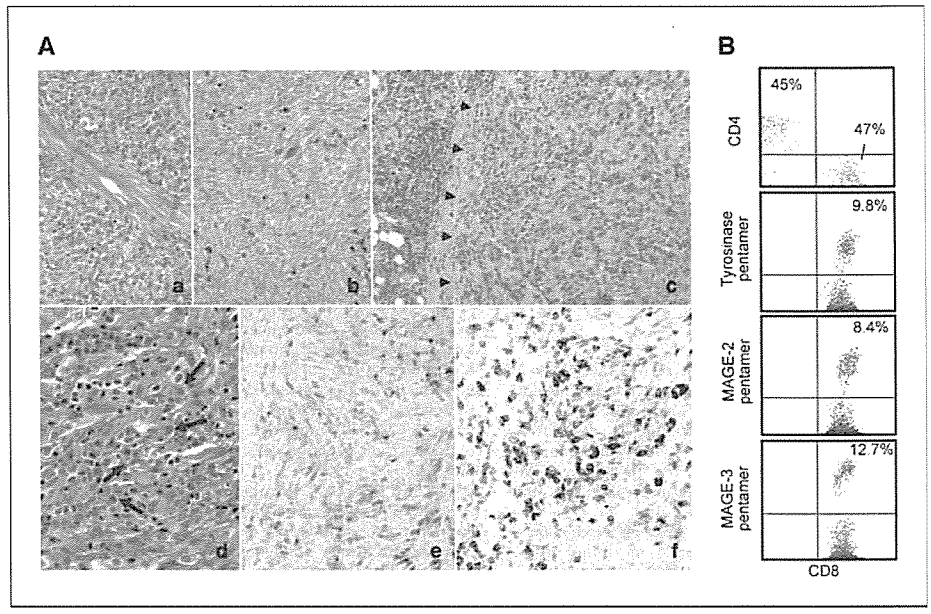

Figure 5. *In vivo* infiltration of CTLs into melanoma lesions induced by PPI. *A*, H&E histology and immunohistology of metastatic skin lesions. Skin lesions from P4 after PPI (*a*, magnification, ×200), from P2 before (*b*, magnification, ×200) and after PPI (*c*, *arrowheads*, tumor mass; magnification, ×100), with apoptotic and necrotic cells (*d*, *arrows*; magnification, ×400) and immunohistochemical staining showing infiltration of $CD4^+$ cells (*e*, magnification, ×100) and $CD8^+$ cells (*f*, magnification, ×100). *B*, flow cytometric profiles of cell suspensions obtained from a regressing s.c. nodule in P2 during PPI, showing the percentage of $CD4^+$ and $CD8^+$ cells among $CD45^+$ cell-gated leukocytes and the percentage of $pentamer^+$ cells among the $CD8^+$ $CD45^+$ cell-gated populations.

tumors in patients with advanced melanoma, which accompanied the induction of specific CTL activity. None of the patients/controls who received PPI exhibited local or systemic toxicity, or developed any clinical and laboratory findings of autoimmunity except vitiligo in patients with melanoma. We therefore propose that this novel strategy is clinically relevant for application in the treatment of malignancies. Furthermore, the generation of HIV gag–specific CTLs indicates the prophylactic strategy of PPI against viral and helminth infections.

Methods of needle-free vaccination delivery have attracted a global interest because of the urgent need for eradication of pandemic disease and treatment of growing numbers of cancer patients. This new approach has several advantages regarding ease and speed of delivery, safety and compliance, and costs, over needle delivery. Reported needle-free strategies to manipulate primarily the skin immune system include transcutaneous immunization (TCI; refs. 19, 39, 40), penetration via hair follicles (41), cutaneous bombardment (42), epidermal powder immunization (43, 44), and immunization with microenhancer arrays (45). All of these protocols target antigen to skin DCs in association with their activation and emigration from the skin, regulating the magnitudes, types, and directions of the immune responses. In particular, TCI is close to PPI in the methodologic respect. Both TCI and PPI are characterized by the application of antigen to the skin surface, thereby treating pathologic processes at a location distant from the application site. The difference between these two methods is the use of adjuvant. To obtain satisfactory immune responses, TCI requires adjuvant such as cholera toxin added to a vaccine antigen. On the other hand, barrier disruption is mandatory to allow the antigen to penetrate and activate the skin immune system in PPI without adjuvant. Although comparison of the effectiveness of CTL induction between PPI and other transcutaneous methods is difficult at present, the current study is the first one that clearly shows the clinical efficacy of PPI-induced CTLs in the human system.

Among many variables in the protocols and technologies of DC immunotherapy, quality control of vaccines in relation to the maturation status of DCs is a key determinant for the regulation of immune responses, and thus, clinical efficacy (4). Barrier disruption with the strong glue in PPI constantly removed a definite amount of the horny layers, irrespective of age, gender, and treated sites of the recipient. Such reproducibility of barrier perturbation enabled us to use *in situ* activated and fully matured LCs as therapeutic vectors. Repeated manipulations were essential to induce CTL responses with clinical efficacy in PPI as in prevailing melanoma vaccine with DC preparations (6). Such a time-consuming strategy might pose a problem when rapid protective responses are required. The application of appropriate adjuvants to the sites of barrier disruption has been shown to enhance immune responses both in mice (19, 39) and in humans (40). Systemic and local incorporation of T cell adjuvants such as interleukin-2, IFNs, and CD4 epitopes to PPI may potentiate CTL induction with less frequent immunization.

In the present pilot study, PPI was in fact effective in patients with advanced melanoma because tumor size was reduced in four of six patients, and apparent tumor burden and tumor development seemed to be abrogated in another. These beneficial effects coincided with the emergence of CTLs with strong cytolytic activity in the blood of some patients. Regressing lesions following PPI was associated with preferential infiltration of CTLs in a responder patient. In contrast, one patient with multiple metastases, and another with primary esophageal melanoma, did not clinically respond to PPI despite the presence of circulating CTLs. The possible reasons for treatment failure seemed to be related to the lack of cellular infiltrate in lesions and the impaired ability of CTLs to propagate *in vitro* under antigenic stimulation. A variety of immunologic mechanisms to evade tumor cell killing might underlie such T cell defects in these patients (46–48).

The safety issue is an important concern because the development of autoimmunity has been reported with the introduction of antigens directly into the body (49). Vitiligo, a well-known feature of autoimmunity targeting melanocytes, develops in association with DC-based immunotherapy in melanoma cases (49, 50). Because the antigens used for the immunization are autoantigens, there would be no expectation of epitope spreading. Although no study participants undergoing PPI

showed any signs of autoimmunity other than vitiligo, careful and repeated follow-up of the recipient's physical condition is indispensable for clinical trials.

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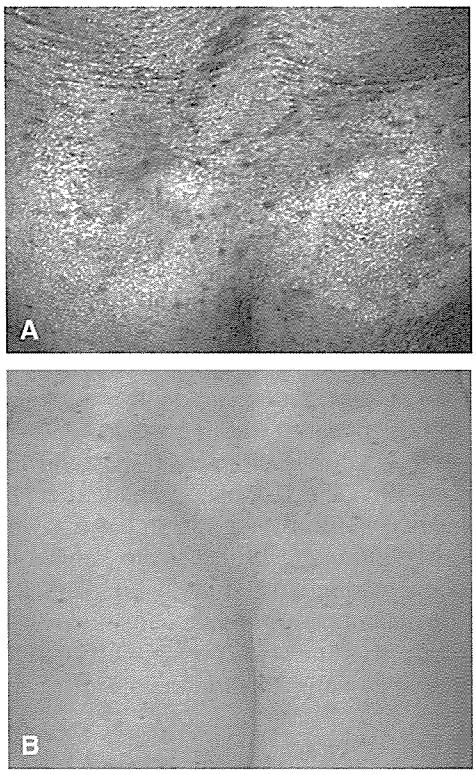

Fig 1. Buttocks with extensive psoriasis before (**A**) and 3 months after (**B**) etanercept therapy.

than 1,000,000 copies/mL viral RNA (normal < 1000). Considerable improvement of psoriasis was observed after only 2 weeks of treatment. Heartened by dramatic improvement of skin and joint disease by the second month of etanercept therapy, methotrexate was gradually withdrawn. Mild to moderate reactions at the injection site were alleviated by topical steroids. No other side effects or abnormal laboratory parameters were observed. After 5 and 12 months of etanercept therapy, the pretreatment HCV viral load remained unchanged. The patient continued to be free of significant papulosquamous activity during the follow-up period (Fig 1, *B*).

The discovery that tumor necrosis factor-alpha played an important role in the immunopathogenesis of both psoriasis and HCV[1-3] led us to pursue a trial of etanercept for a patient with intractable generalized psoriasis, psoriatic arthritis mutilans, and HCV. Etanercept in our patient effectively controlled skin and joint disease while eliminating a concomitant dependence on methotrexate therapy. This report is consistent with a recent case study of Zein[4] showing that etanercept is safe and effective as adjuvant therapy to interferon and ribavirin for the treatment of HCV.[4] Moreover, our findings amplify the conclusions of one other report in which etanercept was used successfully without complications in the setting of psoriasis and HCV.[5]

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Marked and restricted cutaneous pigmentation induced by selective intra-arterial cisplatin infusion

To the Editor: Super-selective intra-arterial high-dose chemotherapy has achieved remarkably good results in the treatment of head and neck cancer.[1] We report here a case of marked cutaneous pigmentation in the infusion area of super-selective intra-arterial cisplatin treatment.

We treated a buccal squamous cell carcinoma in a 66-year-old Japanese male with neoadjuvant chemotherapy followed by a radical operation. The chemotherapy consisted of intra-arterial infusion of 100 mg/m^2 cisplatin. Two days after chemotherapy, dark brown cutaneous pigmentation, different from the blue color of indigotin disulfonate, appeared around the tumor. The pigmentation was restricted to the infusion area for the super-selective

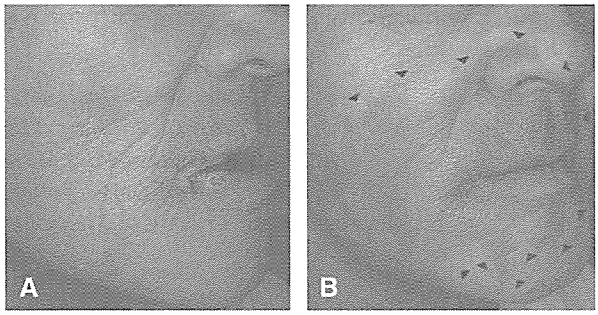

Fig 1. Cisplatin infusion chemotherapy induced cutaneous pigmentation restricted to the right cheek. **A**, Skin around the tumor before intra-arterial chemotherapy. **B**, Cutaneous pigmentation 10 days after the chemotherapy. Marked, dark-brown pigmentation was seen in the right cheek and the lips (*arrowheads*).

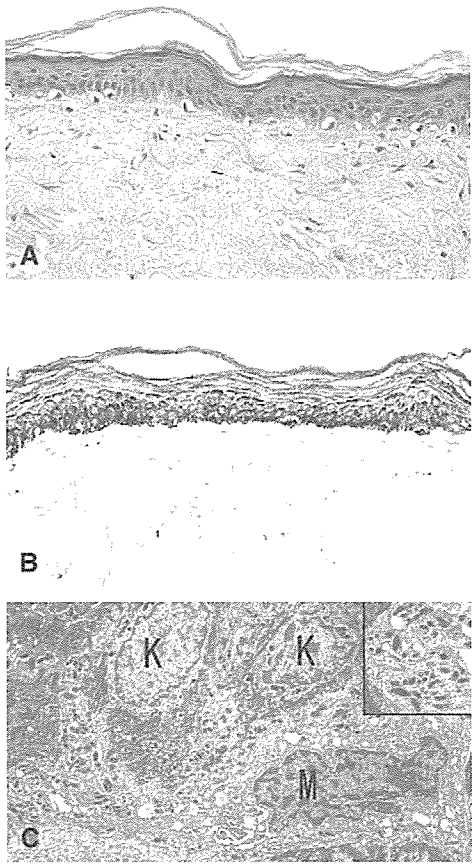

Fig 2. Skin biopsy specimen from the pigmented area on the right cheek. **A**, An increased number of melanocytes in the basal layer and basal melanosis were observed. **B**, Melanin staining clearly demonstrated the basal melanosis. **C**, Transmission electron microscopy demonstrated showed that melanocytes in the basal cell layer were producing a large number of melanosomes. *Inset*, Mature melanosomes were abundant in the cytoplasmic processes of a melanocyte. (**A**, Hematoxylin-eosin stain; **B**, Fontana-Masson stain; original magnifications: **A**, ×100; **B**, ×100; **C**, ×7000; *inset*, ×15,000.)

chemotherapy (Fig 1). No apparent inflammatory symptoms were seen in this region. A skin biopsy specimen showed that the number of melanocytes and amount of melanin pigment were increased in the basal layer (Fig 2, *A* and *B*). Neither inflammatory cell infiltration nor dermal melanocytes were detected. Electron microscopic observation revealed that enlarged melanocytes in the basal layer contained a large number of mature melanosomes in their cytoplasm (Fig 2, *C*). From these findings, the diagnosis of the cutaneous pigmentation caused by an intra-arterial cisplatin infusion was made. The cutaneous pigmentation gradually decreased in color and disappeared within 5 weeks.

Cutaneous complications with cisplatin include cutaneous allergic reactions, but marked cutaneous pigmentation induced by cisplatin has not yet been reported.[2,3] Although the exact mechanism of this hyperpigmentation has not yet been clarified, cutaneous pigmentation around an infusion area should be recognized as one of the possible adverse effect of intra-arterial cisplatin infusion therapy.

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Cutaneous metastases as the first manifestation of pleural malignant mesothelioma

To the Editor: Metastases of malignant mesothelioma (MM) are uncommon, but they have been reported

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Macrophage migration inhibitory factor in zinc-allergic systemic contact dermatitis

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Abstract

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine whose expression has been found to be critical to the generation of the antigen-specific immune response. Recent studies suggested that MIF plays a role in the initiation and maintenance of allergic disease. The aim of this study was to investigate whether MIF is involved in the pathogenesis of zinc-allergic systemic contact dermatitis. A 49-year-old Japanese woman developed facial edema, blepharedema and pruritic edematous erythema with papules over the entire body. Based of the results of a metal patch test, drug lymphocyte stimulating test and drug challenge test, diagnosis of zinc-allergic systemic contact dermatitis was made. Serum MIF and TNF-α levels of the patient, 20 healthy controls and other 6 patients who showed positive reaction to metal patch test were measured by an ELISA. Moreover we examined MIF production of peripheral blood mononuclear cells (PBMCs) from our patient, 3 healthy controls and other 2 patients who showed positive reaction to metal patch test at various metal concentrations. The patient's serum showed high MIF and TNF-α levels compared to healthy controls and other metal allergy patients. Furthermore, zinc stimulation of patient's PBMC showed higher MIF and TNF-α secretion compared with healthy subjects. The MIF content of 2 patients with other metal allergy was not significantly increased after metal stimulation. Our data suggest that zinc in the peripheral blood of zinc-allergic patients induce PBMCs to produce increased MIF levels, which could lead to systemic contact dermatitis.

Keywords: Contact dermatitis; Macrophage migration inhibitory factor; Zinc allergy

1. Introduction

Macrophage migration inhibitory factor (MIF)[1] functions as a pleiotropic protein, participating in both inflammation and the immune response [1]. The central role of MIF in delayed-type hypersensitivity (DTH) has recently been verified in a study of tuberculin-DTH in mice: after tuberculin challenge, anti-MIF antiserum treatment specifically blocked the tuberculin-DTH response [2]. We previously reported that MIF appeared to be important in the regulation of general cutaneous contact hypersensitivity immune responses and to play a central role in the contact hypersensitivity response [3].Systemic contact dermatitis induced by zinc allergy was reported before, but the mechanism has not completely clarified [4]. There have been no reports about a relationship between systemic contact dermatitis induced by essential trace element and MIF. Here, we show that zinc enhances MIF secretion from peripheral blood mononuclear cells in a patient with zinc-allergic systemic contact dermatitis. This result shows that MIF plays an important role in zinc-allergic systemic contact

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[1] *Abbreviations used:* ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MIF, macrophage migration inhibitory factor; TNF, tumor necrosis factor; DTH, delayed-type hypersensitivity; LPS, lipopolysaccharide; DLST, drug lymphocyte stimulating test; SI, stimulating index; PBMCs, peripheral blood mononuclear cells.

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dermatitis, similar to other allergic reactions such as asthma and atopic dermatitis.

2. Materials and methods

2.1. Materials

The following materials were obtained from commercial sources. Nitrocellulose membrane filters were purchased from Millipore (Bedford, MA); Ficoll–Paque Plus from Pharmacia (Uppsala, Sweden); polymyxin B from Sigma (St Louis, Mo); $ZnSO_4$ from Wako pure chemical industries (Osaka, Japan); RPMI 1640, ampicillin, streptomycin, and Moloney murine leukemia virus reverse transcriptase from Gibco (Grand Island, NY). All other chemicals were of reagent grade.

2.2. Activation of peripheral blood mononuclear cells (PBMCs)

PBMCs from our patient, healthy controls ($n = 3$) and patients with other metal allergy ($n = 2$) were prepared from heparinized blood by Ficoll–Paque plus density gradient centrifugation. In brief, we collected a cell layer at the density of 1.077 ± 0.001 g/mL, in which minimal eosinophil contamination was observed by microscopic examination. The PBMC layer was washed three times with sterile PBS. PBMCs (1×10^6/mL) were cultured in RPMI 1640 containing ampicillin (100 IU/mL), streptomycin (50 μg/mL), and polymyxin B (30 μg/mL) with use of 24-well plates at 37 °C in a humidified atmosphere with 5% carbon dioxide. Cells were stimulated with various concentration of $ZnSO_4$ (ranging from 0.05 mM to 0.10 mM) or left unstimulated. After 48-h culture, supernatants were collected and frozen until the assay for ELISA [5]. We sampled the patient's PBMCs before our treatment.

2.3. Enzyme-linked immunosorbent assay (ELISA)

We examined MIF levels from the sera of the patient, 20 healthy controls and other six patients who showed positive reaction to metal patch test as previously described [5]. We sampled the patient's plasma before our treatment. Twenty healthy controls received an annual health check-up and served as control subjects. The supernatants of cultured PBMCs or serum MIF were subjected to ELISA for measurement of MIF. For this assay we used recombinant human MIF to obtain a standard curve, in which good linearity was demonstrated between MIF contents (1–200 ng/mL) and absorbency. TNF-α content of the sera was measured with a TNF-α ELISA kit (Genzyme, Cambridge, MA) according to the manufacture's protocol.

2.4. Statistical analysis

Differences between the various treatments were statistically tested using the Mann–Whitney *U*-tests. *P* values of < 0.05 were considered statistically significant. Data in the figures are shown as the means ± SE.

3. Case report and results

A 49-year-old Japanese woman was referred to our department with a three-month history of multiple pruritic eruptions over her entire body. Physical examination showed diffuse edematous erythema with papules over her entire body. Facial edema and blepharedema were present. We suspected contact dermatitis, drug or viral eruption or erythema multiforme. Her skin lesions had been refractory to topical steroids and anti-histamine drugs for four months. Complete blood counts and laboratory data including serum zinc concentrations were within normal limits. Systemic examination including enhanced computed tomography, gallium scintigraphy and endoscopies, no internal abnormality was detected. Then we performed a metal patch test with a standard metal series (Torii Pharmaceutical Co., Ltd., Tokyo, Japan), including aluminium chloride, chromium sulphate, cobalt chloride, copper sulphate, ferric chloride, gold chloride, indium trichloride, iridium tetrachloride, manganese chloride, mercury bichloride, nickel sulphate, platinum chloride, palladium chloride, potassium dichloride, stannous chloride, silver bromide and zinc chloride on her arms. After 96 h, all metals were negative upon patch testing, except for one positive reaction with Zinc chloride. Furthermore drug lymphocyte stimulating tests (DLST) revealed a strong reaction to zinc sulphate with a stimulating index (SI) 396% (SI in normal range <180%). Drug challenge tests using zinc sulfate (300 mg/day 3 days) caused the eruptions on her ears, palms and arms to worsen including itching edematous erythema. (Fig. 1). Skin biopsy specimens from the right arm showed acanthosis, spongiosis, exocytosis, perivascular lymphocytic infiltration and mild edema on the papillary dermis (Fig. 2). Based on the clinical history and positive patch test, DLST index, zinc challenge test, in addition to pathological observation, we concluded that her refractory pruritic eruption was induced by zinc-allergic systemic contact dermatitis. By dental inspection, she had four teeth that were treated by metal filling which likely to contain zinc. All of her dental fillings of four teeth were completely removed. Her diet was changed to a zinc restricted diet. Moreover oral disodium cromoglycate (900 mg/day) was administered daily. Two weeks later, the majority of the skin lesions which lasted for four months had subsided rapidly.

We examined serum proinflammatory cytokines in the patient and controls ($n = 20$) (Table 1). MIF levels in the sera were high in the patient with zinc allergy (20.1 ng/mL) compared with healthy control subjects (9.3 ± 1.2 ng/mL). TNF-α levels in the patient's sera (28 pg/ml) were also higher than that in controls (all of the controls were undetectable: <5 pg/ml). On the other hand, we measured the plasma levels of MIF and TNF-α in other 6 patients who showed positive reaction to metal

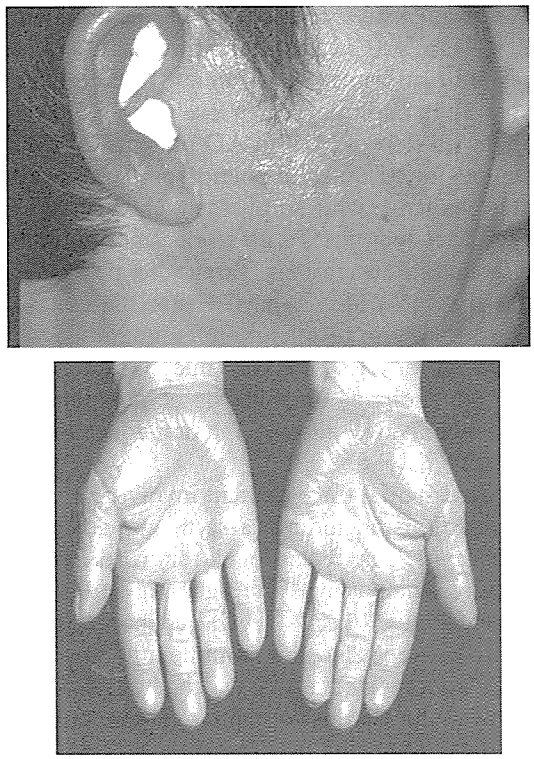

Fig. 1. Drug challenge test by zinc sulfate caused pre-existing eruptions on the ears and palms to worsen including itching edematous erythema.

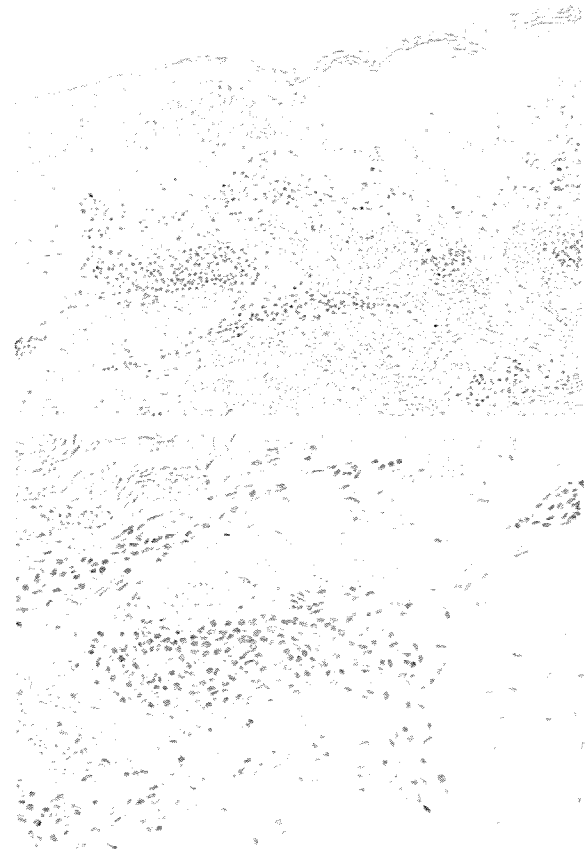

Fig. 2. Skin biopsy specimens showed acanthosis, spongiosis, exocytosis, perivascular lymphocytic infiltration and mild edema in the papillary dermis. (Haematoxylin–eosin stain; original magnification, ×10). High power view showed that lymphocytes surrounding the vessels had slightly atypical nuclei. (Haematoxylin–eosin stain; original magnification, ×100).

patch test (such as nickel, cobalt and so on). MIF levels in the sera (6.3 ± 2.0 ng/ml) and TNF-α levels in the sera (all of them were undetectable: <5 pg/ml) were not significantly different from healthy controls. Moreover we examined MIF and TNF-α production by PBMCs from the patient and healthy controls ($n = 3$) (Table 2). PBMCs from healthy control subjects, the MIF content was 6.5 ± 0.3, 5.8 ± 0.2, 6.3 ± 0.1 ng/ml, the MIF production of patient's PBMCs was 6.9 ± 0.1 ng/ml. These data failed to show a significant difference between the patient and controls. The MIF content of healthy control subjects was not significantly increased after zinc stimulation. Conversely, the MIF content in the medium of PBMCs from the patient with a zinc allergy was significantly increased after zinc stimulation, from 6.9 ± 0.1 ng/ml to 34.2 ± 3.1 ng/ml ($p < 0.005$). Elevated zinc levels were thus shown to specifically increase monokine secretion in patient's PBMC, but not control PBMCs. Similar results were also observed in TNF-α production stimulated by zinc sulfate. Without zinc stimulation, the TNF-α production from the PBMCs of both the patient and three controls were undetectable (<5 pg/ml). The TNF-α content of PBMCs from the three controls with zinc stimulation were also undetectable. However, the TNF-α content of PBMCs from the patient was increased after zinc stimulation, from a low, not detectable level (N.D.) to 82 ± 4.3 pg/ml. Further we examined MIF production by PBMCs from the patients ($n = 2$) with metal allergy who showed positive reaction to nickel and cobalt metal patch test (Table 3). The MIF content of these patients was not significantly increased after metal stimulation.

Table 1
Serum cytokines on the patient and healthy control

	MIF (ng/ml)	TNF-α (pg/ml)
Patient	20.1	28
Control ($n = 20$)	9.3 ± 1.2	N.D. (<5 pg/ml)

N.D.: not detectable.

4. Discussion

In this study, we present (1) a patient with zinc-allergic systemic contact dermatitis and have shown that MIF and TNF-α levels in the serum were higher than those of controls, (2) the zinc-induced increase in MIF and TNF-α levels in the patient were significantly higher than those of non-zinc exposed control cultures, (3) elevated zinc levels were shown to specifically increase monokine secretion our patient's PBMCs, but not control PBMCs, (4) MIF production was not stimulated in other metal allergy

Table 2
Zinc stimulates cytokines from patient's PBMC

(2×10^6)	Zn (mM)	MIF (ng/ml)	TNF-α (pg/ml)
Patient	0	6.9 ± 0.1	N.D.
	0.05	21.0 ± 0.5 *	57 ± 2.7
	0.1	34.2 ± 3.1**	82 ± 4.3
Control 1	0	6.5 ± 0.3	N.D.
	0.05	6.0 ± 0.4	N.D.
	0.1	6.8 ± 0.3	N.D.
Control 2	0	5.8 ± 0.2	N.D.
	0.05	6.2 ± 0.3	N.D.
	0.1	6.6 ± 0.3	N.D.
Control 3	0	6.3 ± 0.1	N.D.
	0.05	6.3 ± 0.4	N.D.
	0.1	6.5 ± 0.5	N.D.

Results are expressed as means ± SE N.D.: not detectable (<5 pg/ml).
* $p < 0.01$.
** $p < 0.005$ vs. Zn (0 mM).

Table 3
MIF from other metal allergy patients's PBMC

(2×10^6)	Nickel or Cobalt (mM)	MIF (ng/ml)
Patient 1	0	5.5 ± 2.7
	Ni0.0020	6.1 ± 1.0
	Ni20.0	6.2 ± 0.7
	Co0.0020	5.6 ± 1.3
	Co20.0	6.0 ± 0.6
Patient 2	0	5.8 ± 1.4
	Ni0.0020	5.3 ± 1.1
	Ni20.0	5.6 ± 1.9
	Co0.0020	5.2 ± 1.1
	Co20.0	5.7 ± 2.9

Results are expressed as means ± SE.

patients' PBMCs. These results suggest that MIF is related to zinc-allergic systemic contact dermatitis. There is evidence suggesting a relationship between the allergic response and MIF. For example some patients with atopic dermatitis, bronchial asthma and allergic rhinitis have been shown to contain significantly elevated levels of MIF, compared to healthy controls [5–7]. Moreover, the serum concentrations of MIF correlate with disease severities in patients with AD [5].In an experimental contact dermatitis model, MIF-deficient gene knockout mice showed a significantly milder clinical symptom compared to wild-type mice [3]. Moreover we reported that MIF and MIF mRNA produced by AD patient's PBMCs were significantly higher than healthy controls, in other words, PBMCs might be an important source of increased serum MIF levels in AD [1]. MIF produced by PBMCs may affect local and systemic pathologic features in AD. In our case zinc-induced MIF expression from patient's PBMCs were higher than those of healthy controls. From our data it is conceivable that peripheral blood zinc in a patient with zinc allergy could induce PBMCs to produce MIF, which might aggravate any of the local dermatitis.

TNF-α was also increased in our experiment. MIF induces TNF-α production in various diseases, such as acute respiratory distress syndrome [8]and contact hypersensitivity [3]. In addition, serum levels of TNF-α decreased in parallel with that of MIF in AD patients [5]. Nakamaru et al. demonstrated that the phenotype of MIF knockout mice after challenge with an allergen, showed significantly decreased nasal symptoms with less eosinophil infiltration, and these findings were very similar to those in TNF-α knockout mice [6]. They reported that the concentration of TNF-α at the site of allergic inflammation was significantly reduced in MIF knockout mice. These results indicate MIF functions as an initiator of inflammation; and that MIF aggravates allergic inflammation through the production of TNF-α.

Zinc is an essential trace element involved in many physiological functions, including catalytic and structural roles in metalloenzymes, as well as regulatory roles in diverse cellular processes such as synaptic signaling and gene expression. There have been several reports about the relationship between zinc and immune function. For example we recently reported a case which showed the relationship between zinc allergy and palmoplantar pustulosis [9]. Studying the relationship between zinc and cytokines, Wellinghausen et al. reported that zinc enhanced the induction of TNF-α and IL-1β in the stimulation of PBMCs with lipopolysaccharide (LPS) [10]. It is also reported that zinc stimulated monokine secretion from PBMCs in a serum- and LPS- free cell culture system [11]. It was showed that zinc increased monokine secretion more efficiently than other related divalent cations including cobalt, nickel and mercury. They concluded that the induction of cytokines in PBMCs was a possible mechanism of zinc-specific immune modulation.

Contact dermatitis is produced by external skin exposure to an allergen, but sometimes a systemically administered allergen may reach the skin and remain concentrated there with aid from the circulatory system to produce a systemic contact dermatitis. While systemic zinc-allergic contact dermatitis induced has been previously reported, the precise mechanism has not been clarified.

Since it has not previously been reported that MIF is involved in zinc-allergic systemic contact dermatitis until now, we examined MIF secretion in systemic contact dermatitis patients with a zinc allergy. We expected that MIF might readily be released into the extracellular space and circulation in response to zinc in zinc-allergic patients. MIF functions as an initiator of inflammation, so other inflammatory cytokines can be produced, such as TNF-α, and an inflammatory and immune response will then occur, in these cases after systemic contact dermatitis.

In conclusion, our data suggest that zinc in the peripheral blood of patients with zinc allergy can induce PBMCs to produce increased MIF, which could lead to systemic contact dermatitis.

Acknowledgement

We thank Dr. James R. McMillan for proofreading and comments concerning this manuscript.

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confluency, and focal pagetoid scatter within the epidermis, resulting in possible diagnostic confusion with melanoma.[5] Even though superimposed LS makes interpretation more challenging, our patient's lesions demonstrated histologic features most in keeping with melanoma, including asymmetry, poor lateral circumscription, junctional confluency resulting in early pseudobullae, prominent pagetoid scatter, aberrant dermal growth, and dermal mitoses.[5] Hassanein et al[3] and Carlson et al[4] provided a hypothesis for the malignant transformation of melanoma in LS as a relational pattern of host immune response to melanoma, and local immune dysregulation in LS. We are unaware of any inherited association between melanoma and LS, and potential genetic contributions to LS have not yet been elucidated.

We report a case of vulvar melanoma with lymph node metastasis in a background of LS occurring in a child who is now alive 32 months after operation. The association of these two conditions requires further investigation. This case illustrates the need for awareness that melanoma, including vulvar melanoma, can and does occur in children.

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Multiple apocrine hidrocystoma showing plane pigmented macules

To the Editor: A 67-year-old Chinese man was referred to our hospital with pigmented asymptomatic eruptions on the face, which was thought to be suggestive of basal cell carcinoma at a previous clinic (Fig 1). He first noticed a small, pigmented lesion in his right nasal side wall 20 years ago. Afterward, similar pigmented macules appeared on the right wing of his nose. Physical examination revealed 4 slightly blackish pigmented macules on the right wing of his nose sized 7 × 6, 5 × 4, 3 × 3, and 2 × 2 mm.

Clinically, the lesions were thought to be basal cell carcinoma, melanocytic nevus, trichoblastic carcinoma, hemangioma, or apocrine hidrocystoma, and a punch biopsy was performed on a characteristic lesion. The biopsy specimen revealed several cystic structures in the papillary dermis. The cavities were lined by cuboidal apocrine secretory cells within flattened myoepithelial cells with some intraluminal papillary projections (Fig 2). Cellular atypia was not seen. From these findings, the diagnosis of multiple apocrine hidrocystoma was finally made. There was not enlarged superficial abnormal sebaceous gland implicating pre-existing nevus sebaceous. Because the patient declined surgical intervention, we performed carbon-dioxide laser therapy. Laser treatment decreased the pigmentation a little, but the patient did not wish to continue treatement. We have observed the patient carefully for a year, and these tumors have shown no remarkable change.

Apocrine hidrocystoma is benign cystic tumor that represents an adenomatous cystic proliferation of apocrine glands. It most commonly occurs as a solitary, dome-shaped, translucent papule around the eye.[1] Puncture of a lesion may result in extravasation of a straw-colored fluid. Although clinically atypical apocrine hidrocystoma showing facial nodules or giant solitary pigmented tumors has been reported, there has been no report of multiple apocrine hidrocystoma appearing as pigmented macules.[2-4] The current case is unique in that the

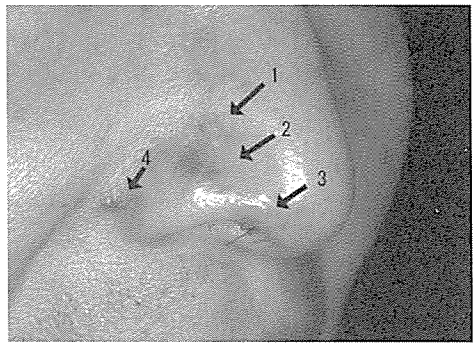

Fig 1. Clinical features: multiple blackish pigmented macules (*arrows 1-4*) on right side of nose.

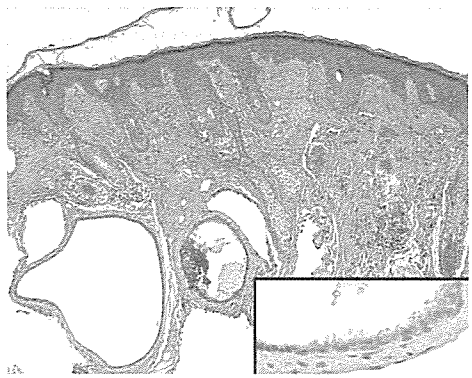

Fig 2. Histopathologic features: several cystic structures in papillary dermis. *Inset*, Cavities were lined by cuboidal apocrine secretory cells within flattened myoepithelial cells with some intraluminal papillary projections. (Hematoxylin-eosin stain; original magnifications: ×10; *inset*, ×100.)

tumors comprised multiple, plane, and nontranslucent macules. The diagnosis of this multiple apocrine hidrocystoma could be made after histopathologic observations. This case further suggests the marked clinical variation of apocrine hidrocystoma mimicking basal cell carcinoma.

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Penile condylomata? Traumatic neuromas!

To the Editor: Traumatic neuroma is a reactive process that leads to regeneration of an injured nerve forming a haphazard nodular proliferation of small nerve bundles. It is usually related to previous operation or trauma.[1] We describe a case of multiple traumatic neuromas clinically suggested to be condylomata acuminata.

A 29-year-old man from Liberia, Africa, had several painless, small papules on his penis, unrelated to local trauma. He claimed no sexual activity with partners during the last 2 years. Physical examination revealed few comedones on an uncircumcised penis and on scrotal skin and several grouped, firm, whitish, translucent papules, 1 to 2 mm in diameter, on the prepuce (Fig 1). A clinical diagnosis of condylomata acuminata was made, and a papule was excised with a 4-mm punch biopsy. Histologic examination of the hematoxylin and eosin—stained slide showed an epidermis with no sign of viral infection. A haphazard proliferation of nerve fibers organized in small fascicles supported by a fibrous stroma was present in the papillary dermis. It appeared to originate from a single nerve located in the reticular dermis (Fig 2). The histologic diagnosis was traumatic neuroma.

Clinical differential diagnosis should also include pearly penile papules,[2] but pearly papules are characterized by plump or stellate fibroblasts embedded in a vascularized connective stroma.

Two cases of traumatic neuroma in genital skin have been reported in association with local trauma.[3,4] Moreover, 3 cases, unrelated to trauma have been described to date.[5] In these latter cases, the authors described concurrent presence of multiple Meissner's corpuscles.[5] The pathogenetic mechanism of these lesions, given their acquired nature and also, in our case, their multiplicity, appears to be traumatic. On the other hand, the previous 3 reported cases were in young, sexually active men, and the authors hypothesized a correlation between the tumor and local microtraumas.[5]

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Epidermodysplasia verruciformis and generalized verrucosis: the same disease?

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Summary

We report a patient with epidermodysplasia verruciformis (EV) who had severe generalized verrucous skin lesions for 50 years without any immunological abnormality. Microscopic examination showed two histopathological features, including seborrhoeic keratosis and common warts. The detected human papilloma virus (HPV) types were found to be HPV 3, 50, 5, and 76, using a degenerate PCR method. EV and generalized verrucosis are distinguished by slight differences in clinical symptoms or HPV types, so there should be no apparent differential points common to both diseases. Therefore, we propose that an abnormal susceptibility specific to HPV, which is the most characteristic feature in EV, should be regarded as a differential point in these two diseases.

Epidermodysplasia verruciformis (EV) is most commonly described as an autosomal recessive disorder, which demonstrates generalized and persistent cutaneous infection with human papillomavirus (HPV). On the other hand, the term generalized verrucosis (GV) has been used for generalized warts in some reports, which were usually associated with immunocompromised conditions.

Report

A 63-year-old Japanese man was referred to our clinic with multiple, slowly growing, hyperkeratotic skin lesions over his feet and arms (Fig. 1a). He had first noticed the warty papules on his hands at the age of 10 years, and they became gradually more generalized and increased in both size and number. The lesions failed to respond to superficial radiotherapy and cryotherapy performed at other clinics. Six months prior to presentation, one of the papules on his right calf had started to grow rapidly. Physical examination revealed a 70 × 60 mm, dark-reddish, dome-shaped tumour with odour on the right calf (Fig. 1b). On the dorsa of the hands and feet, multiple, markedly papillomatous nodules with severe hyperkeratosis were present (Fig. 2a). There was no skin lesion on the other sun-exposed areas, including the patient's face and scalp. He had no past history of repeated infectious diseases implicating an immunocompromised condition, and no family history of recalcitrant warts.

For diagnostic and therapeutic purposes, the reddish tumour showing rapid growth on the right calf was surgically removed. Simultaneously, biopsies were performed from the hyperkeratotic erythematous plaque on the right forearm (Fig. 1c), and the nonhyperkeratotic pigmented macule on his upper back (Fig. 1d). Microscopic examination of sections of all the specimens, stained with haematoxylin and eosin, showed two distinct histopathological features. The first was an acanthotic epidermal layer with sharply defined nests of basaloid cells, which is seen in seborrhoeic keratosis (Fig. 3a,b). The other was the typical histopathological changes seen in common warts, showing papillomatosis,

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Conflict of interest: none declared.

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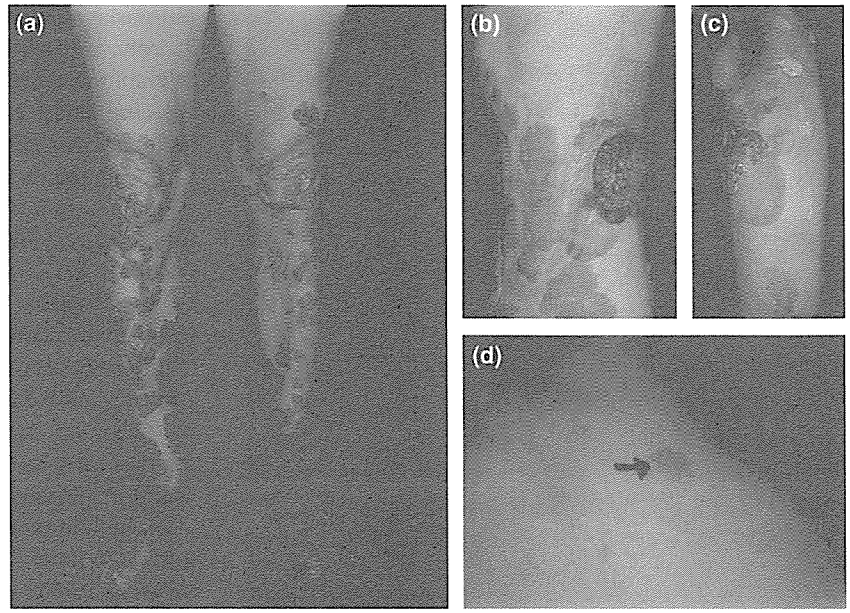

Figure 1 (a) Multiple hyperkeratotic skin lesions on the feet. (b) A 70 × 60 mm, dark-reddish, dome-shaped tumour with an odour on the right calf. HPV 3, 57 and 76 were detected. (c) Hyperkeratotic erythematous plaque on the right forearm. (d) The pigmented macule without hyperkeratosis on his upper back. HPV 3, 50, 57 and76 were detected.

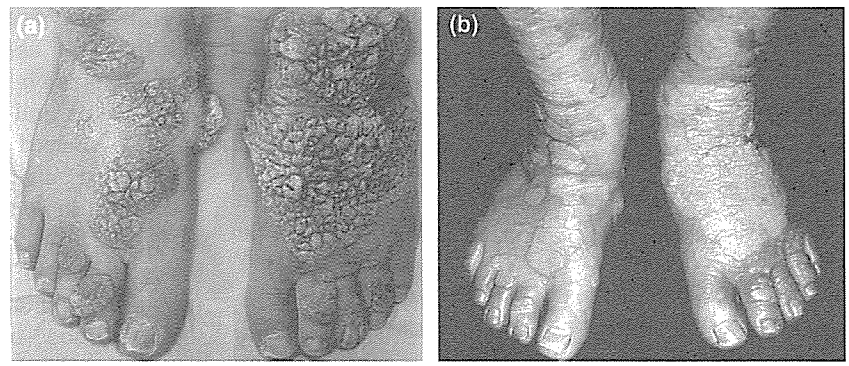

Figure 2 (a) Multiple markedly papillomatous nodules with severe hyperkeratosis were present on the dorsa of the feet; (b) improvement of the patient's cutaneous lesions was noted after 6 months of oral etretinate therapy.

hyperkeratosis and vacuolization of the cytoplasm of granular cells with coarse keratohyalin granules (Fig. 3c). Neither large keratinocytes showing clear cytoplasm, specific for EV, nor malignant changes were found. Laboratory evaluation revealed a normal, complete blood count and a normal absolute lymphocyte count [2000/mm^2, with 79% CD2 (normal range 60–80%), 56% CD4 (normal range 29–53%) and 15% CD8 (normal range 17–49%)]. All immunoglobulin levels were within the normal range. A serological test for human immunodeficiency virus was negative. Specimens from the pigmented macules and the large reddish tumour were investigated for human papilloma virus (HPV) typing, using a degenerating PCR method.[1] The HPV types detected in the specimens were HPV 3, 50, 57 and 76. Of these, HPV 76 belongs to species 3 of the genus Beta-papillomavirus, which is closely associated with EV.[2] HPV 3 and 57 belong to the Alpha-papillomavirus genus (species 2 and 4, respectively), and HPV 50 belongs to species 3 of the genus Gamma-papillomavirus. The patient started treatment with a combination of systemic etretinate, 50 mg/day, and topical maxacalcitol. All the skin lesions dramatically improved after 4 weeks of this treatment. His condition was relatively well controlled with this combination therapy at 6-month follow-up (Fig. 3b), but as the etretinate dose was reduced, his skin lesions relapsed.

Differences between EV and GV are so slight that a diagnosis is often difficult to make. In our case, several clinical features suggested against classic EV. Firstly, there was no parental consanguinity or familial occurrence of similar cutaneous lesions in our patient. Secondly, severely hyperkeratotic papillomatous nodules, which resemble common warts, were the predominant skin lesion in our case. These were different from the typical skin lesions, such as plane warts and

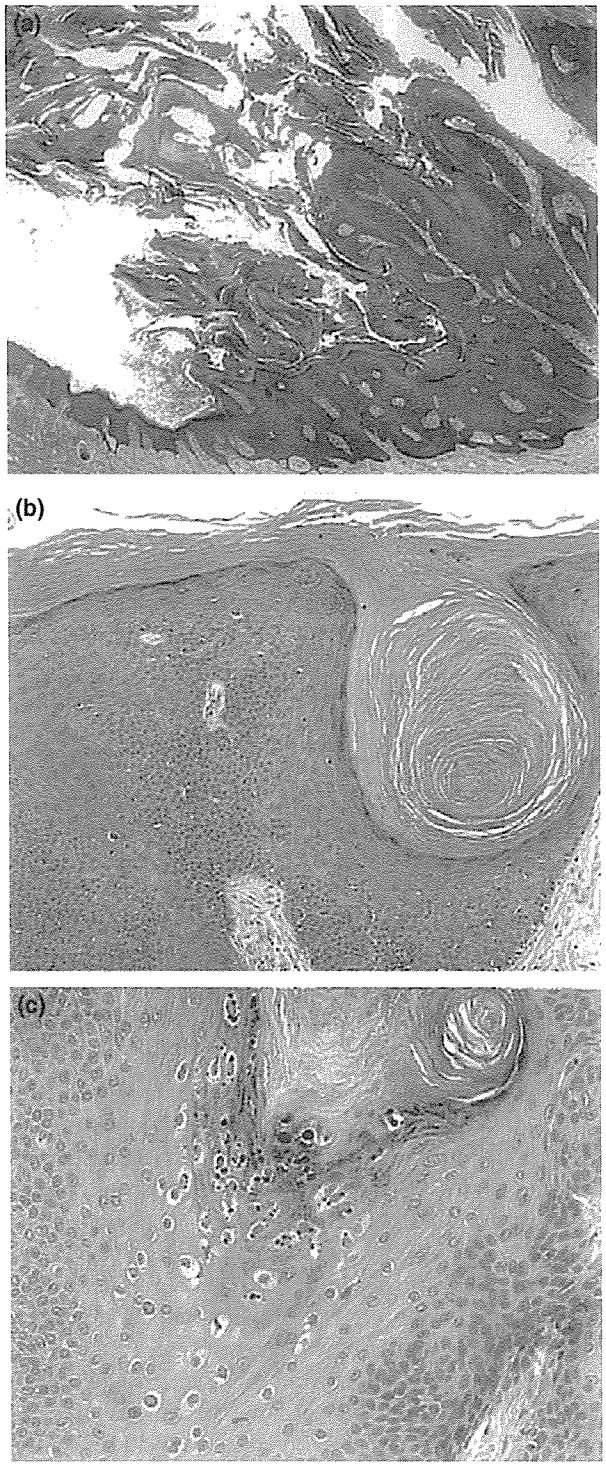

Figure 3 Histological findings (tumour on the right calf). (a) The tumour was exophytic, hyperkeratotic and papillomatous; (b) an acanthotic epidermal layer with sharply defined nests of basaloid cells; (c) vacuolization of the granular cell cytoplasm with coarse keratohyalin granules. Haematoxylin and eosin, original magnification (a) x 10; (b) x 40; (c) x 100.

pityriasis versicolor-like lesions, in an EV patient. Thirdly, histopathological studies showed the features of common warts or seborrhoeic keratosis rather than the characteristic EV lesion pathological features, such as large clear keratinocytes in the upper spinous layer and granular layers. Finally, the lesions in typical EV patients only demonstrate EV-specific HPVs;[3] however, in our patient, HPV typing revealed both EV-specific and non-EV-specific HPVs. Although these points fail to suggest that this case is classic EV, we thought it might be a unique variant of EV.

EV was originally described as an autosomal recessive disorder, characterized by a widespread and persistent cutaneous human papillomavirus infection without any apparent defects in both cellular and humoral immune systems, i.e., EV shows specific immunotolerance to HPVs. Patients with EV suffer from life-long, generalized verrucous skin lesions associated with various types of HPVs, some of which cause malignant transformation when infected with high-risk HPVs. Conversely, GV, in some reports, has been more typically associated with generalized warts, which are associated with immunocompromised conditions such as primary immunodeficiency, cyclic neutropenia and combined immune deficiency.[4–6] Our patient showed no history of repeated infections implicating an immunocompromised condition, therefore we propose that he had an abnormal specific susceptibility to HPV since early childhood. Previously, Requena *et al.* reported a patient with severe verrucosis similar to our case as GV.[4] They indicated that the mode of inheritance, typical clinical appearance including lesion distribution, characteristic histopathological findings and the presence of EV-specific HPV are critical points for the proper differential diagnosis of EV from GV. However, this is the only report that has demonstrated an immunocompetent patient with an abnormal susceptibility specific to HPV who was diagnosed as GV. EV and GV are distinguished by slight differences in clinical symptoms or HPV types, so there should be no apparent differential points common to both diseases. Therefore, we propose that an abnormal susceptibility specific to HPV, which is the most characteristic feature in EV, should be regarded as a differential point in these two diseases, both of which comprise systemic verrucosis.

Recently, EV-associated mutations were reported. Ramoz *et al.* reported nonsense mutations in two genes, *EVER1* and *EVER2*.[7] There is also one case report that demonstrates an *EVER1* mutation in an EV patient.[8] The *EVER1* and *EVER2* gene products show features of integral membrane proteins that are localized to the

endoplasmic reticulum. The function of these molecules is unknown, as is the relationship between the mutation and the abnormal HPV restricted susceptibility. Further follow-up studies and investigations of these gene products are required to clarify the underlying pathomechanisms resulting in the unique abnormal susceptibility to HPV, and to formulate a more precise diagnostic definition of EV.

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Positive Comments Made by Patients:

- "I get the feeling that I have a more active role and I like that. I am more aware of my complaint and my expectations."
- "I don't know who this person (teledermatologist) is, but I assume he has enough knowledge and experience to provide a good judgment."
- "You have to describe your complaint in detail, which can probably improve the diagnostics."
- "This service is accessible 24 hours, 7 days a week."
- "It is distant (pleasant)."

Negative Comments Made by Patients:

- "I don't speak Latin."
- "I really think somebody should see it face-to-face."
- "It's only a judgment from the images."
- "The answers are short and impersonal."

Figure. Positive and negative comments made by patients as an explanation for their scores.

after the teleconsultation, which was higher than before the teleconsultation (71%; 60 of 84). Patients were as positive about a teledermatologist providing a diagnosis, treatment plan, and detailed information before the teleconsultation as after.

Half of the patients (42 of 84) were willing to pay up to €25 for the service; 24% (20 of 84) were willing to pay between €26 and €50 for a consultation; 20% (17 of 84) were willing to pay between €51 and €100; and 6% (5 of 84) would have paid more than €100.

Comment. In general, patients responded positively to patient-assisted teledermatology. A quick response from the teledermatologist was considered an important advantage. A disadvantage was the remote character of the service with lack of personal interaction. However, this remoteness might be seen as an advantage by some patients, for example those who are shy or embarrassed.

To our knowledge, this is the first study on this type of patient-assisted teleconsultation service with a secondary care provider. Internet communication in health care is becoming more accepted and more frequently initiated by patients.[4,5] It is important to know patient perceptions and willingness to pay before such services are introduced.

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VIGNETTES

Peginterferon Alfa-2b for Mycosis Fungoides

Currently, peginterferon alfa-2b plus ribavirin has been shown to be the best available therapy for patients with chronic hepatitis C virus (HCV) infection.[1] Peginterferon alfa-2b (a 12-kDa linear polyethylene glycol moiety) plus ribavirin produces a significantly improved and sustained antiviral response compared with interferon alfa-2b plus ribavirin.[1] Peginterferon alfa-2b has an extended serum half-life that provides constant viral suppression for 7 days, thus allowing once-weekly dosing and an enhanced clinical efficacy.[1] While interferon alfa-2b has been used to treat T-cell lymphoma,[2,3] we report herein the significant efficacy of peginterferon alfa-2b for treatment of skin lesions caused by mycosis fungoides (MF).

A 67-year-old Japanese man was referred to our hospital with a 6-month history of multiple, asymptomatic brown-red–colored scaly erythematous macules and plaques over his arms and legs (**Figure**, A and B). He had not received any medications prior to the onset of his skin eruption. A skin biopsy specimen from his lower leg showed epidermotropism and dense upper dermal infiltration of atypical lymphocytes, which is consistent with MF (Figure, C). T-cell receptor rearrangement (both Jγ and Cβ chains) were recognized in his biopsy specimens. Peripheral blood analysis revealed a normal flow cytometric pattern. Biochemical tests revealed high soluble interleukin (IL) 2 receptor (1795 U/mL; normal range, 135-483 U/mL); however, his human T-cell lymphotropic virus type 1 antibody was negative. After complete systemic examination, including enhanced computed tomography, gallium scintigraphy, and bone marrow aspiration test, no internal invasion was detected. We confirmed the diagnosis of MF plaque stage. We started treatment with topical steroid ointment, systemic psoralen UV-A (PUVA), and etretinate. However, the lesions had been refractory against this treatment for approximately 5 months.

Our patient also had chronic HCV infection, and laboratory data showed an aspartate aminotransferase level of 33 IU/L, an alanine aminotransferase level of 19 IU/L, a total bilirubin level of 0.9 mg/dL (15.4 μmol/L), and an HCV RNA level greater than 5000 kIU/mL. He started treatment for chronic HCV infection with peginterferon alfa-2b plus ribavirin. Peginterferon alfa-2b (80 μg) was injected subcutaneously once a week, and oral ribavirin, 600 mg, was administered daily. At week 28, his serum HCV RNA level had decreased, so his therapy was continued.

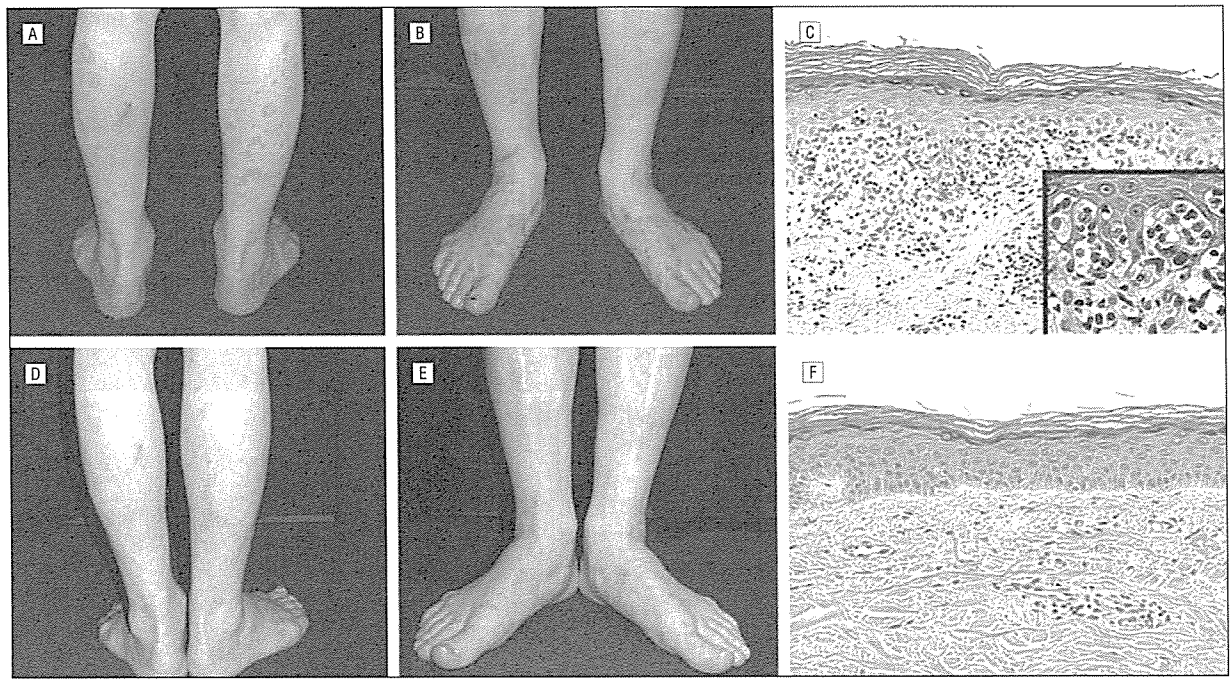

Figure. Mycosis fungoides skin lesions before the treatment with peginterferon alfa-2b (A and B) and after 4 weeks of therapy (D and E). Light microscopic finding of a mycosis fungoides skin lesion before treatment with peginterferon alfa-2b (C) (inset [original magnification ×100] shows the atypical lymphocytes) and after 4 weeks of therapy (F) (hematoxylin-eosin, original magnification ×40).

Interestingly, soon after the initiation of peginterferon alfa-2b, not only the HCV infection but also the patient's skin lesions showed a dramatic improvement, and in only 4 weeks most of the skin lesions had clinically subsided (Figure, D and E). When peginterferon alfa-2b and ribavirin treatments were started, he had been receiving treatment with oral etretinate (10 mg/d) and systemic PUVA (1.3 J once weekly) over a 3-month period, but we stopped these treatments at week 4 because of his skin improvement. To further evaluate whether his MF skin lesions had improved at the microscopic level, we performed a skin biopsy at the same site as the previous biopsy. While dense atypical lymphocyte infiltrates were present in the previous biopsy specimen, no significant numbers of atypical lymphocytes were recognized in the later biopsy specimen (Figure, F). Biochemical tests revealed that the level of soluble IL-2 receptor was decreased (1107 U/mL at week 20). Recurrence of MF lesions was not noted during the treatment period. He has remained in remission and off all skin therapy for the past 24 weeks.

The efficacy of interferon alfa-2b has been well reported and includes MF treatment.[2,3] Interferon alfa has been shown to inhibit IL-4 and IL-5 production by T cells in patients with Sézary syndrome.[3,4] Interferon molecules have been shown to induce cell-mediated cytotoxic effects and stimulate natural killer cell activity in vivo.[3] In addition, interferon alfa-2a plus PUVA or extracorporeal phototherapy have been reported.[5,6] On the other hand, peginterferon alfa-2b and ribavirin treatment of HCV-related, low-grade, B-cell, non-Hodgkin lymphoma has been reported.[7] Although it is reported that MF is not associated with HCV infection today, our patient's improvement might be related with his HCV infection.[8] Moreover, peginterferon is known to exert immunomodulatory and antiangiogenic effects and has been used to treat multiple myeloma.[9]

Peginterferon alfa-2b is a new drug for HCV treatment, and therefore its effects on MF have not been well studied. To our knowledge, this is the first MF report in which the marked efficacy of peginterferon alfa-2b has been confirmed both clinically and histopathologically. This example of 1 case cannot provide evidence whether peginterferon is superior to standard interferon treatment, but it does suggest that further studies are warranted.

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Oral Involvement in Hydroa Vacciniforme

Hydroa vacciniforme (HV) is a rare photosensitivity disorder with onset in childhood. Oral mucosal involvement seems to be rare. We report a case of a 14-year-old boy who had a recurrent papulovesicular necrotic facial eruption. One year prior to presentation at our clinic, his skin symptoms appeared after sun exposure. The eruption resolved in a few weeks with some varicellalike scars. After he used strict photoprotection (Photoderm Max; Bioderma, Lyon, France), there was no recurrence of his cutaneous disorder. In May 2005, he was referred to our clinic with a new relapse of his skin rash triggered by 2 days of sun exposure without any photoprotection. He complained of severe mouth and lip pain. There were no symptoms of ocular involvement.

At clinical examination, he had multiple clusters of confluent necrotic papulovesicles located on his nose and cheeks (**Figure**, A). He also had necrotic papulovesicles and flesh-colored papules on his ears and neck. His lower lip was edematous and covered with large confluent crusted ulcers extending to the lower vestibule. There were no papillae on the distal atrophic mucosa on his tongue (Figure, B). Findings from his general examination were otherwise normal. Vaccinialike lesions with a necrotic and vesiculopustular aspect as well as relation with sun exposure were highly suggestive of HV.

Histological examination of one of the neck papules showed an inflammatory perivascular infiltrate made of lymphocytes. There was no evident sign of lymphoproliferative disorder. Antinuclear factors were negative. Epstein-Barr virus serologic examination revealed a former infection. The patient was treated with hydroxychloroquine sulfate, 800 mg daily, and he was encouraged to apply a photoprotective cream. The skin and mucosal involvement resolved within 2 weeks.

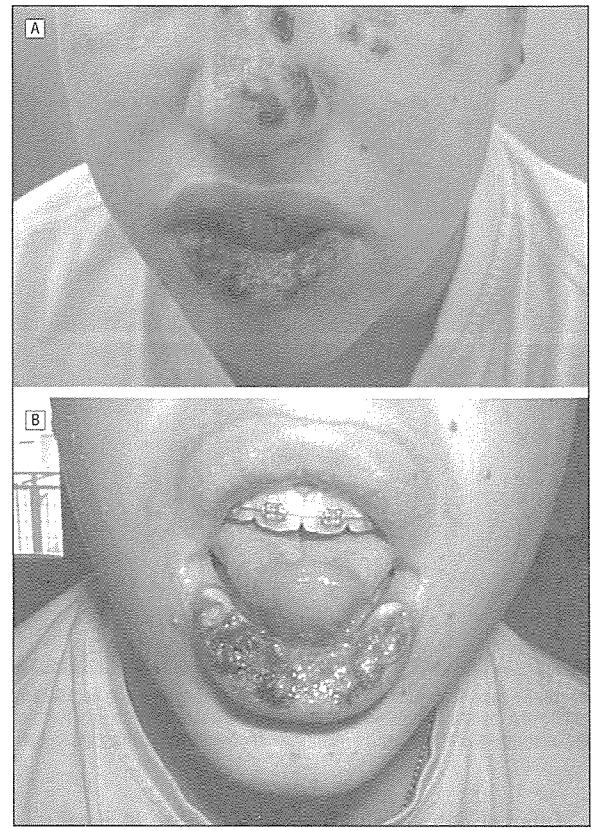

Figure. A, Necrotic papulovesicles located on the nose and cheeks; B, crusted lip ulcers extending to the lower vestibule.

Mucous involvement in HV, which is rare, mostly occurs only on the photoexposed lower lip. In this case, the mucous involvement appears to be a result of contiguous extension of sun-exposed lesions rather than being a distinct site of HV. To our knowledge, only 1 case of mouth ulcers in a 6-year-old girl has been previously described.[1] While the physiopathologic mechanism of HV is not clear, it has been suggested recently that HV could be due to the clonal natural killer cells with latent Epstein-Barr virus infection.[2]

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Epidermolysis bullosa simplex in Japanese and Korean patients: genetic studies in 19 cases

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Conflicts of interest
None declared.

Summary

Background Epidermolysis bullosa simplex (EBS) comprises a group of hereditary bullous diseases characterized by intraepidermal blistering caused by mutations in either keratin gene, KRT5 or KRT14. Significant correlation between the position of mutations within these proteins and the clinical severity of EBS has been noted. A recent report showed EBS cases in Israel had unique genetic features compared with European or U.S.A. associated families, which suggests that the ethnic and geographical features of EBS patients may be different.

Objectives To assess the possibility that EBS may present with certain specific features in Japanese and Koreans and to identify additional EBS mutations for genotype/phenotype correlation.

Methods EBS was clinically diagnosed and confirmed by transmission electron microscopic examination of a skin biopsy. Mutation analysis of KRT5 and KRT14 was performed by direct sequencing in 17 Japanese and two Korean EBS patients.

Results We have identified six novel KRT5 missense mutations (V143D, D158V, V186M, Q191P, R352S, G517D). R352S is the first mutation in the 2A domain. Most of these novel mutations changed amino acids that were evolutionarily conserved. Eight including all five mutations in EBS-Dowling–Meara patients have been previously reported. We were unable to detect mutations in five sporadic EBS-Koebner patients. The proportion of mutations in KRT5 (11 of 14; 78%) is higher than that for KRT14 mutations (3 of 14; 21%) in these Japanese and Korean EBS patients.

Conclusions Japanese and Korean patients with EBS showed very similar phenotype and genotype correlations with patients from Western countries. Whether the higher proportion of KRT5 mutations is a definite characteristic of Japanese and Korean patients with EBS or not, requires further research into mutations in Japanese and Korean people.

Epidermolysis bullosa (EB) encompasses a group of heterogeneous genetic skin diseases characterized by mechanical stress-induced blistering of the skin. EB is classified into three major groups according to the level of the dermoepidermal separation within the basement membrane zone. Epidermolysis bullosa simplex (EBS) results from an intraepidermal blister formation due to cytolysis of the basal keratinocytes. EBS affects approximately one in 30 000–50 000 of the population.[1] EBS can be subdivided into three major subtypes based on the severity of the clinical findings. The mildest subtype is the Weber–Cockayne type EBS (EBS-WC; OMIM 131800) with blistering restricted to the hands and feet, while the moderately severe subtype, the Koebner type (EBS-K; OMIM 13190) shows more generalized blister formation, and the most severe subtype, Dowling–Meara (EBS-DM; OMIM 131760) is characterized by severe herpetiform blistering.[2] In EBS-DM, the keratin intermediate filaments (KIF), major components of basal keratinocyte cytoskeletal network within keratinocytes, are clumped, a finding that serves as a diagnostic feature in electron microscopy.[2,3]

EBS is mostly inherited in an autosomal dominant fashion and is caused by a single mutation in either of the keratin genes, KRT5 or KRT14.[2,3] The majority of mutations are nucleotide substitutions that lead to missense mutations. These

genes encode the keratin 5 (K5) and keratin 14 (K14) proteins which then form heterodimers that assemble into the KIF of the basal cells in the epidermis.[4] Autosomal dominant mutations in *KRT5* or *KRT14* act in a dominant negative manner, i.e. the abnormal protein produced by the mutated allele interferes with the normal protein produced by the normal allele in the process of keratin filament assembly.[3] Both keratin proteins have a similar basic molecular structure to other intermediate filaments (IF), consisting of a central α-helical rod domain of about 310 amino acids, responsible for dimerization and higher order polymerization. This domain consists of four segments (1A, 1B, 2A and 2B) and is interrupted by three nonhelical linkers (L1, L12 and L2).[4]

There are correlations between the EBS phenotype and the K5 or K14 functional domains in which the mutations occur. The mutations responsible for EBS-DM lie within the highly conserved ends of the rod domain, which are critical for K5 and K14 assembly. In contrast, the majority of the EBS-K mutations also lies within the rod domain but are more centrally located, whereas the EBS-WC mutations are mostly within the nonhelical regions.[3,5,6]

Despite many reported mutations in EBS in the Western countries,[3,6] the precise mutation spectrum in Japanese and Korean EBS patients has not been examined. To identify additional EBS mutations for genotype and phenotype correlation studies in Japanese and Korean patients, we performed *KRT5* and *KRT14* mutation analysis by direct sequencing in 17 Japanese and two Korean patients with EBS and compared them to the previously reported mutations in Western patients.

Materials and methods

Patients

The clinical phenotypes of the 17 Japanese and two Korean patients with EBS (Patients 6 and 7) are summarized in Table 1. EBS was at first clinically diagnosed and later confirmed by transmission electron microscopic examination of a skin biopsy, obtained from the leading edge of a fresh blister, that reveals splitting just above the basal cell layer and/or by immunohistochemical antibody/antigen mapping.

Mutation analyses

Genomic DNA extracted from whole blood was used as a template. The nine exons of *KRT5* and the eight exons of *KRT14* as well as each of the intron–exon boundaries were amplified by the methods previously reported.[7,8] DNA sequencing of the polymerase chain reaction (PCR) products was carried out with an ABI 3100 sequencer. For all novel mutations, their presence in 50 unrelated ethnically matched control individuals has been excluded.

Results

Pathogenic mutations were identified in 14 EBS cases out of 19 (Table 1).[7,9–14] In EBS-WC patients, two novel missense mutations (473A → T, D158V; 1054C → T, R352S) were detected in *KRT5*. The D158V mutation (Patient 1) lies within the nonhelical V1 head domain, while the R352S mutation (Patient 2) is in the 2A rod domain. Four out of five *KRT5* missense

Table 1 Clinical phenotypes and mutations found in Japanese and Korean epidermolysis bullosa simplex (EBS) cases included in this study

Case	Age/sex	Phenotype	Inheritance	Gene	Mutation	Effect	References
1	8Y/M	WC	Familial	*KRT5*	473A → T	D158V	Novel
2	24Y/M	WC	Familial	*KRT5*	1054C → T	R352S	Novel
3	38Y/F	WC	Familial	*KRT14*	415A → G	M119V	Cummins *et al.*[9]
4	1Y/M	K	*De novo*	*KRT5*	428T → A	V143D	Novel
5	54Y/M	K	Familial	*KRT5*	558G → A	V186M	Novel
6	23Y/F	K	Familial	*KRT5*	573C → A	Q191P	Novel
7	27Y/M	K	Familial	*KRT5*	1550G → A	G517D	Novel
8	1W/M	K	*De novo*	*KRT5*	558G → T	V186L	Liovic *et al.*[10]
9	1D/M	DM	*De novo*	*KRT5*	527A → G	N176S	Stephens *et al.*[8]
10	42Y/M	DM	*De novo*	*KRT5*	1423G → A	E475K	Schuilenga-Hut *et al.*[7]
11	1Y/F	DM	*De novo*	*KRT5*	1429G → A	E477K	Coulombe *et al.*[11]
12	8Y/F	DM	Familial	*KRT14*	434G → A	R125H	Coulombe *et al.*[11]
13	1W/M	DM	*De novo*	*KRT14*	433C → T	R125C	Coulombe *et al.*[11]
14	30Y/M	MP	Familial	*KRT5*	465C → T	P25L	Uttam *et al.*[12]
15	1W/F	K	*De novo*	ND			
16	1M/F	K	*De novo*	ND			
17	1Y/M	K	*De novo*	ND			
18	30Y/F	K	*De novo*	ND			
19	35Y/F	K	*De novo*	ND			

Y, year; W, week; D, day; M, month; EBS-WC, Weber–Cockayne type EBS; EBS-K, Koebner type EBS; EBS-DM, Dowling–Meara type EBS; EBS-MP, EBS with mottled pigmentation; ND, not detected.