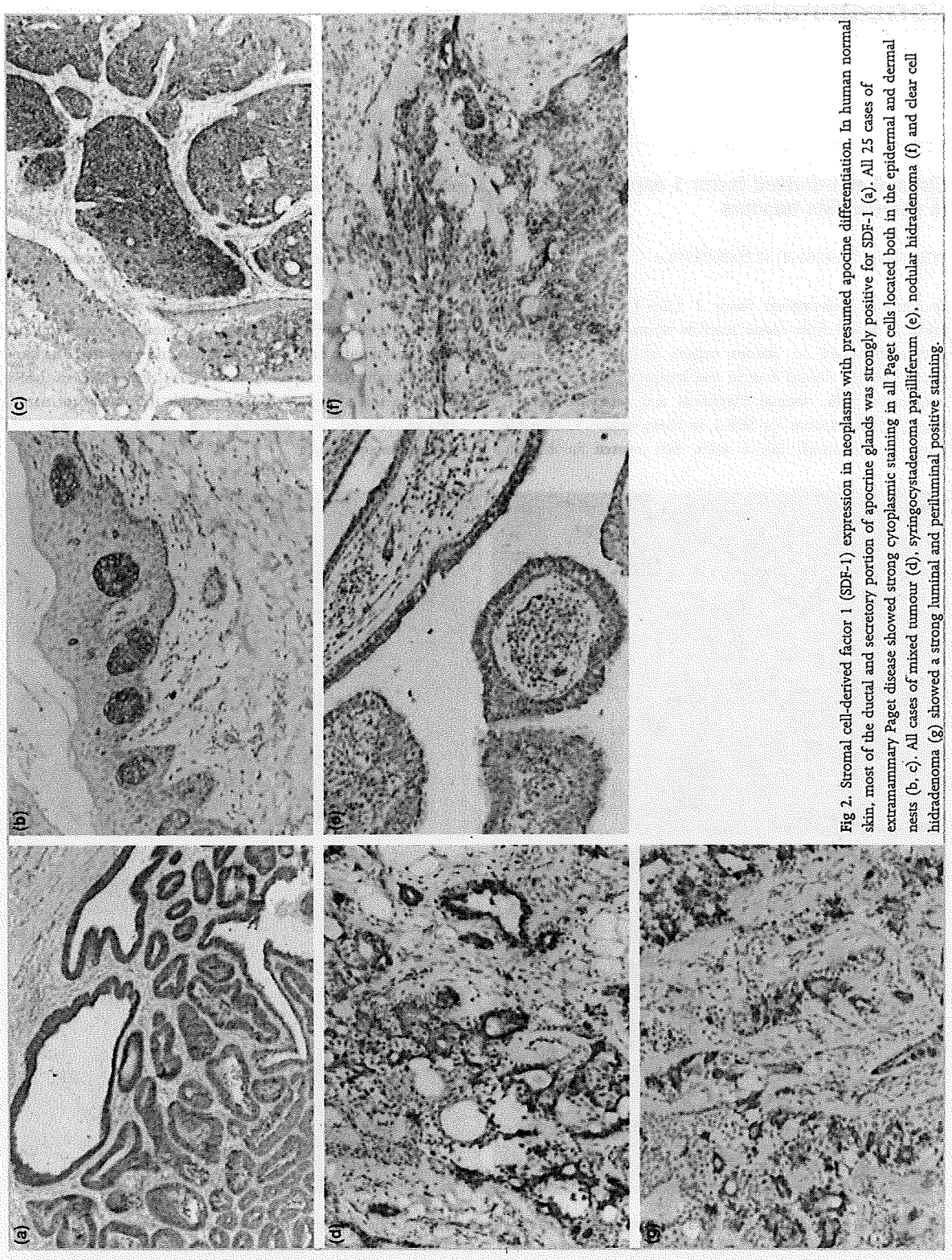

Fig 2. Stromal cell-derived factor 1 (SDF-1) expression in neoplasms with presumed apocine differentiation. In human normal skin, most of the ductal and secretory portion of apocrine glands was strongly positive for SDF-1 (a). All 25 cases of extramammary Paget disease showed strong cytoplasmic staining in all Paget cells located both in the epidermal and dermal nests (b, c). All cases of mixed tumour (d), syringocystadenoma papilliferum (e), nodular hidradenoma (f) and clear cell hidradenoma (g) showed a strong luminal and periluminal positive staining.

One hundred and eleven paraffin sections of skin tumours were subjected to immunohistochemical staining for SDF-1, including 10 cases of squamous cell carcinoma (SCC), eight of basal cell carcinoma (BCC), six pilomatrixoma, six trichoepithelioma, six trichoblastoma and three sebaceous carcinoma; six syringoma, six eccrine poroma, three eccrine hidradenoma and six eccrine porocarcinoma; and 25 extramammary Paget disease (EMPD), eight mixed tumours, five syringocystadenoma papilliferum, five apocrine hidradenoma, three nodular hidradenoma and five clear cell hidradenoma.

In human normal skin, SDF-1 was detected on scattered inflammatory cells and some fibroblasts in the dermis, endothelial layer of blood vessels and perifollicular fibrous sheets as in a previous study.[9] The outer layer of hair follicles was faintly positive for SDF-1. The secretory, but not ductal, portion of eccrine glands was strongly positive for SDF-1 (Fig. 1a). In contrast, most of the ductal and secretory portion of apocrine glands was strongly positive for SDF-1 (Fig. 2a).

Of the 111 skin tumours, clear cytoplasmic positive staining was observed only in two of 10 SCCs. All cases of BCC, pilomatrixoma, trichoepithelioma, trichoblastoma and sebaceous carcinoma showed negative staining in tumour cells. The staining intensity for SDF-1 of stromal fibroblasts and endothelial cells in these tumours was similar to that of normal skin dermis. Eccrine poromas and porocarcinomas were basically negative for SDF-1 in tumour cells (Fig. 1b,c); however, luminal and periluminal positive staining was detected focally in two poromas and one porocarcinoma (Fig. 1b,d). None of the eccrine hidradenomas or syringomas showed any staining for SDF-1 (data not shown). Interestingly, SDF-1 was more strongly and widely distributed in other skin tumours of appendage differentiation. All of the 25 cases of EMPD showed very strong cytoplasmic staining in each Paget cell located both in the epidermal and dermal nests (Fig. 2b,c). All eight cases of mixed tumours (Fig. 2d), five of five syringocystadenoma papilliferum (Fig. 2e), five of five apocrine hidradenoma, three of three nodular hidradenoma (Fig. 2f) and five of five clear cell hidradenoma (Fig. 2g) showed a strong luminal and periluminal positive staining.

SDF-1 is a 68-amino acid small (8 kDa) cytokine that belongs to the CXC chemokine family. SDF-1 is expressed in two isoforms, SDF-1α and SDF-1β, from a single gene that encodes two splice variants.[10] Recent reports have shown that SDF-1 and CXCR4 are expressed in various solid tumours, and are involved in tumour development and metastasis.[4–6] However, to our knowledge, the involvement of this chemokine in cutaneous neoplasms remains unclear. In the present study, the staining pattern of SDF-1 in normal skin is in agreement with previous studies.[7–9] SDF-1 staining was confined to inflammatory cells, some fibroblasts, endothelial cells of dermal vessels, perifollicular fibrous sheets, the secretory portion of eccrine glands, and the ductal and secretory portion of apocrine glands. The entire epidermis was completely negative for SDF-1, suggesting that SDF-1 may be a marker of adnexal differentiation. Thus, we further investigated the expression pattern in cutaneous neoplasms. Strong positive staining for SDF-1 was evident in the tumours of apocrine differentiation such as EMPD, syringocystadenoma papilliferum and apocrine hidradenoma. In addition, the positive staining was largely compartmentalized in the luminal and periluminal area of eccrine poroma, eccrine porocarcinoma, nodular hidradenoma, clear cell hidradenoma and mixed tumours. SDF-1 was not expressed in syringoma and eccrine hidradenoma, which are considered to originate from the ductal portion of eccrine glands. However, except for two of 10 SCCs that showed positive staining for SDF-1 in tumour cells, all the nonglandular epidermal and follicular tumours such as BCC, pilomatrixoma, trichoblastoma and trichoepithelioma showed negative staining. These results indicated that SDF-1 may relate to the glandular differentiation of tumour cells to the secretory portion of eccrine glands or apocrine glands. The role of SDF-1 in these glandular skin tumours and some SCCs still needs further study.

*Department of Dermatology, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashiku, Fukuoka 812-8582, Japan
†Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
Correspondence: Takeshi Nakahara.
E-mail: nakahara@dermatol.med.kyushu-u.ac.jp

S-J. Chen*†
T. Nakahara*
M. Kido*
M. Takahara*
H. Uchi*
S. Takeuchi*
L. Dugu*
Y-T. Tu†
Y. Moroi*
M. Furue*

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Key words: *apocrine glands, eccrine glands, skin tumours, stromal cell-derived factor 1*

Conflicts of interest: none declared.

MUM1 expression does not differentiate primary cutaneous anaplastic large-cell lymphoma and lymphomatoid papulosis

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Sir, We read with interest the article entitled 'MUM1 expression in cutaneous CD30+ lymphoproliferative disorders: a valuable tool for the distinction between lymphomatoid papulosis and primary cutaneous anaplastic large-cell lymphoma' recently published in the *British Journal of Dermatology*.[1]

We have been reviewing and studying the cutaneous CD30+ lymphoproliferative disorders of the Canarian Primary Cutaneous Lymphoma Network. Clinical, demographic, morphological and immunohistochemical data were obtained from 21 selected cases. Futhermore, we examined the expression of MUM1 in these disorders to assess its value as a diagnostic marker, because of the two papers published recently with conflicting results.[1,2]

Twenty-one cases: 13 lymphomatoid papulosis (LyP), seven cutaneous anaplastic large-cell lymphoma (C-ALCL) and one secondary cutaneous anaplastic large-cell lymphoma (S-ALCL) were selected from the database of the Canarian Primary Cutaneous Lymphoma Network. All of them met diagnostic criteria according to the WHO–EORTC classification for cutaneous lymphomas.[3] Clinical, demographic, morphological and immunohistochemical data were reviewed and studied. Formalin-fixed, paraffin-embedded specimens were analysed by immunohistochemistry with a monoclonal antibody against MUM1 (Dako, Glostrup, Denmark).

Clinical, demographic, morphological and immunohistochemical results were similar to those in previously reported series.[3,4] Positive staining for MUM1 was observed in 12 of 13 cases of LyP (92%), seven of seven C-ALCL (100%) and one of one S-ALCL (100%).

In the study reported recently by Kempf *et al.*,[1] 13 of 15 LyP (87%) samples stained strongly positive for MUM1, while only two of 10 C-ALCL (20%) specimens did so. Conversely, Wasco *et al.*[2] found strong expression of MUM1 in five of five cases of C-ALCL (100%) and in 19 of 19 LyP (100%).

As regards the number of patients, our series of cases is similar to both series published. While our results are in agreement with those of Wasco *et al.*, they do not agree with the results of Kempf *et al.* Therefore, MUM1 is not helpful in separating different types of CD30+ lymphoproliferative disorders and we cannot consider MUM1 as a diagnostic marker between LyP and ALCL. Additional studies with larger groups of patients are necessary to confirm the final value of MUM1 in these disorders.

Departments of Dermatology and B. Hernandez-Machin
†*Pathology, Hospital Universitario* R.F. de Misa*
Insular de Gran Canaria, Avda T. Montenegro†‡
Marítima del Sur s/n, J.C. Rivero§
Las Palmas 35016, Spain J. Bastida¶
Departments of *Dermatology and* C. Febles**
***Pathology, Hospital Universitario* E. Pique‡
Nuestra Señora de la Candelaria,
Santa Cruz de Tenerife, Spain
‡*Department of Dermatology,*
Hospital General de Lanzarote,
Lanzarote, Spain
Departments of §*Pathology and*
¶*Dermatology, Hospital Universitario*
Gran Canaria Doctor Negrin,
Las Palmas, Spain
E-mail: buenaventurahdezm@aedv.es;
bhermacx@gobiernodecanarias.org

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Key words: *CD30+ lymphoproliferative disorders, cutaneous lymphoma, immunohistochemistry, MUM1*

Conflicts of interest: none declared.

A case of primary cutaneous diffuse large B-cell lymphoma, leg type monitored with fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography

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Sir, Accumulating evidence has shown that fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomo-

BJD British Journal of Dermatology

Ichthyosiform eruptions in association with primary cutaneous T-cell lymphomas

S. Morizane, N. Setsu, T. Yamamoto, T. Hamada, G. Nakanishi, K. Asagoe and K. Iwatsuki

Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan

Correspondence
Keiji Iwatsuki.
E-mail: keijiiwa@cc.okayama-u.ac.jp

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acquired ichthyosis, anaplastic large cell lymphoma, ichthyosiform mycosis fungoides

Conflicts of interest
None declared.

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Summary

Background Malignant lymphoma is occasionally complicated by ichthyosiform eruptions.

Objectives To analyse histopathologically the ichthyosiform eruptions associated with cutaneous lymphomas.

Methods We reviewed the files of patients with malignant lymphoma seen in our dermatology department between January 2001 and May 2006 to search for patients with ichthyosiform eruptions.

Results In our series, nine of 106 patients with malignant lymphomas had ichthyosiform eruptions during their clinical courses, including three (30%) of 10 patients with anaplastic large cell lymphoma (ALCL) and six (14%) of 44 patients with mycosis fungoides (MF). None of the 18 patients with cutaneous B-cell lymphoma had ichthyosiform eruptions. The three patients with ALCL had ichthyosiform eruptions histopathologically consistent with acquired ichthyosis (AI) in which packed horny layers and thin granular layers were present without lymphocytic infiltration. In contrast, four of the six patients with MF (stages Ib and IIb) had ichthyosiform eruptions with epidermotropic infiltration of atypical lymphocytes, as observed in ichthyosiform MF (IMF). Of the remaining two patients, one showed histopathological features overlapping AI and IMF, and the other had AI alone. These two patients (stages IVa and IIb) had tumours composed of CD30+ cells. Filaggrin expression was markedly diminished in both AI and IMF-like eruptions, similar to that of inherited ichthyosis vulgaris.

Conclusions Ichthyosiform eruptions are often associated with ALCL and MF and can be classified into three groups: AI associated with ALCL and MF expressing CD30, IMF, and their overlap.

Acquired ichthyosis (AI) is a reactive cutaneous manifestation associated with malignant and nonmalignant diseases that occur in adulthood.[1–3] Patients with AI usually present with pityroid and rhomboid scales on the trunk and extremities. The histopathological findings show compact or laminated orthohyperkeratosis and epidermal atrophy with thinning or loss of granular layers, while no cell infiltration is observed in the dermis or epidermis. AI is a frequent complication of Hodgkin lymphoma, characterized by the appearance of CD30+ atypical cells.[3–5] CD30+ lymphoproliferative disorders, such as anaplastic large cell lymphoma (ALCL) and lymphomatoid papulosis (LyP), are also associated with AI.[6–8]

In contrast, ichthyosiform mycosis fungoides (IMF) is a variant of mycosis fungoides (MF), arising in 1·8–3·5% of MF patients.[9,10] Although the clinical features of IMF are indistinguishable from those of AI, the histopathological findings reveal epidermotropic infiltrates composed of cerebriform lymphocytes typical for MF.[9–14] Although ichthyosiform eruptions with epidermotropic infiltrates often coexist with typical MF lesions (designated as IMF-like lesions in the present study), the diagnosis of IMF should be considered when it is the sole manifestation suggestive of MF. There has been controversy as to whether AI also occurs in MF.[1,2] Furthermore, a close association of AI with CD30+ lymphoma is another issue to be clarified. To explore these questions, we have studied ichthyosiform eruptions arising in patients with malignant lymphoma.

Patients and methods

Patients

We reviewed the files of patients with malignant lymphoma seen in our dermatology department between January 2001 and May 2006 to search for patients with ichthyosiform

eruptions. All patients with ichthyosiform eruptions were diagnosed with MF or ALCL based on clinical and histological findings. Extensive staging evaluation was performed by cervicothoracoabdominal computed tomography, gallium scintigraphy and bone marrow aspiration. In addition to routine laboratory tests (full blood cell count and biochemical analyses), we examined serum-soluble interleukin 2 receptor and antihuman T-cell leukaemia virus type 1 antibodies to exclude adult T-cell leukaemia/lymphoma. Staging was determined by classification for MF[15] and by the Ann Arbor Cotswolds classification for ALCL.[16] The presence of T-cell neoplastic clones was confirmed by rearrangement analysis of T-cell receptor genes.

Histopathology

Biopsy specimens were obtained from all patients after we received their informed consent. Formalin-fixed, paraffin-embedded blocks were cut into 4-μm sections and processed for routine haematoxylin and eosin staining and immunohistochemistry.

Clinicopathological criteria for AI and IMF were as follows. In addition to ichthyosiform eruptions, (i) AI demonstrated packed orthohyperkeratosis and thinning or absent granular layers mimicking ichthyosis vulgaris, without lymphocytic infiltrates; (ii) IMF showed epidermotropic infiltration of atypical lymphocytes without ichthyosis vulgaris-like packed hyperkeratosis; and (iii) the overlap of AI and IMF was defined as packed hyperkeratosis mimicking ichthyosis vulgaris, associated with epidermotropic infiltration of atypical lymphocytes.

Immunohistochemistry and *in situ* hybridization

Biopsy specimens were immunohistochemically examined using monoclonal antibodies to CD3, CD4, CD8, CD20, CD30, CD56 and anaplastic lymphoma kinase (ALK). To evaluate the expression of filaggrin, we reacted all specimens with monoclonal antifilaggrin antibody (Lab Vision, Fremont, CA, U.S.A.) and compared the results with those of normal skin (two patients), X-linked ichthyosis (three patients) and ichthyosis vulgaris (three patients). Histochemical visualization was carried out with an LSAB 2 kit (Dako, Carpinteria, CA, U.S.A.), according to the manufacturer's instructions. Epstein–Barr virus-encoded small nuclear RNA (EBER) was examined by *in situ* hybridization as previously reported.[17]

Results

Patient diagnoses

Between January 2001 and May 2006, 106 patients with malignant lymphoma were seen in our dermatology department. These patients included 44 with MF, 10 with ALCL and 18 with B-cell lymphoma. In our series, nine patients with cutaneous T-cell lymphomas had ichthyosiform eruptions, whereas none of the 18 patients with cutaneous B-cell lymphoma did. Of the nine patients with ichthyosiform eruptions, three, patients 1 and 2 (male, aged 57 and 30 years) and patient 3 (female, aged 64 years), were diagnosed as having ALK−, primary cutaneous ALCL based on histological findings and their clinical courses (Fig. 1a). Although the primary lesions were localized on the skin, lymph node involvement occurred within a few months after the initial diagnosis in the three patients. The remaining six patients with ichthyosiform eruptions (patients 4–9) presented with patch or plaque lesions consistent with MF, which was histologically proven (Fig. 1b–d). This group included four male and two female patients with a mean age of 57·0 (range 34–82) years. Of the six patients with MF, two (patients 5 and 9) had skin tumours without lymph node involvement (stage IIb), while one (patient 8) had tumours on the skin and regional lymph nodes (stage IVa). The remaining three patients had widespread patches and plaques without tumour formation or lymph node involvement, indicating stage Ib (Table 1).

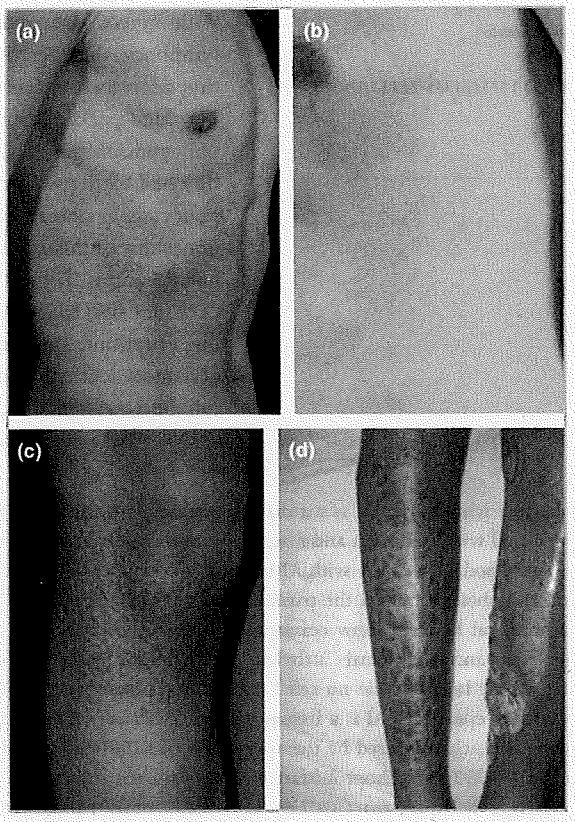

Fig 1. Clinical findings of ichthyosiform eruption. (a) The trunk in patient 1 with AI and anaplastic large cell lymphoma. (b) The trunk in patient 4 with IMF and conventional MF. (c) The knee and the lower leg in patient 8 with both AI and IMF. (d) The lower leg in patient 9 with AI and MF. AI, acquired ichthyosis; MF, mycosis fungoides; IMF, ichthyosiform MF.

Table 1 Clinical data on our nine patients

Patient no.	Age (years)/sex	Medical history	Onset of ichthyosiform eruption (years)	Onset of lymphoma (years)	Site of ichthyosiform eruption	Additional eruptions	Type of ichthyosiform eruption	Type of lymphoma	TCR rearrangement	sIL-2R	Clinical stage of lymphoma	CD30
1	58/M	–	57	57	Trunk	Tumour (nose)	AI	ALCL	Cβ1+ (SB)	1088	II_1	++
2	30/M	–	28	28	Trunk and ext.	Tumour (chest)	AI	ALCL	Cβ1+ (SB)	1434	III	++
3	64/F	–	64	58	Lower ext.	Tumour (L thigh)	AI	ALCL	γ+ (PCR)	991	II_3	++
4	43/M	–	43	43	Trunk and ext.	Plaques (trunk and ext.)	IMF-like eruptions	MF	Negative	2492	Ib	–
5	67/F	Prednisone	64	64	Trunk and ext.	Tumour and subcutaneous nodules (trunk and ext.)	IMF-like eruptions	MF	Cβ1+ (SB)	5172	IIb	++
6	82/M	Ca-blocker	70	70	Trunk and ext.	Plaques (trunk and ext.)	IMF-like eruptions	MF	ND	325	Ib	–
7	55/F	–	20s	30s	Trunk and ext.	Plaques (ext.)	IMF-like eruptions	MF	ND	291	Ib	–
8	34/M	–	28	30	Trunk and ext.	Tumour and plaques (thigh)	Overlap of IMF-like eruptions + AI	MF	Cβ1+ (SB)	776	IVa	++
9	61/M	–	61	56	Trunk and ext.	Tumour and plaques (trunk and ext.)	AI	MF	Cβ1+ (SB)	2029	IIb	+

TCR, T-cell receptor; sIL-2R, serum-soluble interleukin 2 receptor; ALCL, anaplastic large cell lymphoma; MF, mycosis fungoides; Cβ1, T-cell receptor Cβ1 chain; ext., extremities; CD30+, < 25% positive in tumour lesions; CD30++, > 25% positive in tumour lesions; ND, not done; PCR, polymerase chain reaction; SB, Southern blotting. AI demonstrates packed orthohyperkeratosis and thinning or absent granular layers mimicking ichthyosis vulgaris, without lymphocytic infiltrates; IMF, shows epidermotropic infiltration of atypical lymphocytes without ichthyosis vulgaris-like packed hyperkeratosis; the overlap of AI and IMF is defined as packed hyperkeratosis mimicking ichthyosis vulgaris, associated with epidermotropic infiltration of atypical lymphocytes.

Fig 2. Histopathological findings of ichthyosiform eruption. (a) In patient 4 with IMF, orthokeratosis, thinning of the granular layer, mild acanthosis and infiltration of atypical lymphocytes into the epidermis and the superficial dermis were seen. (b) In patient 8 with both AI and IMF, compact hyperkeratosis, thinning of the granular layer, acanthosis and infiltration of atypical lymphocytes to the epidermis and the superficial dermis were seen. (c) In patient 9 with AI, orthohyperkeratosis, thinning of the granular layer and atrophy of epidermis were observed. No infiltrate was present. AI, acquired ichthyosis; IMF, ichthyosiform mycosis fungoides.

Histopathological and immunohistochemical findings

Ichthyosiform eruptions associated with ALCL (patients 1–3) had packed orthokeratotic horny layers with thinning or

absent granular layers. No lymphoma cell infiltration was observed. These histological features were consistent with AI, excluding IMF. The biopsy specimens from ichthyosiform eruptions of four patients with MF (patients 4–7) showed disappearance or thinning of the granular layer, and the infiltration of atypical lymphocytes in both the epidermis and superficial dermis (Fig. 2a). The ichthyosiform lesion of one patient with MF (patient 8) showed compact orthohyperkeratosis with thinning of granular layers, and epidermotropic infiltrates of atypical lymphocytes, which are characteristic of AI and IMF, respectively (Fig. 2b). The ichthyosiform eruption of another patient with MF (patient 9) was histologically consistent with AI, without epidermotropic infiltrates suggestive of MF (Fig. 2c).

In our series, 20 patients, including 10 patients with ALCL, seven patients with LyP, and three patients with MF, had CD30+ cells. Of the 20 patients, three with ALCL and three with MF had ichthyosiform eruptions (Table 1). The phenotypes of all three patients with ALCL associated with AI (patients 1–3) were CD3+, CD4+, CD8−, CD20−, CD30+, CD56− and ALK−. Tumour lesions of MF (patients 5 and 8) included large atypical cells exceeding 25% of the dermal infiltrate, which fulfilled the criteria of large cell transformation (Table 1), whereas in the tumour lesions of patient 9, CD30+ cells made up 3–10% of the infiltrating lymphoid cells. The phenotypes of atypical epidermotropic lymphocytes in all IMF-like lesions (patients 4–7) were CD3+, CD4+ and CD8−, and negative for CD30. No EBER+ cells were observed in all specimens from the nine patients with ichthyosiform eruptions.

Clinical course of ichthyosiform eruptions

The ALCL and ichthyosiform eruptions of patients 1 and 2 were temporarily improved by cyclophosphamide, adriamycin, vincristine and prednisone (CHOP) chemotherapy and electron beam therapy (Fig. 1a). The ichthyosiform eruptions of patient 3 disappeared after six courses of CHOP chemotherapy, although the tumours later recurred. On the other hand, IMF-like eruptions in patients 4–7 also improved or disappeared after the skin-directed treatment for MF (Fig. 1b). Patients 4, 6 and 7 have remained in good condition with ongoing psoralen plus ultraviolet A treatment. A 67-year-old woman with stage IIb (patient 5) who developed large cell transformation was treated with three courses of CHOP chemotherapy with temporary improvement, but she died of complications of an infection of unclear origin.

A 34-year-old man (patient 8) first had MF lesions at the age of 28 years, and later developed tumorous lesions on the thigh associated with widespread ichthyosis (Fig. 1c). The tumour cells of a CD30+ phenotype rapidly progressed to lymph nodes in the inguinal and pelvic areas.

A 61-year-old man with stage IIb (patient 9) had progressive disease, in which neither electron beam nor CHOP therapy improved the tumours and ichthyosiform eruptions (Fig. 1d, Table 2).

Fillagrin expression in ichthyosiform eruptions

In normal skin and X-linked ichthyosis lesions, filaggrin was clearly stained in the granular layer (Fig. 3a, b). On the other hand, in common with findings in ichthyosis vulgaris, AI and IMF, filaggrin expression was markedly diminished in the thin granular layers, which was observed in all specimens of the nine patients (Fig. 3c–f).

Discussion

In our series of patients with cutaneous lymphoma, nine of 106 patients had ichthyosiform eruptions during the clinical

Table 2 Treatments and clinical courses of our nine patients

Patient no.	Therapy	Response of ichthyosiform eruption	Response of tumour/plaque	Outcome
1	CHOP + EBT + salvage therapy	PR	PR	Dead (62 years)
2	CHOP + EBT + salvage therapy	PR	PR	Dead (32 years)
3	CHOP	CR	CR	Alive
4	RT + PUVA	PR	PR	Alive
5	CHOP	PR	PR	Dead (67 years)
6	PUVA	PR	PR	Alive
7	PUVA	CR	CR	Alive
8	CHOP + EBT + salvage therapy	NC	NC	Dead (35 years)
9	CHOP + EBT	NC	NC	Alive

CHOP, cyclophosphamide, adriamycin, vincristine and prednisone; EBT, electron beam therapy; salvage therapy, dexamethasone, cytarabine, etoposide, ifosfamide and cisplatin were used; PR, partial response; CR, complete response; RT, radiotherapy; PUVA, psoralen plus ultraviolet A; NC, no change.

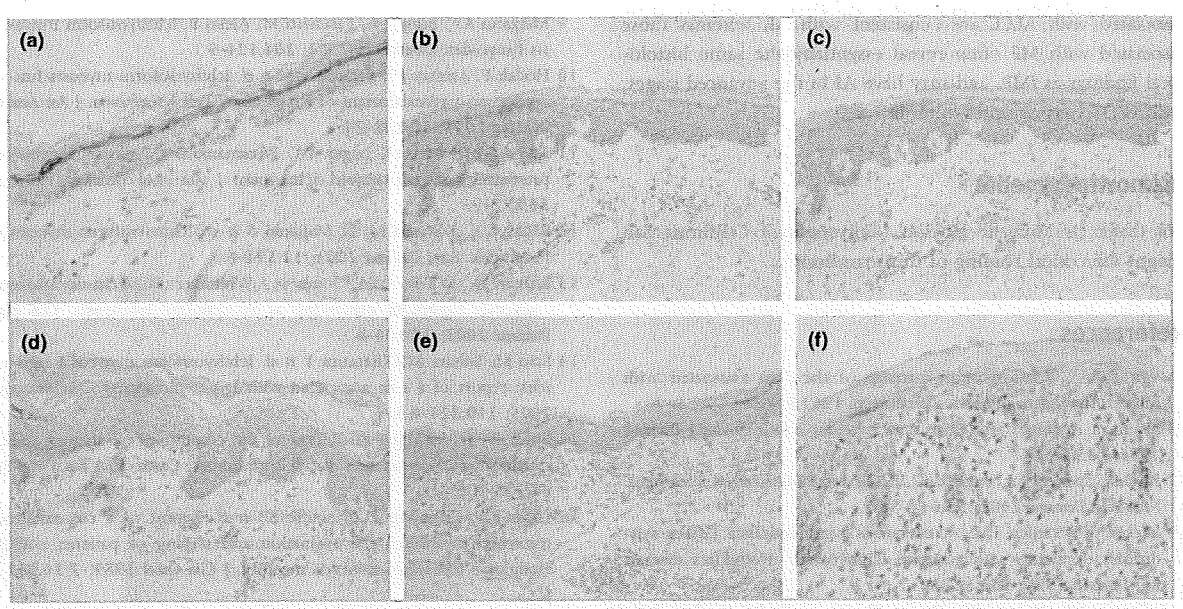

Fig 3. Immunostaining with filaggrin on normal skin and ichthyosiform eruptions. In normal skin (a) and an X-linked ichthyosis lesion (b), filaggrin was stained clearly in the granular layer. On the other hand, in ichthyosis vulgaris (c), patient 1 with AI (d), patient 4 with IMF (e), and patient 8 with both AI and IMF (f), filaggrin was observed only slightly in the thin granular layers (c–f). AI, acquired ichthyosis; IMF, ichthyosiform mycosis fungoides.

course, including three of 10 patients (30%) with ALCL and six of 44 patients (14%) with MF. None of the 18 patients with cutaneous B-cell lymphoma had ichthyosiform eruptions or CD30 cells. The complication of ichthyosiform eruptions in B-cell lymphoma has not thus far been reported in the English literature.

Based on clinicopathological findings, we diagnosed all ichthyosiform eruptions associated with ALCL as being AI. It is well known that AI is a frequent complication in patients with Hodgkin lymphoma. In addition, some reports have described the coexistence of AI associated with ALCL or LyP.[6–8] In our case series, three patients with ALCL were in the advanced stages with involvement of internal lymph nodes. The expression of CD30 antigens is a hallmark of atypical lymphocytes composing Hodgkin lymphoma, ALCL and LyP lesions. The CD30 antigen, or Ki-1 antigen, is a transmembrane glycoprotein with a molecular weight of 105 kDa and is expressed by not only Hodgkin cells and ALCL/LyP cells but also activated T cells and B cells.[18] The CD30 molecule is a member of the tumour necrosis factor receptor superfamily and has a CD15 molecule as a ligand.[18] The binding of the ligand transmits multifunctional signalling, which induces proliferation, activation, differentiation and cell death, depending on the situation. These pleiotropic functions, mediated by CD30 signalling, might be associated with the development of AI.

In most cases reported as IMF, the ichthyosiform eruption was the sole manifestation suggestive of MF, whereas Hodak *et al.*[10] have described three patients with typical MF associated with ichthyosiform eruptions under a diagnosis of IMF. In the present study, we observed ichthyosiform eruptions in six of 44 patients with MF. The ichthyosiform eruptions were consistent with histopathological findings of IMF in four (patients 4–7), the overlap of AI and IMF in one (patient 8), and AI alone in one (patient 9). These observations indicate that patients with MF may develop ichthyosiform eruptions consistent with AI, IMF or their overlap during their clinical courses. IMF in a strict sense usually occurs in patients with early MF.[9] However, similar lesions may develop in patients with advanced stages, such as patients 5 and 8, whose tumours had large cell transformations and who died of MF-related complications. AI associated with MF was observed in two patients with more advanced MF (patients 8 and 9). As seen in patient 8, ichthyosiform eruptions having overlapping histological features of IMF and AI may occur in the same individual. The two patients with AI and MF had CD30+ atypical lymphocytes in tumours to various degrees. Therefore, the development of AI might be associated with CD30 expression in MF as well as in Hodgkin lymphoma and ALCL.

Filaggrin is essential for the formation of stratum corneum and is a key protein in epidermal differentiation and the maintenance of barrier function.[19] Recently it has been demonstrated that two functional mutations in the gene encoding filaggrin cause ichthyosis vulgaris, the most common disorder in inherited ichthyoses.[20] In our series, the expression of filaggrin was markedly diminished in the thin granular layers in all our patients who had either AI, IMF-like eruptions, or both, as is usually observed in ichthyosis vulgaris.

AI is considered a cutaneous sign of other diseases, whereas IMF represents a specific cutaneous manifestation of MF. The present observations indicate that ichthyosiform eruptions

associated with ALCL are consistent with AI, whereas those associated with MF often reveal essentially the same histological findings as IMF, and may have AI in the advanced stages, with CD30 expression by tumour cells.

Acknowledgments

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皮膚病診療：31(5)；601～604, 2009

◇リンフォーマ<臨床例>——⑮

insect bite-like reaction
——mantle cell lymphomaに伴って生じた例——

浅越　健治*　　岩月　啓氏*

Key words
insect bite-like reaction，mantle cell lymphoma，exaggerated arthropod-bite reaction，慢性リンパ球性白血病

- Insect bite-like reactionは，慢性リンパ球性白血病(chronic lymphocytic leukemia，以下，CLL)，mantle cell lymphoma(以下，MCL)をはじめとする血液系の悪性腫瘍に合併するまれな非特異的皮膚反応である．
- 虫刺の既往がないにもかかわらず，臨床的にも病理組織学的にも虫刺症と区別のつかない皮疹を生じる．
- MCLに伴ってinsect bite-like reactionを生じた1例を報告する．

症例　69歳，男．

初診　2003年12月．

主訴　顔面，体幹，四肢の瘙痒を伴う丘疹，小結節．

家族歴　特記すべきことなし．

既往歴　甲状腺癌手術(59歳時)．

現病歴　初診の約2カ月前，胆石にて他院入院中にCTにて全身のリンパ節腫脹を指摘された．生検にてMCL(stage IVa)と診断．加療目的で当院血液腫瘍内科に入院中．初診の7カ月ほど前より全身に瘙痒感を伴う虫刺様皮疹を繰り返し生じていたが，皮疹が悪化し，MCLの皮膚浸潤を疑われて当科を紹介され受診した．患者は虫刺を受けるような機会はなかったといい，入院後も皮疹の出没を繰り返している．

現症　初診時，顔面，体幹を中心に米粒大から大豆大までの，表面平滑で光沢を有する紅色の丘疹，小結節が散在する(図1)．痂皮や滲出液を伴う部もある．また，ピンポン玉大までの表在リンパ節腫脹を認める．

検査所見

血液・生化学検査：WBC 7,300/μl(異型リンパ球を認めない)，RBC 383万/μl，PLT 23.5万/μl，TP 8.05g/dl↑，alb 47.4%↓，γ-glb 32.0%↑，A/G 0.9↓，IgG 2551.0mg/dl↑，IgA 706.2mg/dl↑，IgM 227.8mg/dl，CRP 0.7mg/dl，sIL-2R 1,677 U/ml↑．

胸腹部CT：縦隔，気管周囲，大動脈周囲，腸管膜沿いに1～2cm程度の腫脹リンパ節を多数認める．

骨髄生検：MCLの浸潤(+)．

上部，下部消化管内視鏡：ポリープ様の結節病変を多数認め，生検にてMCLの消化管浸潤と診断．

病理組織学的所見

角層は錯角化．表皮にはそのほかに大きな変化を認めない．表皮から1層の正常な膠原線維層を隔

* Asagoe, Kenji(講師)／Iwatsuki, Keiji(教授)
岡山大学大学院医歯薬学総合研究科・皮膚科学分野(〒700-8558 岡山市北区鹿田町2-5-1)

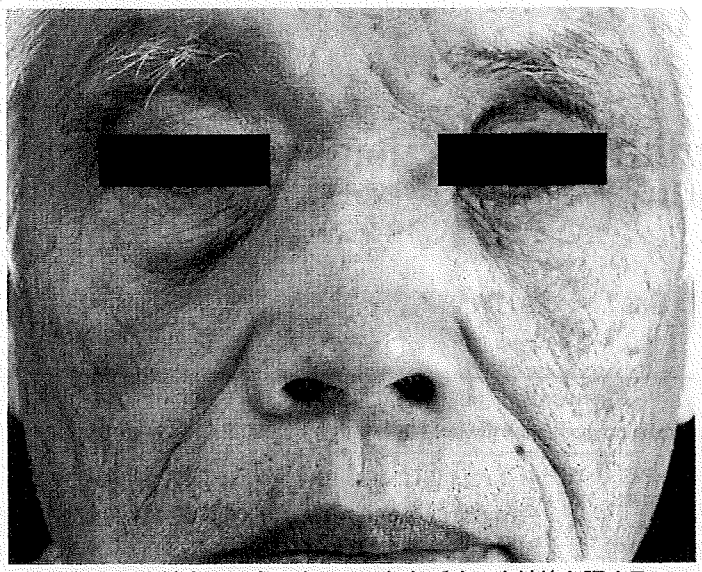

図1　顔面に米粒大から大豆大までの紅色丘疹，小結節を認める．

てて，真皮浅層から中層の血管および付属器周囲に結節性の細胞浸潤を認める．浸潤する細胞は小～中型のリンパ球様単核球が主体で，明らかな異型性を認めない．好酸球を多数混じ，組織の一部に好中球浸潤と核塵を認める．血管炎像は認めない(図2，3)．

免疫染色では浸潤細胞はCD3(CD4＞CD8)，CD5が陽性，CD79a，cyclin D1，CD30は陰性であった．また*in situ* hybridization法にてEBERは陰性であった．

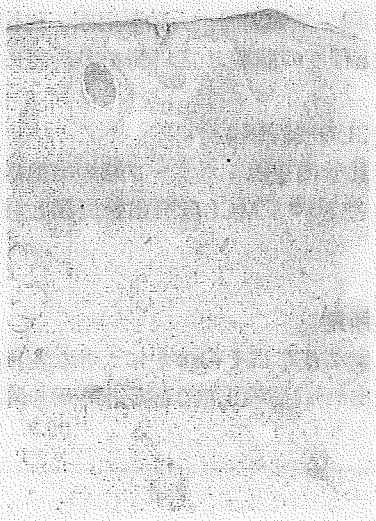

図2　真皮浅層～中層の血管および付属器周囲に結節性の細胞浸潤を認める(H-E染色，×40)．

鑑別診断

虫刺症：自験例では，本人に虫刺を受けた自覚がないこと，入院中，外来通院中にかかわらず皮疹が出没を繰り返すこと，露出部，非露出部に関係なく全身に皮疹が分布することなどから否定的と考えられた．

疥癬：検鏡にて疥癬虫を認めなかった．また，皮疹の分布も典型的ではない．

蚊刺過敏症：大型の硬結や潰瘍は認めず，全身症状も伴っていない．また，EBERも陰性．

MCLの皮膚浸潤：MCLではまれに皮膚浸潤を生じ，多発性もしくは単発性の結節，斑状丘疹，局面を呈する．病理組織学的には表皮向性がなくgrenz zoneを経て，血管周囲性，付属器周囲性の細胞浸潤を認める．多くはblastoid MCLで，好酸球や形質細胞を混ずることがある[1]．MCLの皮膚浸潤でも小～中型の異型性の目立たない細胞が主体のことがあり，自験例では鑑別上重要であった．H-E染色だけでは否定できなかったが，免疫染色にてB細胞マーカーとcyclin D1が陰性であったことから否定．

リンパ腫様丘疹症：病理組織学的に大型の異型細胞を認めず，浸潤細胞はCD30陰性であったこと

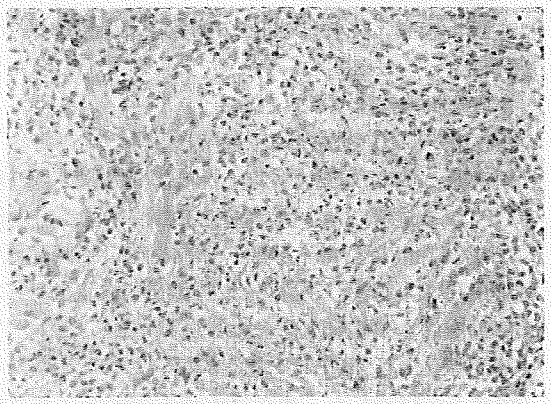

図3　浸潤する細胞の主体は小～中型のリンパ球様単核球で好酸球を多数混じる．組織の一部には好中球の浸潤と核塵を認める．血管炎の像は認めない(H-E染色，×400)．

から否定.

pseudolymphoma：病理組織学的にはT-cell pseudolymphomaとの鑑別が必要だが，自験例の臨床像は多発性の丘疹であり，短期間に皮疹が出没を繰り返すことから否定的と考えた.

診断確定

特徴的な経過，上記鑑別疾患が否定されたことからMCLに合併したinsect bite-like reactionと診断した.

治療と経過

MCLに対し化学療法(hyper CVAD＋HDMA)を施行．化学療法中に，大量のステロイド(デキサメタゾン40mg/日 3日間)の投与を受けると一時的に皮疹は消褪するが，1～2週間で再燃．個疹は約1週間で自然消褪するが，同様の皮疹を繰り返し生じていた(図4，5)．皮疹に対してプロピオン酸クロベタゾールなどのステロイド剤を外用したが，十分な治療効果は得られなかった．末梢血幹細胞移植を受け，完全寛解した後は皮疹を認めない.

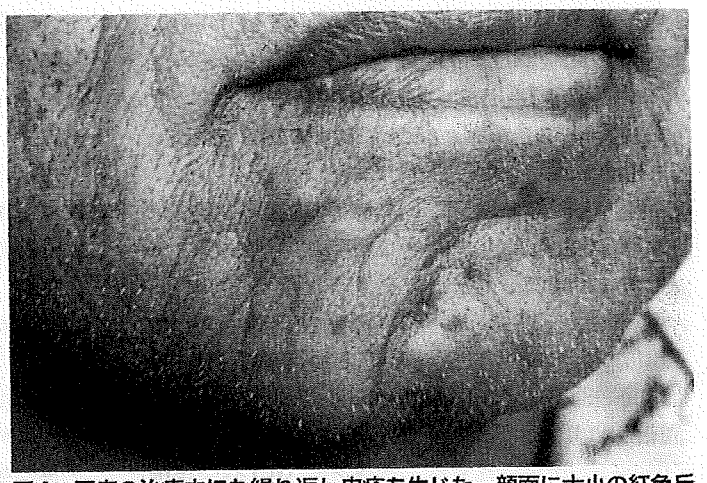

図4　原病の治療中にも繰り返し皮疹を生じた．顔面に大小の紅色丘疹を認め，痂皮や滲出液を伴う.

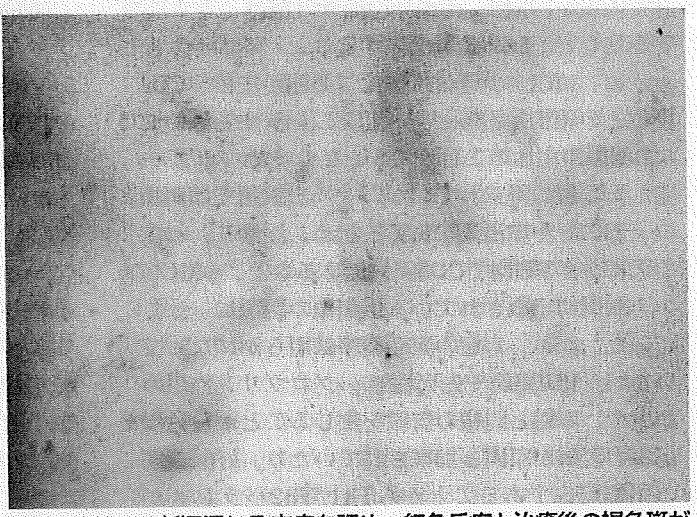

図5　体幹には新旧混じる皮疹を認め，紅色丘疹と治癒後の褐色斑が混在

考　按

Insect bite-like reaction という病名での報告は，1999年のBarzilaiらの報告が初めてであるが[2]，それ以前より，CLL患者では虫刺症の症状が増強することが知られ，“exaggerated arthropod-bite reaction”として報告されていた[3-7]．しかし，報告例の過半数は虫刺の既往がはっきりしておらず．Barzilaiらは，血液系悪性腫瘍の患者ではまれに，明らかな虫刺の既往がなくても虫刺されと区別のしようがない特徴的な皮疹を生じることに注目し，insect bite-like reactionとして報告した[2]．虫刺の既往がないこと以外に，皮疹の分布に偏りはなく全身どこにでも生じること，季節性がなく年余にわたって皮疹を生じることも虫刺症との鑑別点となる．皮疹は，瘙痒を伴う紅色丘疹，結節，局面などで，水疱を伴ったり潰瘍化したりすることもある．個疹は1週間程度で消褪して慢性化することはないが，繰り返し皮疹を生じる．また，血液系悪性腫瘍の診断に先行して皮疹が出現することもあり，本症が原病の診断のきっかけともなりうる．自験例もMCLと診断される約半年前より皮疹を生じており，血液系悪性腫瘍発症後の比較的早期から本症を合併する可能性があると思われる．原因となる

血液系悪性腫瘍はCLLの報告がもっとも多いが，Barzilaiらはそれ以外に急性リンパ球性白血病，急性単球性白血病，MCLなどに合併して生じることを報告した．その後，MCLに伴って生じたinsect bite-like reactionが相次いで報告され[8,9]，CLLに次ぐ頻度で報告されている．本症は血液系悪性腫瘍，とくにCLLとMCLに特異性の高いデルマドロームとして認識する必要があると思われる．

臨床症状と同様，病理組織像も虫刺症と極めて類似しており，真皮浅層～中層から深層，しばしば皮下脂肪織に至る稠密な細胞浸潤を認める．浸潤細胞はリンパ球が主体で多数の好酸球を混じ，flame figureが存在することもある．MCLへの合併例では，それ以外の症例と比べ好中球浸潤や核塵を伴うことが多いとされる[9]．真皮乳頭の著明な浮腫や表皮下水疱，赤血球の血管外漏出，ときに血管炎像なども認める．浸潤するリンパ球のサブセットは，MCLへの合併例ではT細胞が主体でCD4優位との報告がある一方，CLLに合併するものではB細胞が主体のことが多いともいわれ，まだ一定した見解は得られていない[7,9]．自験例では組織の一部に好中球浸潤や核塵を認め，浸潤リンパ球の大部分がT細胞でCD4優位であるなど，MCLへの合併例に記載されている組織像の特徴に一致していた．なお，本症の発症機序は明らかになっていない．HIV感染や先天性無γ-グロブリン血症の患者で，本症と同様の皮疹を生じることから免疫応答の変調が原因と推察されていたり，好酸球性の組織反応であることからTh1/Th2バランスがTh2に傾いていると推察されていたりもするが，それを証明するだけの根拠は得られていない[2,7,9]．

鑑別疾患としては，虫刺症，疥癬，蚊刺過敏症，痒疹，リンパ腫様丘疹症，pseudolymphoma．リンパ腫の皮膚浸潤などがあげられる．虫刺症とは臨床的，病理組織学的に区別がつかないが，前述のごとく病歴や皮疹の分布などから鑑別する．痒疹は従来，悪性腫瘍，とくに血液系悪性腫瘍に合併するといわれており，古くには慢性痒疹の1型としてリンパ節腫性痒疹という概念もある[10]．その記載によるとやはりCLLに合併する頻度が高く，これはinsect bite-like reactionと同一の疾患をみていた可能性がある．ただ，insect bite-like reactionは，いわゆる慢性痒疹とは違い個疹が慢性化することは少なく，比較的新鮮な丘疹を繰り返し生じるのが特徴である．そのほかの疾患との鑑別点は，症例報告の「鑑別診断」の項に記載したとおりである．

また治療に関しては，ステロイドの外用，抗ヒスタミン剤，紫外線療法などが行われているが，反応は悪く治療抵抗性である．抗腫瘍剤投与の際にステロイドの大量投与(プレドニン換算量40mg/日程度)を行うと皮疹は改善するが，投与終了後しばらくすると再燃する．そのほかに，最近DDSが本症に有効との報告がある[11,12]．本症の病態形成には好酸球が関与しており，DDSは好酸球ペルオキシダーゼ(以下，EPO)活性を抑制するため，EPOによる組織傷害と炎症の波及を軽減して本症の症状を改善している可能性が推察される．なお，原病の臨床症状や検査データと皮疹の程度は相関しないといわれている．治療を受けて原病が完全寛解しても皮疹が持続する場合には，原病が完治していない可能性があり注意が必要である．自験例は化学療法中に大量のステロイドが投与されると皮疹は消褪し，ステロイドの投与中止後1～2週間で再燃するという典型的な経過を繰り返したが，自己末梢血幹細胞移植を受けた後は，皮疹を生じなくなり原病の再燃もない．

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CASE REPORT

Leukemia cutis in a patient with acute monocytic leukemia presenting as unique facial erythema

Tomoyasu HATTORI, Hiroo AMANO, Yayoi NAGAI, Osamu ISHIKAWA

Department of Dermatology, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

ABSTRACT

A 67-year-old woman was referred to our department with a 1-month history of facial exanthemas. She had been diagnosed as having acute monocytic leukemia (French–American–British classification, M5b) based on the histological findings of bone marrow. Physical examination revealed diffuse edematous erythema on her cheeks, eyelids and glabella with scattered reddish papules. Histological examination demonstrated dense infiltration of atypical mononuclear cells in the dermis. Specific cutaneous lesions could occur in acute monocytic leukemia more frequently than in other types of leukemia, but rarely show symmetrical edematous erythema limited to the face.

Key words: acute monocytic leukemia, edematous erythema, leukemia cutis.

INTRODUCTION

Leukemia cutis, cutaneous infiltration of leukemic cells, can occur in all types of leukemia. It has been reported that a high frequency of leukemia cutis in acute myelogenous leukemia (AML) is a reflection of the aggressive disease course.[1] We herein report a patient with acute monocytic leukemia (AMoL), who revealed unique skin involvement and a distinctive clinical course.

CASE REPORT

A 67-year-old Japanese woman presented with a 1-month history of general fatigue. Laboratory studies disclosed the following abnormalities: hemoglobin, 6.5 g/dL; white blood cells, 6.72×10^4/uL; platelets, 7.2×10^4/uL; and lactate dehydrogenase, 766 IU/L. She was hospitalized in our hematology department under the diagnosis of AMoL, M5b in the French–American–British (FAB) classification, based on the histological findings of bone marrow. She had also noticed the skin eruption on her face for 1 month. Physical examination revealed diffuse edematous erythema distributing symmetrically on her cheeks, eyelids and glabella (Fig. 1a). Her eyelids were swollen and small reddish papules were scattered on the erythema. On the border of the right lower lip, red papules and vesicles with crust were also noted. We diagnosed the skin lesions on her lip as herpes labialis. As for other eruptions, we suspected leukemia cutis or Sweet's syndrome, and performed a skin biopsy from her cheek. Histological examination revealed dense infiltration of large atypical mononuclear cells in the dermis (Fig. 1b). Some cells were extended into the subcutaneous tissue, although epidermotropism was absent. The nuclei of those cells were large with irregular contours, and a few mitotic figures were observed (Fig. 1c). These atypical cells were immunohistochemically positive for CD68 (KP-1) (Fig. 1d). The diagnosis of leukemia cutis was established. Skin lesions regressed after combined chemotherapy with enocitabine and daunorubicin hydrochloride.

One month after the chemotherapy, erythema suddenly developed on her lateral trunk. Physical

Correspondence: Tomoyasu Hattori, M.D., Ph.D., Department of Dermatology, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan. Email: tohatt@showa.gunma-u.ac.jp

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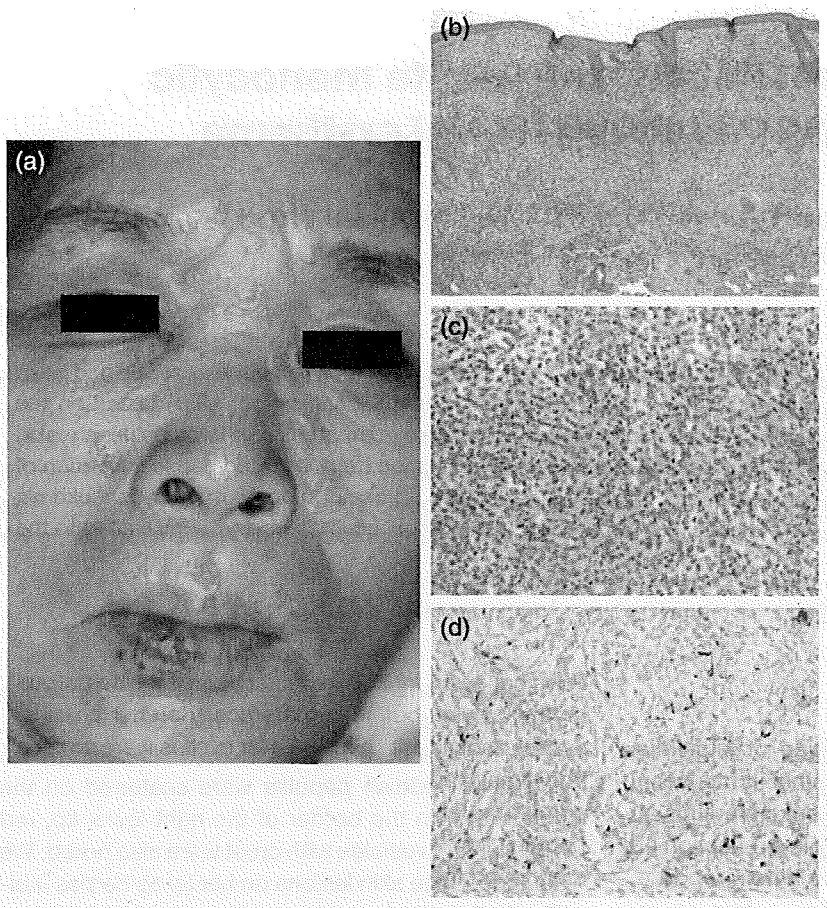

Figure 1. (a) Symmetrical diffuse edematous erythema on the cheeks, eyelids and glabella, with red papules, vesicles and crust on the lower lip. (b,c) Photomicrograph showing dense infiltration of atypical mononuclear cells in the dermis (HE stain, original magnification: (b) ×100; (c) ×400). (d) Immunohistochemical staining for CD68 (original magnification ×400).

examination revealed multiple small reddish erythemas (Fig. 2a). She had not taken any drugs. The peripheral blood showed 0.5×10^3/uL white blood cells with 89% lymphocytes, 7% eosinophils, 2% neutrophils, 1% basophils and 1% atypical lymphocytes. Bone marrow examination showed hypocellularity, indicating no relapse of leukemia. Histological examination of the skin lesions revealed perivascular infiltrate of various cells in the upper dermis with epidermotropism (Fig. 2b). Infiltrated cells were composed of lymphocytes, plasma cells and eosinophils, intermingled with a few large atypical cells. Large atypical cells were positive for both CD68 (Fig. 2c) and lysozyme. Diagnosis of leukemia cutis was suspected on these findings, however, skin lesions regressed spontaneously without chemotherapy. Subsequent bone marrow examination confirmed remission, and consolidation chemotherapy was therefore administered.

DISCUSSION

Acute monocytic leukemia is classified as M5 in the FAB classification, in which AML has been classified as eight subtypes (M0–M7). A further subclassification (M5a and M5b) can be made depending on whether the monocytic cells are predominantly monoblasts or a mixture of monoblasts and promonocytes. In Japan, the frequency of M5 was estimated to be 4% (M5a, 1%; M5b, 3%) in adult acute leukemia.[2] While M5 is relatively rare, specific cutaneous lesions occur more often in monocytic leukemia than in other forms. It has been reported that approximately 20% of patients with M5 have skin lesions.[3]

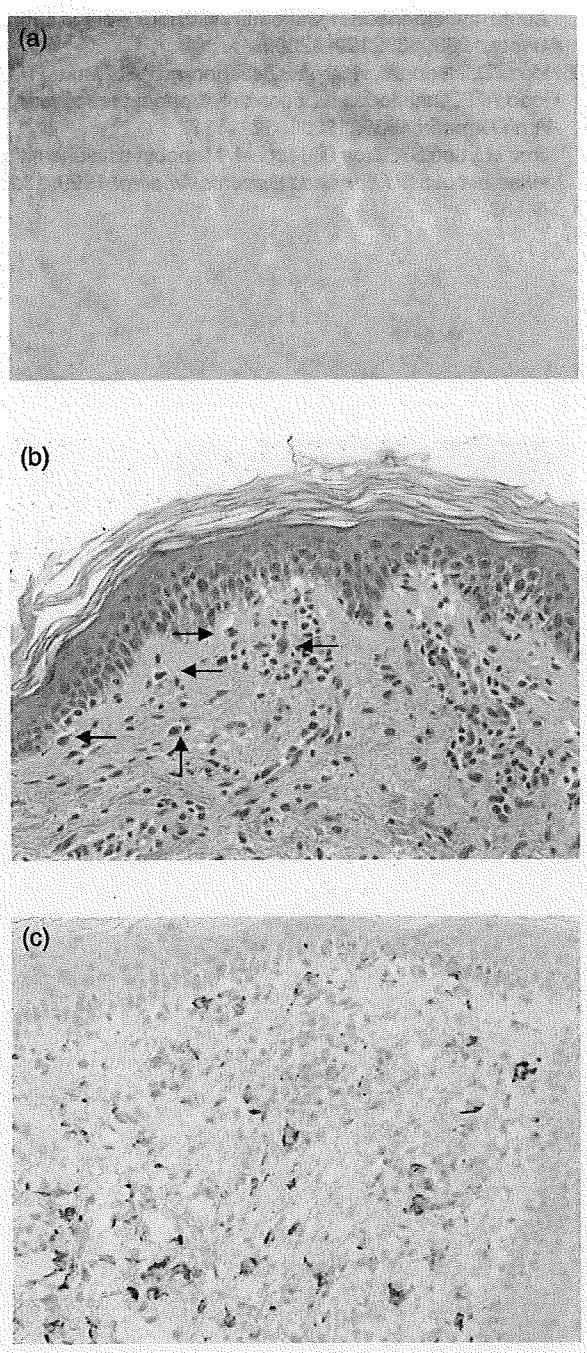

Figure 2. (a) Multiple pink to reddish erythemas on the lateral trunk. (b) Photomicrograph showing perivascular infiltrate of mononuclear cells composed of lymphocytes, plasma cells and eosinophils, intermingled with a few large atypical cells (arrow) (HE stain, original magnification ×400). (c) Immunohistochemical staining for CD68 (original magnification ×400).

In the previous reports, skin manifestations of AML were variably described, such as reddish to violaceous papules, infiltrates, plaques, ulcerations, nodules and exfoliative erythroderma.[4] The clinical appearance of our patient was quite unusual as the cutaneous manifestation presented as edematous erythema limited to the face. We could find no similar case in leukemia.

Skin can be the initial site of relapse in some leukemic patients, even if unaccompanied by leukemic changes in bone marrow or other sites.[5] In this context, it was pointed out that systemic chemotherapy adequate to induce and maintain bone marrow remission could not control leukemia cutis.[5] In our case, the initial skin lesions regressed after the initial chemotherapy, whereas subsequent skin eruptions developed without bone marrow relapse and regressed spontaneously in a short period. It is unclear whether the subsequent skin lesions were specific or non-specific skin manifestations of leukemia because the number of atypical cells was relatively small. We assume that the induction chemotherapy could regress the initial lesions, but a small number of surviving leukemic cells might have caused transient skin lesions. Immune reaction against the leukemic cells presumably resulted in a transient development and spontaneous regression of the skin lesions. We also suspected it might be cutaneous eruptions of lymphocyte recovery,[6] but the chemotherapy-induced nadir of the leukocyte count was sustained after the eruption disappeared.

Leukemia cutis, a specific cutaneous lesion, reveals distinctive clinicopathological features that allow the diagnosis of leukemia to be established. Such skin manifestations often predict an aggressive course and poor prognosis in patients with AML.[1] We should recognize the various clinical presentations of leukemia cutis, as skin eruption might be the initial manifestation of leukemia.[7]

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Original Article

Tumor Cell Expression of Programmed Cell Death-1 Ligand 1 Is a Prognostic Factor for Malignant Melanoma

Ryosuke Hino, MD[1]; Kenji Kabashima, MD[1,2,3]; Yu Kato, MD[4]; Hiroaki Yagi, MD[5]; Motonobu Nakamura, MD[1]; Tasuku Honjo, MD[4]; Taku Okazaki, MD[4,6]; and Yoshiki Tokura, MD[1]

BACKGROUND: Melanoma tends to be refractory to various immunotherapies because of tumor-induced immunosuppression. To investigate the mechanism underlining the immunosuppression of melanoma patients, the authors focused on programmed cell death-1 (PD-1)/PD-1 ligand 1 (PD-L1) interaction between tumor cells and T cells. **METHODS:** Melanoma specimens were collected from 59 primary tumors, 16 lymph nodes, and 4 lesions of in-transit metastasis. Specimens stained with anti-PD-L1 monoclonal antibodies were digitalized to jpg files. To evaluate the intensity of PD-L1 expression, histograms were used, and the red density (RD) was measured. PD-1 expression on T cells was analyzed in blood samples from 10 patients who had stage IV melanoma and in 4 samples of in-transit metastases. **RESULTS:** Twenty-five patients comprised the "low" PD-L1 expression group (RD value, <90), and 34 patients comprised the "high" group (RD value, ≥90). Breslow tumor thickness in the high-expression group was significantly higher than in the low-expression group. Univariate and multivariate analyses revealed that the overall survival rate of the high-expression group was significantly lower than that of the low-expression group. In all patients with stage IV disease who were examined, both CD8-positive and CD4-positive T cells had significantly higher PD-1 expression levels in the peripheral blood. Tumor-infiltrating T cells expressed high levels of PD-1, and its expression was elevated further during the clinical course. **CONCLUSIONS:** The current results indicated that there is a correlation between the degree of PD-L1 expression and the vertical growth of primary tumors in melanoma. Multivariate analysis demonstrated that PD-L1 expression is an independent prognostic factor for melanoma. ***Cancer*** **2010;116:1757–66.** © *2010 American Cancer Society.*

KEYWORDS: melanoma, peripheral blood mononuclear cells, programmed cell death, tumor-infiltrating lymphocytes.

Although malignant melanoma is a representative immunogenic tumor among various neoplasms,[1] it tends to be refractory to immunotherapy.[2,3] The presence or absence of tumor-infiltrating lymphocytes is 1 of several hallmarks that predict prognosis for patients with melanoma.[4] High frequencies of tumor-infiltrating, CD8-positive lymphocytes that are specific to melanoma antigens can be identified at tumor sites[5] or in peripheral blood from patients.[6] Conversely, an immunosuppressive status often is observed in patients with advanced malignant melanoma,[7] and many immunotherapies have been unsuccessful because of such immunosuppression. The number or function of CD4-positive/CD25-positive/forkhead box P3 (Foxp3)-positive regulatory T (Treg) cells[8-11] or interleukin 10 (IL-10)-producing immunosuppressive dendritic cells[12-14] is increased or promoted during the progression of malignant melanoma, even when patients receive some tumor vaccination therapies.[3] Investigation of the mechanisms underlying this tumor-induced immunosuppression may provide clues about how to overcome malignant melanoma therapeutically.

Recently, it has been established that programmed cell death-1 (PD-1), an immunoinhibitory receptor that belongs to the CD28 family, plays a critical role in tumor immune escape.[15,16] Two ligands for PD-1, PD-1 ligand 1 (PD-L1) and

Corresponding author: Ryosuke Hino, MD, Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; Fax: (011) 81 93 691 0907; hinoti@med.uoeh-u.ac.jp

[1]Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan; [2]Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]Center for Innovation in Immunoregulative Therapeutics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [4]Department of Immunology and Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan; [5]Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan; [6]Division of Immune Regulation, Institute for Genome Research, The University of Tokushima, Tokushima, Japan

See editorial on pages 1623-5, this issue.

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PD-1 ligand 2 (PD-L2) (also known as B7-DC) are involved in the negative regulation of cellular and humoral immune responses by engaging the PD-1 receptor.[15] PD-L1 is expressed on resting T cells, B cells, dendritic cells, macrophages, and parenchymal cells, including vascular endothelial cells and pancreatic islet cells.[15,16] Conversely, the expression of PD-L2 is limited to macrophages and dendritic cells.[17] Previous studies have demonstrated that PD-1/PD-L interaction inhibits T-cell growth and cytokine secretion[18] and that tumor cell-borne PD-L1 induces the apoptosis of tumor-specific T-cell clones in vitro,[19] suggesting the potential involvement of PD-Ls in tumor immunity. The expression of PD-L1 in tumors has been reported in melanoma[19,20]; in cancers of the lung,[19] breast,[21] ovary,[22] kidney,[23] pancreas,[24] esophageus,[25] and bladder[26]; and even in adult T-cell leukemia/lymphoma.[27] In addition, the involvement of PD-L1 has been demonstrated in the protection of cancer cells from cell lysis by activated T lymphocytes.[28] However, the expression of PD-L1 on melanoma cells in relation to tumor cell behavior and prognosis remains to be elucidated. In the current study, we investigated PD-L1 expression in human malignant melanoma to define its clinical significance and relevance to the prognosis for patients with these tumors.

MATERIALS AND METHODS

Patients and Samples

Patients who were enrolled in this study were treated and followed from 2000 to 2007 by the Department of Dermatology, University of Occupational and Environmental Health (Kitakyushu, Japan). Tumors were classified according to the American Joint Committee on Cancer (AJCC) staging system.[29] Patients were followed at regular intervals for evaluation of recurrence by physical examination and radiologic studies. Melanoma specimens were collected from 59 primary tumors, 16 lymph nodes (LNs) (9 metastatic LNs and 7 nonmetastatic LNs), and 4 in-transit metastases.

Each specimen was fixed with 20% formalin and embedded in paraffin, and serial sections were stained with hematoxylin and eosin for histologic evaluation. The specimens were digitized by using the NanoZoomer Digital Pathology C9600 (Hamamatsu Photonics, Hamamatsu, Japan), and Breslow tumor thickness (BTT) was analyzed with NDP View software (Hamamatsu Photonics).

Table 1. Relation Between Programmed Cell Death-1 Ligand 1 Expression in Primary Tumors and Other Clinicopathologic Factors

Variable	No. of Patients: Low Expression	No. of Patients: High Expression	*P*
Total no.	25	34	
Sex			
Men	15	23	.5981[a]
Women	10	11	
Age: Mean±SD, y	68.84±2.85	69.94±2.1	.7509[b]
Tumor type			
NM	4	6	
ALM	18	21	
SSM	3	5	
LMM	0	2	
Tumor site			
Extremity	20	26	
Trunk	5	4	
Head and neck	0	4	
BTT: Mean±SD	1.92±0.42	3.1±0.33	.0298[b]
Tumor classification			
T0-T2	16	9	
T3-T4	9	25	.0072[a]
Ulceration			
Absent	19	22	
Present	6	12	.4031[a]
LN metastasis			
N0	22	21	
N1-N3	3	13	.0375[a]
Clinical stage			
0	5	3	
IA	5	1	
IB	5	4	
IIA	2	6	
IIB	3	4	
IIC	2	3	
IIIA	0	3	
IIIB	2	3	
IIIC	0	7	
IV	1	0	

SD indicates standard deviation; NM, nodular melanoma; ALM, acral lentiginous melanoma; SSM, superficial spreading melanoma; LMM, lentigo maligna melanoma; BTT, Breslow tumor thickness; LN, lymph node.
[a]Fisher exact test.
[b]Student *t* test for unpaired data.

Immunohistochemistry

Immunohistochemical staining for PD-L1 was achieved by using a monoclonal antibody (MoAb) capable of detecting PD-L1 on formalin-fixed, paraffin-embedded specimens.[22] Sample specimens were cut into 4-mm-thick sections that were deparaffinized in xylene (3 times for 10 minutes each) and dehydrated through graded alcohols

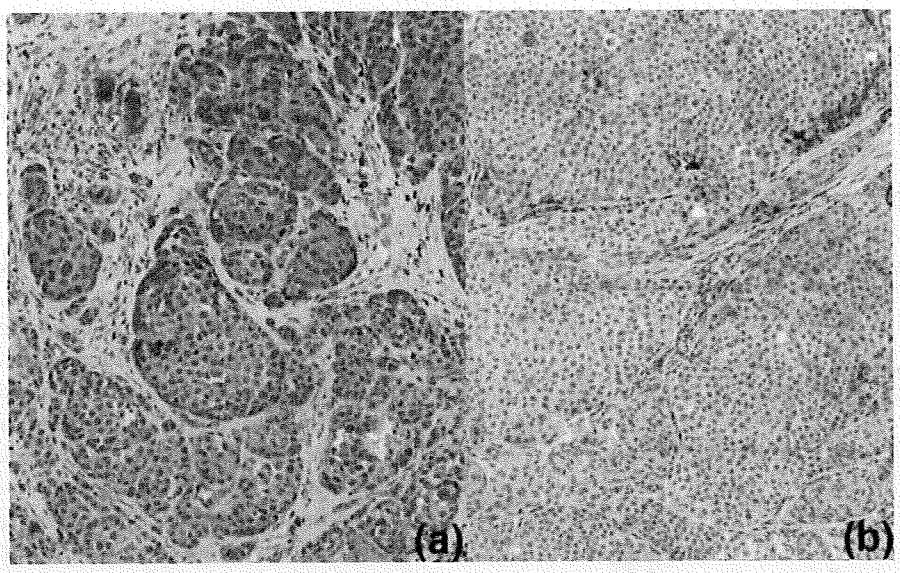

Figure 1. These photomicrographs illustrate immunohistochemical staining of programmed cell death-1 ligand 1 (PD-L1) in malignant melanoma. (a) An acral lentiginous melanoma on the left first toe in a human aged 78 years had a Breslow tumor thickness (BTT) of 5 mm and a red density (RD) of 26.57. (b) A nodular melanoma on the back of a human aged 72 years had a BTT of 2.8 mm and an RD of 153.81.

(99%, 99%, and 70%) to water. Antigens were retrieved by boiling in citrate buffer, pH,6.0, using microwaves. To block endogenous peroxidase activity, all sections were treated with 100% methanol containing 0.3% H_2O_2 for 15 minutes. Nonspecific binding of immunoglobulin G was blocked by using normal rabbit serum (Nichirei, Tokyo, Japan). The sections were incubated with mouse anti-PD-L1 MoAbs (clone 27A2; MBL, Nagoya, Japan)[22] overnight at 4°C. Then, they were incubated with biotinylated rabbit-antimouse secondary antibody (Nichirei) and subsequently incubated in a streptavidin-peroxidase complex solution for 30 minutes. Signals were generated by incubation with 3-amino-9-ethyl carbazole. Finally, the sections were counterstained with hematoxylin.

Analysis of Expression Intensity in Histologic Specimens

Digitized specimens were exported to JPG files by using NDP View software (Hamamatsu Photonics). The following processes were performed in Adobe Photoshop CS (J) (Adobe Systems, Inc. San Jose, Calif). Three different areas from the tumor cell cytoplasm were selected and expressed as Red channel histograms. In the bar graphs that were produced, the horizontal and vertical axes represented tone and quantity, respectively. Histograms revealed 255 different shades from pitch black (0) to pure white (255), and a number represented the level of brightness of each color. We analyzed the mean intensity of the histogram in the cytoplasm and averaged the value of 3 different areas. To obtain density, we calculated the 255-"mean" of each color. We called these values "red density"(RD) values and used them for further investigation. Specimens with an RD value <90 were defined as the low expression group, and specimens with an RD value ≥ 90 were defined as the high expression group.

Flow Cytometric Analysis

Blood samples were collected from 10 patients who had stage IV melanoma and from 5 normal, healthy volunteers to evaluate PD-1 expression on T cells. Peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized venous blood of patients by using Ficoll-Hypaque (Sigma Chemical Company, St. Louis, Mo) density-gradient centrifugation. Four local metastatic skin lesions were removed surgically, dissociated by teasing, and subjected to flow cytometric analysis. Single cell suspensions were obtained from the excised metastatic skin tumors by teasing and filtering and were subjected to flow cytometric analysis. Cells were double stained with fluorescein isothiocyanate-conjugated anti-PD-1, anti-PD-L1, or anti-PD-L2 MoAb and phycoerythrin (PE)-conjugated anti-CD4 or anti-CD8 MoAb (all from BD Biosciences, San Diego, Calif) at 2 μg/10^6 cells in Hanks balanced salt solution containing 0.1%