### 2.6. Immunohistochemistry

Formalin-fixed and paraffin-embedded 4-μm sections of specimens were deparaffinized and rehydrated through descending alcohol series and in phosphate buffered saline (PBS, pH 7.4). Antigen was retrieved with Antigen Retrieval Citra (BioGenex Laboratories, San Ramon, CA), and endogenous peroxidase was quenched with 1.5% hydrogen peroxide in methanol. After blocking nonspecific antigen binding sites with 3% nonimmune serum, anti-Sema3A rabbit polyclonal IgG antibodies (sc-28867; 1:200; Santa Cruz) was applied overnight at 4 °C. After rinsing with PBS, sections were incubated with biotinylated secondary antibody and then with peroxidase conjugated avidin solution (Vector Laboratories Inc., Burlingame, CA). Peroxidase was visualized with 3-amino-9-ethylcarbazole chromogen, and the sections were lightly counterstained with hematoxylin.

### 2.7. Statistical analysis

Data were analyzed using an unpaired two-tailed *t*-test. *P*-value of less than 0.05 was considered to be significant.

## 3. Results

### 3.1. High calcium augments the expression of SEMA3A but not NGF in NHEK

Keratinocytes proliferate at a low calcium concentration, such as 0.15 mM, and differentiate at a high calcium concentration in culture [12]. The proliferating and differentiating cells represent the basal cell in the lower most epidermis and the prickle cell in the upper epidermis, respectively. To address the physiological production of Sema3A and NGF by epidermal keratinocytes, NHEK were cultured under varying calcium concentrations, ranging from 0.15 to 0.9 mM, and after 2-h incubation, the expression of *SEMA3A* and *NGF* was assessed by real-time PCR. Whereas *SEMA3A* expression was low at 0.15 or 0.3 mM calcium, its expression was upregulated at higher concentrations of 0.45–0.75 mM (Fig. 1a). Calcium at 0.9 mM or more reduced the expression of *SEMA3A*. In contrast, calcium concentration did not affect the expression of *NGF* in NHEK (Fig. 1b). The incubation period of 24 h produced the comparable levels of *SEMA3A* and *NGF* expression to the 2-h incubation.

To confirm the above finding at the protein level, we performed a Western blot analysis for Sema3A, NGF, and differentiation marker loricrin in NHEK cultured with 0.15 or 0.6 mM calcium. NHEK incubated with 0.6 mM calcium showed higher levels of Sema3A as well as loricrin than those with 0.15 mM calcium, while the levels of NGF were comparable between them (Fig. 1c). These results indicate that the production of Sema3A but not NGF is dependent on calcium concentration.

### 3.2. Immunohistochemical staining for Sema3A in normal skin and cutaneous tumors

The above finding suggested that Sema3A is highly expressed in the upper epidermis where keratinocytes are differentiated by high calcium concentration. We therefore investigated Sema3A expression by immunohistochemistry in normal skin, squamous cell carcinoma (SCC), and basal cell carcinoma (BCC). In normal

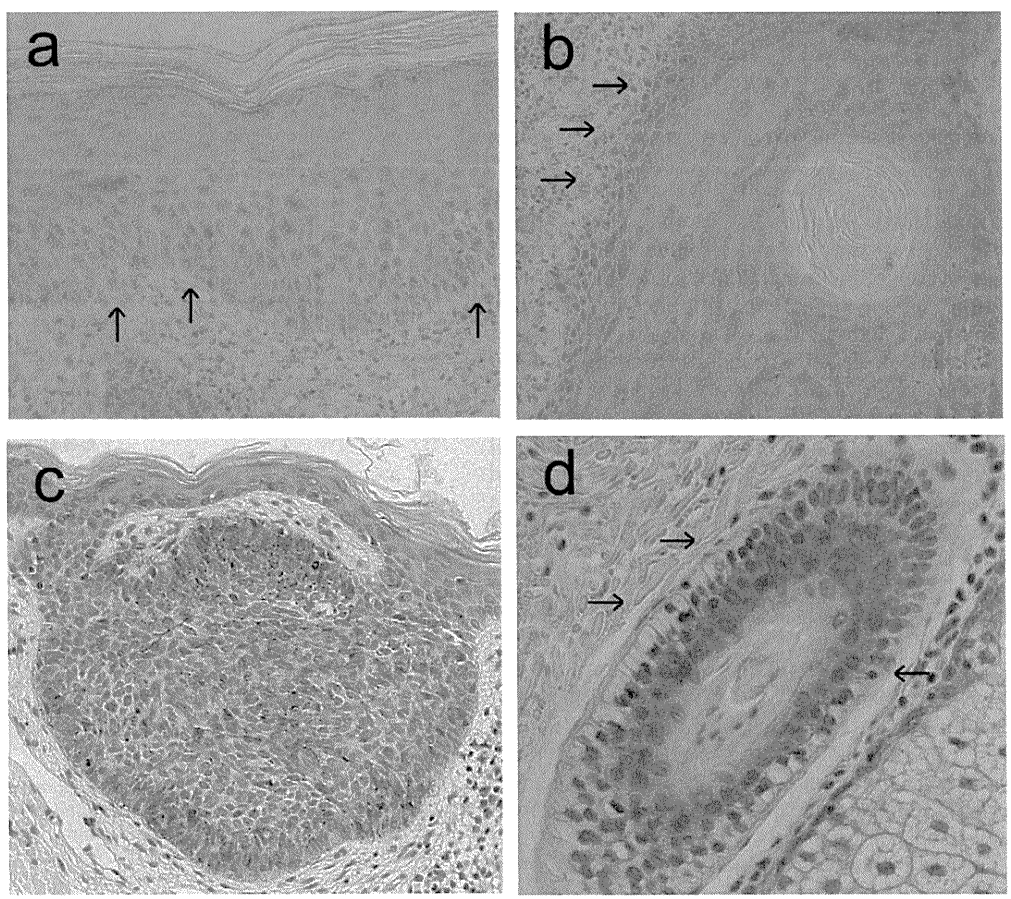


**Fig. 2.** Sema3A expression in normal skin and SCC. Immunohistochemical staining for Sema3A in normal epidermis (a), tumor nest of SCC (b), tumor nest of BCC (c), and hair follicle (d). Arrows indicate basal and suprabasal cells stained negatively. Original magnification: ×200.

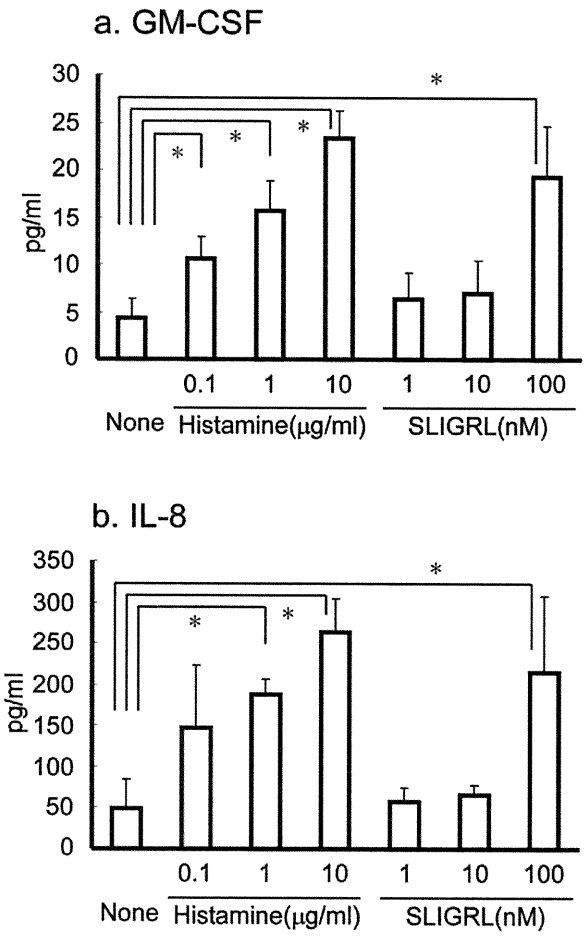


**Fig. 3.** Effects of histamine and SLIGRL on cytokine/chemokine production by NHEK. NHEK were cultured with various concentrations of histamine or SLIGRL for 48 h. The amounts of IL-8 and GM-CSF in the supernatants was measured using CBA. Bars represent the means ± SD of IL-8/GM-CSF concentration in triplicate. $^*P < 0.05$.

skin, keratinocytes of the prickle layer were positive for Sema3A, while cells of the basal and suprabasal layers and the granular layer were negative (Fig. 2a). Likewise, in SCC tissues, squamoid tumor cells were strongly positive for Sema3A except for basal and suprabasal cells of the tumor nests (Fig. 2b). The epidermal cells of BCC were negative for Sema3A (Fig. 2c). In hair follicles of normal skin, inner cells in the outer root sheath were strongly Sema3A-positive, but basal and suprabasal cells in this part were not positively stained (Fig. 2d).

### 3.3. Histamine and SLIGRL are bioactive for keratinocytes

Since histamine is one of the critical pruritus-related molecules involved in allergic skin disorders, we examined its effect on Sema3A and NGF production by keratinocytes. SLIGRL was also tested in parallel, because signaling *via* PAR-2 is important as well as signaling *via* H1 receptor in C-fiber stimulation [15], and keratinocytes express both the receptors [16,17].

In advance of testing the effects of histamine and SLIGRL on the production of Sema3A and NGF, we confirmed their biological activities on keratinocytes and estimated their optimal concentrations in our culture system. NHEK were cultured at 0.15 mM calcium with histamine (0.1–100 μg/ml) and SLIGRL (1–1000 nM) for 48 h. In our preliminary study, we examined the effects of histamine and SLIGRL on the production by NHEK of GM-CSF, IL-8, CXCL9/MIG, CXCL10/IP-10, CCL5/RANTES, CCL22/MDC, and vascular endothelial growth factor. Among these cytokines and chemokines, GM-CSF and IL-8 were increased by histamine and SLIGRL. We therefore chose these two cytokines in the following experiments. When these two cytokines were quantified in the supernatants, histamine at 0.1 μg/ml or more and SLIGRL at 100 nM increased the production of GM-CSF and IL-8 by NHEK (Fig. 3). Histamine 100 μg/ml and SLIGRL 1000 nM were toxic to NHEK as they abolished cell viability (data not shown). Therefore, histamine at 10 μg/ml and SLIGRL 100 nM were used in the following studies.

### 3.4. Histamine upmodulates SEMA3A expression and downmodulates NGF expression in keratinocytes

Incubation of NHEK with histamine (10 μg/ml) for 2 h upregulated the expression of *SEMA3A* under either 0.15 mM or 0.6 mM of calcium concentration, while stimulation with SLIGRL (100 nM) unaffected *SEMA3A* expression at both calcium concentrations (Fig. 4a). SLIGRL at higher concentrations (up to 10 μM) was also tested, but *SEMA3A* expression was not increased (data not shown). On the contrary, the expression of *NGF* was significantly decreased by histamine at 10 μg/ml or SLIGRL at 100 nM, although the former's suppressive activity was higher than the latter's (Fig. 4b). Thus, histamine but not SLIGRL augmented the expression of Sema3A, and both stimulants inhibited *NGF* expression in keratinocytes. Thus, the opposite effects of histamine on Sema3A and NGF productions are remarkable.

### 3.5. Histamine and SLIGRL downregulate the expression of both SEMA3A and NGF in fibroblasts

In addition to the epidermis, the dermis is a critical microenvironment where peripheral sensory nerve elongates. Dermal fibroblasts express H1 receptor [18] and PAR-2 [19]. The expression of the axon guidance factors by fibroblasts and the effects of histamine and SLIGRL on their expressions are issues to be explored. By using NHFb, we first examined the fibroblast expression of *SEMA3A* and *NGF* and the dependency on calcium concentration. NHFb expressed mRNAs for both factors, and there was no modulatory effect of calcium concentration (data not shown). Next, NHFb were incubated with histamine (1 or 10 μg/ml) or SLIGRL (10 or 100 nM) for 2 h. The expression levels of *SEMA3A* (Fig. 5a) and *NGF* (Fig. 5b) were significantly reduced by histamine at 10 μg/ml or SLIGRL at 100 nM, suggesting that the fibroblast production of both axon guidance factors with opposite capacities are depressed by these mast cell-released mediators.

## 4. Discussion

In this study, we demonstrated the modulation of *SEMA3A* and *NGF* expressions by calcium concentration and histamine or SLIGRL. The expression of *SEMA3A* depended on calcium concentration in NHEK, but not in NHFb. Whereas NHEK cultured at low calcium concentrations of 0.15–0.3 mM, inducible for keratinocyte proliferation, expressed low levels of *SEMA3A*, cells cultured at high calcium concentrations of 0.45–0.75 mM, suitable for keratinocyte differentiation, expressed high levels of *SEMA3A*. On the contrary, NGF was constitutively expressed by NHEK irrespective of calcium concentration. Therefore, the expression of *SEMA3A* but not *NGF* is markedly regulated by calcium concentration in epidermal keratinocytes, implying that the chemorepellent may control C-fiber elongation in the physiological condition. The mechanisms by which Sema3A production is dependent on the extracellular calcium concentration is not clear. One possible explanation is that $Ca^{2+}$ signaling participates in the transcription of Sema3A gene. The second messenger inositol triphosphate and intracellular

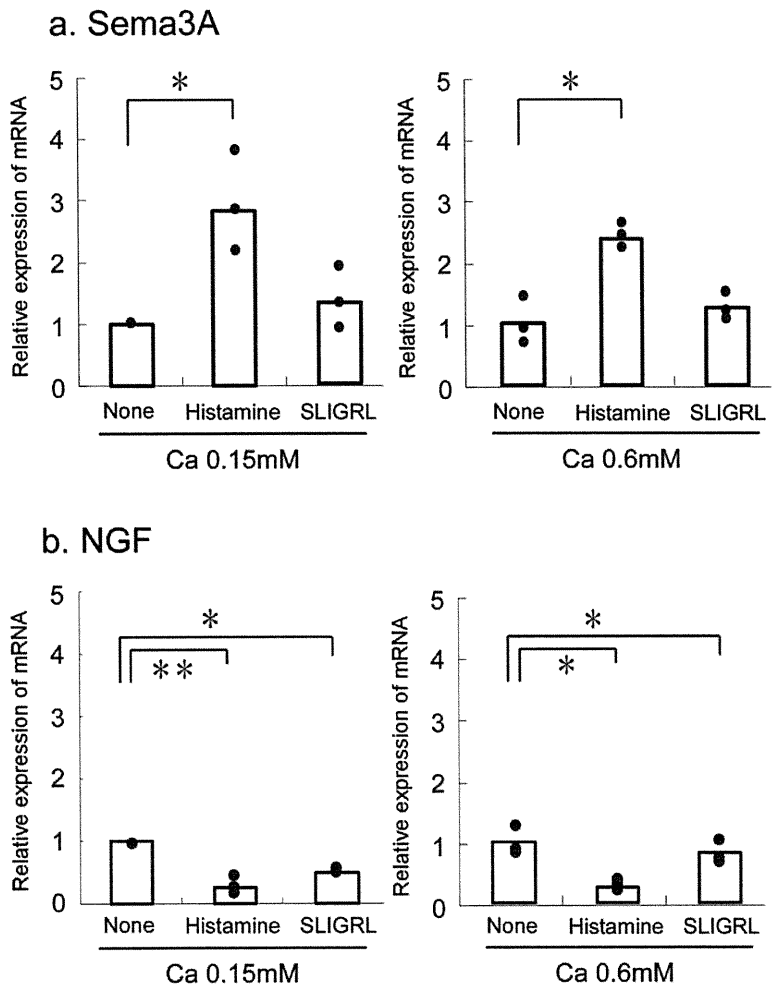


**Fig. 4.** Effects of histamine and SLIGRL on the expression of *SEMA3A* and *NGF* in NHEK. NHEK were cultured with histamine or SLIGRL under different Ca concentrations (0.15 or 0.6 mM) for 2 h. After harvesting, the expression of Sema3A and NGF was assessed by real-time PCR. The data are expressed as (expression level of stimulated group)/(expression level of no addition control). Bars represent the means of three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

calcium are generated in that $Ca^{2+}$-induced differentiation [20]. Sema3A production might share the signaling pathway with many differntiation-asscoaited molecules. It has been reported that NGF secreted by basal keratinocytes causes hypertrophy of the peripheral nerve [7,21]. Our finding suggests that NGF is homogenously produced throughout the epidermis, and Sema3A determines the location of nerve endings and inhibits excess C-fiber elongation into the epidermis.

In accordance with the results from the NHEK culture study, our immunohistochemical study showed that keratinocytes in the prickle layer of the epidermis and the outer root sheath of the hair follicle were positive for Sema3A, while basal cells and suprabasal cells were negative in these tissues. The similar staining pattern was obtained in SCC, where Sema3A was expressed by squamoid epithelial cells in the tumor nests, sparing two to three layers of basal or adjacent suprabasal cells. Notably, cells of the sebaceous glands and arector pilli bore Sema3A, providing a possibility that sensory nerves cannot invade these apparatus tissues.

Histamine and SLIGRL not only stimulated NHEK to produce IL-8 and GM-CSF, but also modulated the expression of Sema3A and NGF in NHEK and NHFb. In NHEK, histamine increased Sema3A expression but decreased NGF expression, and SLIGRL did not increase Sema3A expression and decreased NGF expression to some degree. Thus, the modulatory effects of histamine on NHEK are greater than those of SLIGRL. These *in vitro* results suggest that histamine unexpectedly inhibits C-fiber elongation in the epidermis. The release of histamine from mast cells occurs upon antigenic stimulation *via* IgE and Fcε receptors and upon substance P stimulation *via* NK1 receptor [3,4,22]. Since these stimulatory events take place in itch-related allergic diseases, the histamine-enhanced *SEMA3A* and -reduced *NGF* expressions in keratinocytes might be a feedback phenomenon to suppress exaggerated pruritus. However, a recent paper has reported that H1 antagonist olopatadine hydrochloride increased the expression of Sema3A in the skin of NC/Nga mice with atopic dermatitis [23], providing a contradictory finding. Considering that the histamine-augmented Sema3A expression in NHEK was not inhibited by H1 blocker pyrilamine maleate salt (data not shown), the discrepancy between their and our findings might be due to different usage of the types of histamine receptors. In NHFb, histamine and SLIGRL depressed the expression of both *SEMA3A* and *NGF*. Considering the opposite actions of these two axon guidance factors, the final outcome remains unclear in this study. Concerning the chemorepellent, however, histamine seems to allow sensory fibers to elongate in the dermis. It should be noted that the opposite effects of histamine on NHEK and NHFb in the expression of *SEMA3A* may induce positive and negative sensory fiber elongation in the dermis and epidermis, respectively.

Sema3A may play a crucial inhibitory role for C-fiber elongation/sprouting in the upper layers of the epidermis. Disruption of the physiological calcium gradient may induce the disordered Sema3A production by keratinocytes, resulting in C-fiber elonga-

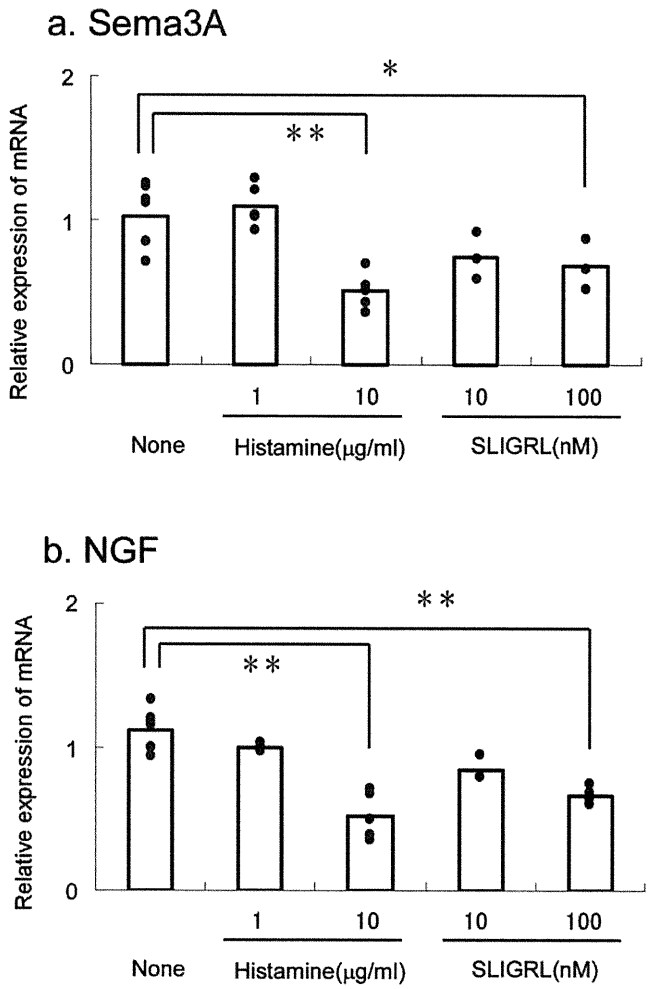


**Fig. 5.** Effects of histamine and SLIGRL on the expression of *SEMA3A* and *NGF* in NHFb. NHFb were cultured with histamine and SLIGRL at the indicated concentration for 2 h. After harvesting, the expression of *SEMA3A* (a) and *NGF* (b) was assessed by real-time PCR. The data are expressed as (expression level of stimulated group)/(expression level of no addition control). The bars represent the means of three to five independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$.

tion. In the epidermis of patients receiving hemodialysis and suffering from uremic pruritus, calcium concentration is not elevated from the inside to the outside of the skin, but distributed equally in all layers of the epidermis [24]. Considering that Sema3A production is regulated by calcium concentration, the disordered calcium gradient in such a pruritic disease might result in the elongation of C-fiber in the epidermis. In atopic dermatitis, C-fiber elongates into the upper epidermis as a result of a reduced production of Sema3A [9–11]. Our finding suggests that this epidermal elongation and sprouting of nerve endings is not promoted by histamine. It has been reported that mast cell-derived tumor necrosis factor (TNF) promotes nerve fiber elongation in the epidermis and dermis during contact hypersensitivity in mice [25]. This provides a possibility that mast cells contribute to nerve elongation by secreting TNF but not histamine. Future investigation may clarify this important issue.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2010.11.012.

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Eur J Dermatol 2011 (epub ahead of print)

CORRESPONDENCE

# Blocking of CTLA-4 on lymphocytes improves the sensitivity of lymphocyte transformation tests in a patient with nickel allergy

Allergic contact dermatitis is a T cell-mediated cutaneous inflammatory response typically induced by contact with low-molecular weight chemicals. The patch test remains the gold standard method of confirming allergic contact dermatitis and to identify the specific causative allergen, although it is time-consuming, subjective, and not completely safe since it can induce sensitization to allergens during the test. The frequency and morbidity of allergic contact dermatitis and the problems related to the patch test necessitate the development of an alternative test.

Several *in vitro* alternative tests have been described in the literature, and the lymphocyte transformation test (LTT) is one of the most promising possibilities [1]. The low sensitivity of the LTT limits its usefulness as a diagnostic tool, however. We describe a patient with nickel allergy in whom the LTT was initially negative but turned positive after the addition of a neutralizing monoclonal antibody (mAb) to cytotoxic T lymphocyte antigen 4 (CTLA-4).

A 56-year-old woman with a past history of metal-induced allergic contact dermatitis following ear piercing was referred to our department. Patch testing was performed for various metal allergens, including 19 ready-made patch test reagents (Torii Pharmaceutical Corporation, Tokyo, Japan) [2]. The patch test was positive for nickel (2+, according to the International Contact Dermatitis Research Group scale), confirming that the patient had a metal allergy to nickel.

To evaluate the LTT as a diagnostic tool for contact dermatitis, peripheral blood mononuclear cells (PBMCs) of the patient and a healthy control donor were freshly isolated and incubated with 50 mM of nickel sulfate (Ni) and 50 mM of cobalt (Co) as previously reported [3]. Briefly, PBMCs were incubated in RPMI1640 medium supplemented with 10% heat-inactivated fetal calf serum. Cells were stimulated with 3 μg/ml of concanavalin A as a positive control. Cultures were performed in triplicate in 96-well flat bottomed plates for six days. Eighteen hours before harvesting, 1 μCi of $^{3}$H-thymidine was added to each culture, and T cell proliferation was measured based on $^{3}$H-thymidine incorporation.

It has been demonstrated that IL-10-, TGF-b-, and CTLA-4-dependent mechanisms may contribute to the suppression of T cell proliferation by regulatory T cells (Tregs) [4]. Therefore, we hypothesized that lymphocyte proliferation was likewise suppressed by Tregs, despite the presence of effector T cells for Ni in the culture. To address this issue, we added neutralizing mAb to IL-10 (10 μg/ml) (eBioscience, San Diego, CA) and CTLA-4 (10 μg/ml) (BD Biosciences, San Jose, CA) and a selective inhibitor of TGF-b (1 μM) (Sigma-Aldrich, St.-Louis, MO, USA). Intriguingly, when anti-CTLA-4 mAb was added to the PBMCs, lymphocytes proliferated well in response to Ni *(figure 1A)*. In contrast, no such enhanced proliferation was observed in response to Co in the patient's PBMCs *(figure 1A)*. Cavani *et al.* reported that CD4$^{+}$ T cells purified from the peripheral blood of healthy subjects proliferate to Ni *in vitro* [5]. In our study, however, no responses to Ni or Co were found in three healthy controls, as represented by an individual shown in *figure 1B*.

It is possible that the CTLA-4-mediated suppressive mechanism depends on contact allergens, but our results suggest that CTLA-4 is involved in the mechanism responsible for suppression by Tregs, at least in the LTT assay for certain antigenic molecules. Since CTLA-4 is important in Ni-specific T cell proliferation, Treg-antigen presenting cell interaction could be a possible mechanism in our case.

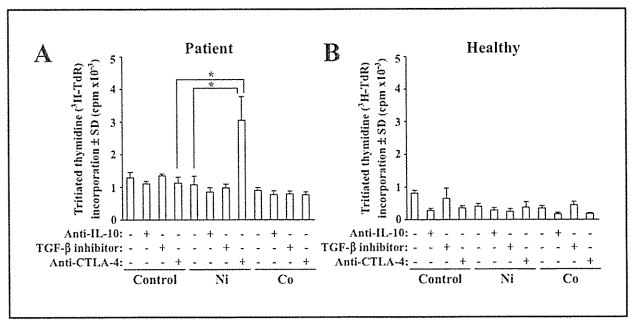


**Figure 1. LTT assay for Ni and Co.**
PBMCs were isolated from the patient **(A)** and a healthy donor **(B)**, and incubated ($5\times10^{5}$ cells/well) with or without metal as indicated for six days. Lymphocyte proliferation is indicated as thymidine incorporation for the last 18 h of culture. Data are representative of two patients (A) and three healthy donors (B) and are presented as the mean ± SD from triplicated wells. *, $P<0.05$ *versus* a corresponding group (one-way ANOVA followed by the Dunnett multiple comparison test).

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Although the number of cases is limited in this study, our method represents a modified LTT with improved sensitivity for Ni, thus warranting further investigation. ■

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[1] Department of Dermatology, University of Occupational and Environmental Health, Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan
[2] Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan
[3] Department of Immunobiology, Institute of Development, Aging and Cancer, Tohoku University Sendai, Japan

**Kazunari SUGITA**[1]
**Kenji KABASHIMA**[2]
**Yu SAWADA**[1]
**Sanehito HARUYAMA**[1]
**Manabu YOSHIOKA**[1]
**Tomoko MORI**[1]
**Miwa KOBAYASHI**[1]
**Kouetsu OGASAWARA**[3]
**Yoshiki TOKURA**[4]

[4] Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan
<k-sugita@med.uoeh-u.ac.jp>

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# Type of skin eruption is an independent prognostic indicator for adult T-cell leukemia/lymphoma

Yu Sawada, Ryosuke Hino, Kayo Hama, Shun Ohmori, Haruna Fueki, Shigenori Yamada, Shoko Fukamachi, Makiko Tajiri, Rieko Kubo, Manabu Yoshioka, Daiki Nakashima, Kazunari Sugita, Ryutaro Yoshiki, Takatoshi Shimauchi, Tomoko Mori, Kunio Izu, Miwa Kobayashi, Motonobu Nakamura and Yoshiki Tokura



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# Type of skin eruption is an independent prognostic indicator for adult T-cell leukemia/lymphoma

Yu Sawada,[1] Ryosuke Hino,[1] Kayo Hama,[1] Shun Ohmori,[1] Haruna Fueki,[1] Shigenori Yamada,[1] Shoko Fukamachi,[1] Makiko Tajiri,[1] Rieko Kubo,[1] Manabu Yoshioka,[1] Daiki Nakashima,[1] Kazunari Sugita,[1] Ryutaro Yoshiki,[1] Takatoshi Shimauchi,[1] Tomoko Mori,[1] Kunio Izu,[2] Miwa Kobayashi,[1] Motonobu Nakamura,[1] and Yoshiki Tokura[1,3]

[1]Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan; [2]Department of Dermatology, Kyushu Kosei Nenkin Hospital, Kitakyushu, Fukuoka, Japan; and [3]Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan

**Cutaneous involvement is seen in ~ 50% of adult T-cell leukemia/lymphoma (ATLL) patients. We investigated the association between skin eruption type and prognosis in 119 ATLL patients. ATLL eruptions were categorized into patch (6.7%), plaque (26.9%), multipapular (19.3%), nodulotumoral (38.7%), erythrodermic (4.2%), and purpuric (4.2%) types. When the T stage of the tumor-node-metastasis-blood (TNMB) classification of mycosis fungoides/Sézary syndrome was applied to ATLL staging, 16.0% were T1, 17.7% T2, 38.7% T3, and 4.2% T4, and the remaining 23.5% were of the multipapular and purpuric types. For the patch type, the mean survival time (median survival time could not be estimated) was 188.4 months. The median survival times (in months) for the remaining types were as follows: plaque, 114.9; multipapular, 17.3; nodulotumoral, 17.3; erythrodermic, 3.0; and purpuric, 4.4. Kaplan-Meier curves of overall survival showed that the erythrodermic type had the poorest prognosis, followed by the nodulotumoral and multipapular types. The patch and plaque types were associated with better survival rates. Multivariate analysis demonstrated that the hazard ratios of the erythrodermic and nodulotumoral types were significantly higher than that of the patch type, and that the eruption type is an independent prognostic factor for ATLL. The overall survival was worse as the T stage became more advanced: the multipapular type and T2 were comparable, and the purpuric type had a significantly poorer prognosis than T1. (*Blood.* 2011;117(15): 3961-3967)**

## Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a malignancy of mature $CD4^+$ T cells caused by the human T-cell lymphotropic virus type I (HTLV-1).[1-3] HTLV-1 infection is prevalent in southern Japan, especially in Kyushu,[4,5] and in the Caribbean region and Africa.[6,7] Based on the number of abnormal lymphocytes, organ involvement, and severity, ATLL is divided into 4 clinical categories: acute, lymphoma, chronic, and smoldering (Shimoyama classification).[8] This classification is the most common tool used for estimating the prognosis of ATLL patients. The smoldering type has the best prognosis, followed by the chronic type, lymphoma type, and acute type. The median survival times (MSTs) of the acute, lymphoma, and chronic types are 6.2, 10.2, and 24.3 months, respectively.[8] Thus, the acute and lymphoma types of ATLL are associated with remarkably poor prognoses despite advances in chemotherapy and allogeneic hematopoietic stem cell transplantation.[9-11] In contrast, the chronic and smoldering types are relatively indolent and can usually be managed with "watchful waiting" until the disease progresses to acute crisis, just as smoldering (asymptomatic) myeloma is managed.[12]

Studies have attempted to identify other prognostic factors for survival of ATLL patients. Advanced performance status, high blood lactate dehydrogenase (LDH) level, age of 40 years or more, more than 3 involved lesions, and hypercalcemia have all been associated with shortened survival.[13] The existence of hepatosplenomegaly and lymphadenopathy also indicates poor prognosis.[8,14] However, there has been no large study on the correlation between the type and spread of skin eruptions and the prognosis of ATLL.

Because cutaneous involvement can be recognized in approximately 50% of ATLL patients,[15,16] the evaluation of skin lesions in relation to prognosis is important. Tumor cells infiltrating the skin exhibit several differences in phenotype and function.[17,18] ATLL patients can develop various types of eruptions, including nodules, tumors, plaques, erythrodermas, and even purpuric lesions,[19,20] and the categorization of these eruption types remains unclear. In this study, we retrospectively analyzed the prognosis of ATLL on the basis of the skin manifestations. We classified the skin eruptions and applied the T stage of the tumor-node-metastasis-blood (TNMB) classification for mycosis fungoides (MF) and Sézary syndrome (SS) to the type of skin lesions of ATLL. Our results indicate that eruption type is a predictor for prognosis.

## Methods

### Patients

We analyzed 119 patients with newly diagnosed, untreated ATLL who had skin eruptions and were seen at the University of Occupational and Environmental Health and Kyushu Kosei Nenkin Hospital from April 1979 to December 2009. The cutoff date for analysis was June 2010. The diagnosis of ATLL was based on clinical features, histopathologically and cytologically proven mature T-cell malignancy, presence of anti–HTLV-1 antibody, and monoclonal integration of HTLV-1 proviral DNA into the blood and/or skin tumor cells, as described previously.[2,8,21,22] The subtypes

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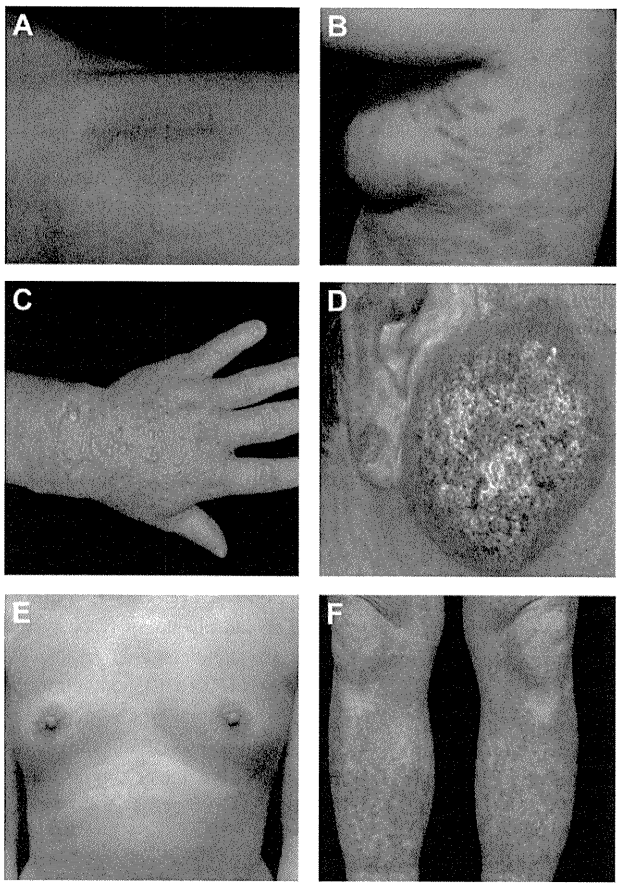


**Figure 1. Clinical features of ATLL with skin eruptions.** (A) Patch type, (B) plaque type, (C) multipapular type, (D) nodulotumoral type, (E) erythrodermic type, and (F) purpuric type.

of ATLL were classified according to the criteria established by the Lymphoma Study Group of Japan Clinical Oncology Group (Shimoyama classification).[8] Our retrospective, nonrandomized, observational study using existing data was granted an exemption from the institutional review board and was exempt from the requirement for written informed consent in accordance with the Declaration of Helsinki.

### Clinical evaluation and definitions

The patients were categorized into 2 age groups: younger than 60 years and 60 years or older. Complications at diagnosis were classified into present and absent. Leukocytosis and lymphocytosis were defined as white blood cell count more than $12 \times 10^9$/L, and total lymphocyte count more than $6.5 \times 10^9$/L, respectively. LDH and calcium levels were classified into 2 groups according to a standard index.[13] We categorized skin eruptions of ATLL into 6 different types: patch, plaque, multipapular, nodulotumoral, erythrodermic, and purpuric (Figure 1). We defined the criteria for categorizing ATLL-related skin involvement into the patch type as no infiltrated erythema, the plaque type as infiltrated erythema, the multipapular type as multiple papules with diameter less than 1 cm, the nodulotumoral type as nodules or tumors with diameters more than 1 cm, the erythrodermic type as generalized erythema involving 80% or more of the patient's skin, and the purpuric type as red or purple discolorations that did not change with diascopy.

### Statistical analyses

Overall survival (OS) was defined as the time from the date of first diagnosis to the date of death or the latest contact with the patient. Survival curves were drawn using the Kaplan-Meier method and were compared with the log-rank test. *P* values were calculated using the generalized Wilcoxon test. MST was defined as the time point at which the Kaplan-Meier survival curves crossed 50%. Mean survival time was provided when MST could not be calculated. To examine the multiple comparisons of the factors and of the pairs of groups, univariate and multivariate Cox regression analyses were applied to evaluate prognosis factors for survival. The effects of clinical parameters were evaluated as hazard ratios (HRs) and their 95% confidence intervals. All statistical analyses were performed using Dr SPSS II software (SPSS). A $P$ value $< .05$ was considered statistically significant.

## Results

### Patient clinical characteristics

The clinical data of 119 patients with skin eruptions (ratio of male: female = 1.2:1) are summarized in Table 1. The mean age of the patients was 64.0 years (range, 23-91 years; SD, 12.00 years). According to Shimoyama classification, 40 (33.6%) patients were diagnosed with the acute type of ATLL, 6 (5.0%) with the chronic type, 17 (14.3%) with the lymphoma type, and 56 (47.1%) with the smoldering type. Twenty-three patients had complications at the time of diagnosis, including 7 patients with diabetes mellitus, 10 with hypertension, 3 with stroke, and 9 with opportunistic infections. Blood examination revealed that 36 patients (30.3%) had leukocytosis, 26 (21.9%) had lymphocytosis, and 49 (41.2%) had high LDH levels. Hypercalcemia was found in 70 patients (58.8%).

### Patient skin lesions

We categorized the skin eruptions into the patch, plaque, multipapular, nodulotumoral, erythrodermic, and purpuric types (Figure 1). The most highly incident was the nodulotumoral type in 46 patients (38.7%), followed by the plaque type in 32 patients (26.9%), the multipapular type in 23 patients (19.3%), the patch type in 8 patients (6.7%), the erythrodermic type in 5 patients (4.2%), and the purpuric type in 5 patients (4.2%). Because the categorized skin eruptions of ATLL have similarities to those of MF/SS (with the exception of the multipapular and purpuric types), and because the TNMB classification for MF/SS[16] has been widely used, we attempted to apply the T stage of the TNMB classification to ATLL skin lesions. According to the MF/SS classification,[16] eruptions are classified into: T1 (patch/plaque, less than 10% of body surface), T2 (patch/plaque, more than 10% of body surface area), T3 (nodulotumoral type), and T4 (erythrodermic type). Ninety-one (76.5%) of our 119 patients could be classified using this system: 19 patients (16.0%) belonged to T1, 21 (17.7%) to T2, 46 (38.7%) to T3, and 5 (4.2%) to T4. The remaining 28 patients (23.5%) had multipapular (19.3%) and purpuric (4.2%) types, which are peculiar for ATLL and are not described in the T classification of MF/SS. We also evaluated these 2 types to investigate whether they are comparable with either the T1 or T4 category of the MF/SS classification system.

We examined the frequencies of the clinical subtypes of Shimoyama classification in each of the eruption types and T stages (Table 2). All patients with the erythrodermic type belonged to the acute type, whereas most of the patients with the patch type were grouped into the smoldering subtype. As the T stage advanced, the frequencies of the aggressive types (the acute and lymphoma types) increased, whereas those of the smoldering type decreased.

### Survival by baseline clinical factors

Sixty-nine of our 119 patients died during the observation period, with a median follow-up duration of 3.0 years (range, 30 days-20.3 years). The MSTs of the acute, lymphoma, chronic, and

**Table 1. Survival by baseline clinical factors**

| Factor | No. of evaluated cases | No. of deaths | MST, mos | P |
|---|---|---|---|---|
| Total | 119 | 69 | | |
| **Clinical subtype** | | | | < .001 |
| Acute type | 40 | 30 | 7.7 | |
| Lymphoma type | 17 | 12 | 15.0 | |
| Chronic type | 6 | 5 | 16.6 | |
| Smoldering type | 56 | 22 | 154.0 | |
| **Patient-related factors** | | | | |
| Sex | | | | .956 |
| Male | 66 | 38 | 20.3 | |
| Female | 53 | 31 | 24.9 | |
| Age, y | | | | .702 |
| ≥ 60 | 81 | 46 | 24.5 | |
| < 60 | 38 | 23 | 18.4 | |
| **Complications at diagnosis** | | | | .114 |
| Absent | 96 | 59 | 21.0 | |
| Present | | | | |
| Diabetes mellitus | 7 | 3 | 14.8 | |
| Hypertension | 10 | 4 | 141.4* | |
| Stroke | 3 | 2 | 17.2 | |
| Opportunistic infections | 9 | 4 | 49.3 | |
| **Hematologic factors** | | | | |
| WBC count, × $10^9$/L | | | | < .001 |
| ≥ 12.0 | 36 | 25 | 9.5 | |
| < 12.0 | 83 | 44 | 47.8 | |
| Total lymphocyte count, × $10^9$/L | | | | < .001 |
| ≥ 6.5 | 26 | 20 | 10.4 | |
| < 6.5 | 93 | 49 | 47.8 | |
| **Laboratory factors** | | | | |
| LDH | | | | < .001 |
| ≤ NI | 70 | 37 | 47.9 | |
| > NI | 49 | 32 | 9.5 | |
| Calcium | | | | .420 |
| ≤ NI | 49 | 28 | 27.8 | |
| > NI | 70 | 41 | 18.6 | |
| **Skin lesions** | | | | < .001 |
| Patch type | 8 | 2 | 188.4* | |
| Plaque type | 32 | 9 | 114.9 | |
| Multipapular type | 23 | 12 | 17.3 | |
| Nodulotumoral type | 46 | 38 | 17.3 | |
| Erythrodermic type | 5 | 5 | 3.0 | |
| Purpuric type | 5 | 3 | 4.4 | |
| **T stage** | | | | < .001 |
| T1 | 19 | 3 | 192.6* | |
| T2 | 21 | 8 | 47.9 | |
| T3 | 46 | 38 | 17.3 | |
| T4 | 5 | 5 | 3.0 | |

The cumulative probability of the survival rate was estimated using the Kaplan-Meier method and the *P* value was calculated using the generalized Wilcoxon test.

MST indicates median survival time; and NI, normal index.

*Mean survival time is given because the MST cannot be calculated.

smoldering types were 7.7, 15.0, 16.6, and 154.0 months, respectively (Table 1). Of the 69 fatal cases during the observation, 45 patients died of acute ATLL, 17 of acute crisis from the other subtypes, 5 of other diseases (3 of chronic pulmonary diseases and 2 of acute respiratory disease syndrome [ARDS]), and 2 patients of unknown causes.

The effects of various clinical factors on prognosis in the 119 patients were analyzed using the Kaplan-Meier method (Table 1). There was no statistically significant difference in survival rates between the absence and presence of any complication ($P = .114$), between the ≥ 60 years and < 60 years age groups ($P = .702$), or between males and females ($P = .956$). The survival rate was poor in patients with leukocytosis ($P < .001$), lymphocytosis ($P < .001$), and higher LDH levels ($P < .001$). Blood calcium level did not significantly affect survival in this study.

### Survival and multivariate analyses in each eruption type

The MSTs were different between the types of skin eruptions. In the erythrodermic type, all 5 patients died of the disease with 3.0 months of MST. In the nodulotumoral type, the MST was 17.3 months, and 38 of 46 patients died, 17 of acute ATLL, 16 of acute crisis, 1 of ARDS, 2 of chronic pulmonary disease, and 2 of unknown causes. In the plaque type, the MST was 114.9 months, and 9 of 32 died of the disease. The multipapular type showed the same MST (17.3 months) as the nodulotumoral type, and 9 died of acute ATLL, 1 of acute crisis, 1 of ARDS, and 1 of chronic pulmonary disease. The patch type exhibited a good prognosis, with 188.4 months of mean survival time (the MST was not estimable). The purpuric type was found to have a poor prognosis, with an MST of 4.4 months and 3 of 5 patients dying of the disease.

Kaplan-Meier curves of the OS for each eruption type are shown in Figure 2A. The OS rate of the erythrodermic type was significantly lower than those of the other eruption types ($P < .001$, erythrodermic type vs the nodulotumoral, multipapular, plaque, or patch types). The OS rate of the nodulotumoral type was significantly lower than those of the multipapular, plaque, or patch types ($P = .010$, nodulotumoral type vs multipapular type; $P < .001$, nodulotumoral type vs plaque or patch type). The OS rate of the multipapular type was significantly lower than that of the patch type ($P = .045$). Therefore, the erythrodermic type of ATLL is associated with the poorest prognosis, followed by the nodulotumoral and multipapular types. The patch and plaque types showed better survival rates.

We performed univariate and multivariate analyses of the eruption types in a comparison with Shimoyama classification, sex, age, complications, leukocyte counts, lymphocyte counts, LDH level, and calcium level (Table 3). In the multivariate analysis, the smoldering type proved to be a good prognostic factor. We fixed the HR of the patch type to be 1, and then compared it with those of the other eruption types. In the univariate analysis, the HRs of the other eruption types were significantly higher than that of the patch type. In the multivariate analysis, the HRs of the nodulotumoral and erythrodermic types were significantly higher than that of the patch type. The purpuric type also showed such a tendency; however, this result provided limited power for tests against the other groups. The analysis demonstrated that the eruption type is an independent prognostic factor for ATLL.

### Survival and univariate and multivariate analyses in each T stage

We also performed the univariate and multivariate analyses of T stage and other clinical and laboratory parameters for OS. Of 19 patients in the T1 stage, 3 died of the disease, and the mean survival time (the MST was not estimable) was 192.6 months (Table 1). In the T2 stage, 8 of 21 died of the disease and the MST was 47.9 months. In the T3 stage, the MST was 17.3 months and 38 of 46 patients died: 17 of acute ATLL, 16 of acute crisis, 1 of ARDS, 2 of chronic pulmonary disease, and 2 of unknown etiology. In the T4 stage, 5 patients died of the disease with 3.0 months of MST. The OS of the patients was worse as the T stage became more advanced (Figure 2B). Patients in the T1 stage had the longest OS, followed by patients in the T2-T4 stages ($P = .034$, T1 vs T2; $P < .001$, T1 vs T3 or T4; $P < .001$ T2 vs T3 or T4; and $P < .001$, T3 vs T4).

The multipapular and purpuric types are missing in the T stage of the MF/SS system due to their peculiarity. We therefore compared the OS of

**Table 2. Frequencies of the clinical types of Shimoyama classification in each eruption type and T stage**

| | Acute type | Lymphoma type | Chronic type | Smoldering type | *P* |
|---|---|---|---|---|---|
| **Eruption type** | | | | | .015 |
| Patch type | 0 | 0 | 1 (12.8%) | 7 (87.2%) | |
| Plaque type | 9 (28.1%) | 4 (12.5%) | 0 | 19 (59.4%) | |
| Multipapular type | 10 (43.5%) | 2 (8.7%) | 0 | 11 (47.8%) | |
| Nodulotumoral type | 14 (30.4%) | 10 (21.7%) | 5 (10.9%) | 17 (37.0%) | |
| Erythrodermic type | 5 (100%) | 0 | 0 | 0 | |
| Purpuric type | 2 (40.0%) | 1 (20.0%) | 0 | 2 (40.0%) | |
| **T stage** | | | | | .004 |
| T1 | 2 (10.5%) | 1 (5.3%) | 0 | 16 (84.2%) | |
| T2 | 7 (33.3%) | 3 (14.3%) | 1 (4.8%) | 10 (47.6%) | |
| T3 | 14 (30.4%) | 10 (21.7%) | 5 (10.9%) | 17 (37.0%) | |
| T4 | 5 (100%) | 0 | 0 | 0 | |

these 2 eruption types with those of the T stages. Patients with the multipapular type and T2 had a similar outcome (Figure 2C), and there was no statistical significance ($P = .415$). Patients with the purpuric type had a significantly poorer prognosis than those with T1 ($P = .001$); Figure 2D). The differences in OS between the purpuric type and the other T stages were not statistically significant ($P = .412$, purpuric type vs T2; $P = .257$; purpuric type vs T3; $P = .099$, purpuric type vs T4).

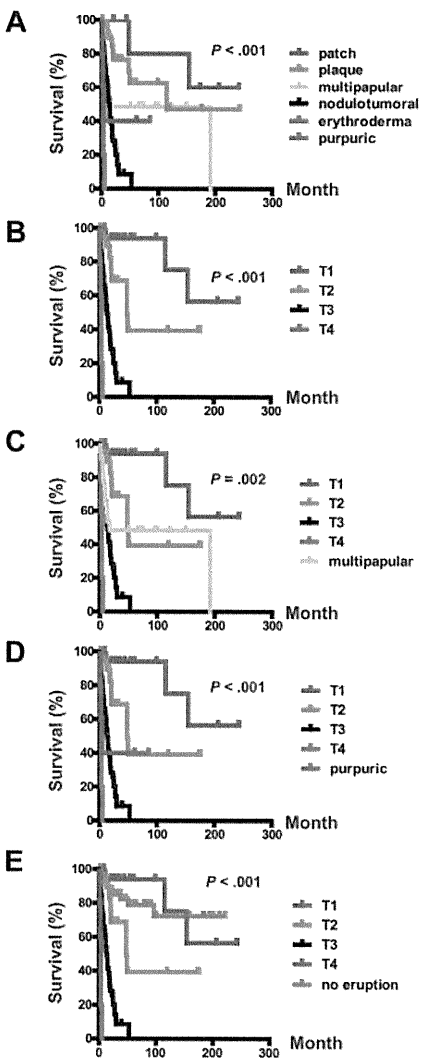


**Figure 2. OS of ATLL patients with skin eruptions.** (A) OS rates of skin eruption types. (B) OS rate of T stage. (C) OS rate of the T stage and the multipapular type. (D) OS rate of the T stage and the purpuric type. (E) OS rate of the T stage and the no-eruption type.

We performed univariate and multivariate analyses of T stage and clinical and laboratory parameters with the HR of T1 set as 1 (Table 3). The univariate analysis revealed that the prognoses of T2, T3, T4, and the multipapular and purpuric types were significantly higher than that of T1. In the multivariate analysis, the HR of T3 and T4 and the multipapular and purpuric types were significantly higher than that of T1.

### Survival and univariate and multivariate analyses in each T stage and in the no-eruption group

We performed univariate and multivariate analyses of T stage by comparing them with the no-eruption group and other clinical and laboratory parameters for OS. Of 51 patients without skin eruptions, 10 died of the disease and the mean survival time (the MST was not estimable) was 66.5 months. When classifying the no-eruption patients into each clinical Shimoyama subtype, 7 patients (13.7%) belonged to the acute type, 5 (9.8%) to the lymphoma type, 12 (23.5%) to the chronic type, and 27 (52.9%) to the smoldering type. The OS of the patients without eruption was better than those at T2-T4 (Figure 2E; no-eruption group vs T2, $P = .033$; no eruption group vs T3 or T4, $P < .001$). There was no statistically significant difference in OS between the no-eruption group and T1.

We performed univariate and multivariate analyses of T stage, including the no-eruption group and clinical and laboratory parameters, by assigning a value of 1 to the HR of T1 (Tables 4 and 5). The univariate and multivariate analyses revealed that the prognoses of T3 and T4 were significantly worse than that of T1.

## Discussion

In the present study, we investigated the association of each type of skin eruption with prognosis in ATLL patients and attempted to apply the T stage of MF/SS classification to the assessment of ATLL skin lesions. We classified ATLL skin eruptions into 6 categories: patch, plaque, multipapular, nodulotumoral, erythrodermic, and purpuric. Table 2 shows that the frequencies of the clinical subtypes of Shimoyama classification were different for each eruption type and T stage. All erythrodermic patients belonged to the acute type, whereas most of patients with the patch type were of the smoldering type. This raised the possibility that prognosis is different among the individual eruption types. Our results revealed the poorest prognosis in the erythrodermic type, followed by the nodulotumoral and multipapular types. The patch and plaque types exhibited better survival rates. Moreover, our multivariate analysis demonstrated that the HRs of the erythrodermic and nodulotumoral

**Table 3. Cox analysis of eruption type and T stage for clinical factors and OS**

| | Univariate | | Multivariate (eruption type and clinical factors) | | Multivariate (T stage and clinical factors) | |
|---|---|---|---|---|---|---|
| **Clinical factor** | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** | **HR (95% CI)** | ***P*** |
| **Clinical subtype** | | | | | | |
| Acute type | 1 | | 1 | | 1 | |
| Lymphoma type | 0.5 (0.1-0.8) | .013 | 0.9 (0.3-2.5) | .852 | 0.9 (0.1-1.3)) | .852 |
| Chronic type | 0.1 (0.3-1.1) | .082 | 0.4 (0.1-1.4) | .167 | 0.4 (0.3-2.4) | .140 |
| Smoldering type | 0.1 (0.1-0.2) | < .001 | 0.2 (0.8-0.6) | .002 | 0.2 (0.1-0.6) | .003 |
| **Patient-related factors** | | | | | | |
| Sex | | | | | | |
| Male | 1 | | 1 | | 1 | |
| Female | 1.0 (0.6-1.6) | .903 | 1.2 (0.7-2.1) | .576 | 1.2 (0.7-2.1) | .576 |
| Age, y | | | | | | |
| < 60 | 1 | | 1 | | 1 | |
| ≥ 60 | 1.0 (0.6-1.7) | .922 | 0.9 (0.5-1.6) | .658 | 0.9 (0.5-1.5) | .578 |
| **Complications at diagnosis** | | | | | | |
| Absent | 1 | | 1 | | 1 | |
| Present | | | | | | |
| Diabetes mellitus | 0.6 (0.2-2.0) | .427 | 0.5 (0.1-1.9) | .314 | 0.5 (0.1-1.8) | .274 |
| Hypertension | 0.4 (0.2-1.2) | .119 | 1.0 (0.3-3.9) | .958 | 1.1 (0.3-4.2) | .905 |
| Stroke | 1.8 (0.4-7.3) | .425 | 3.5 (0.6-19.4) | .161 | 2.9 (0.5-17.3) | .256 |
| Opportunistic infection | 1.0 (0.4-2.6) | .927 | 1.0 (0.3-3.1) | .958 | 1.1 (0.3-3.4) | .938 |
| **Hematologic factors** | | | | | | |
| WBC count, × $10^9$/L | | | | | | |
| < 12.0 | 1 | | 1 | | 1 | |
| ≥ 12.0 | 3.6 (2.1-6.2) | < .001 | 1.8 (0.6-5.2) | .279 | 1.7 (0.6-4.9) | .325 |
| Total lymphocyte count, × $10^9$/L | | | | | | |
| < 6.5 | 1 | | 1 | | 1 | |
| ≥ 6.5 | 3.7 (2.0-6.5) | < .001 | 1.1 (0.5-2.6) | .803 | 1.1 (0.5-2.7) | .768 |
| **Laboratory factors** | | | | | | |
| LDH | | | | | | |
| ≤ NI | 1 | | 1 | | 1 | |
| > NI | 3.0 (1.8-4.9) | < .001 | 1.2 (0.6-2.2) | .581 | 1.2 (0.6-2.2) | .599 |
| Calcium | | | | | | |
| ≤ NI | 1 | | 1 | | 1 | |
| > NI | 1.3 (0.8-2.1) | .381 | 1.0 (0.6-1.8) | .960 | 1.0 (0.6-1.8) | .914 |
| **Eruption type** | | | | | | |
| Patch | 1 | | 1 | | | |
| Plaque | 2.2 (0.5-10.9) | .321 | 1.4 (0.3-8.0) | .680 | | |
| Nodulotumoral | 12.5 (2.7-57.1) | .001 | 8.8 (1.6-48.0) | .012 | | |
| Erythrodermic | 68.4 (11.5-405.9) | < .001 | 21.2 (3.0-150.3) | .002 | | |
| Multipapular | 4.8 (1.0-22.6) | .045 | 3.5 (0.6-20.1) | .159 | | |
| Purpuric | 7.1 (1.1-45.7) | .039 | 6.8 (0.9-53.7) | .071 | | |
| **T stage** | | | | | | |
| T1 | 1 | | | | 1 | |
| T2 | 4.0 (1.0-15.1) | .047 | | | 2.2 (0.5-9.8) | .304 |
| T3 | 15.3 (4.4-52.8) | < .001 | | | 11.3 (2.8-46.0) | .001 |
| T4 | 83.9 (17.8-394.6) | < .001 | | | 27.5 (5.0-151.8) | < .001 |
| Multipapular type | 5.8 (1.6-20.9) | .007 | | | 8.1 (1.4-47.1) | .045 |
| Purpuric type | 8.6 (1.7-44.5) | .010 | | | 8.1 (1.4-47.1) | .020 |

CI indicates confidence interval; and NI, normal index.

types were significantly higher than that of the patch type, and that skin eruption is an independent prognostic factor for ATLL.

**Table 4. Univariate analyses of T stage compared with patients having no skin eruptions**

| | Univariate | |
|---|---|---|
| **Clinical factor** | **HR (95% CI)** | ***P*** |
| **T stage** | | |
| T1 | 1 | |
| T2 | 3.6 (0.9-13.6) | .059 |
| T3 | 16.4 (4.9-55.1) | < .001 |
| T4 | 127.4 (26.2-618.1) | < .001 |
| No eruption | 1.3 (0.3-4.8) | .670 |

It has been reported that the smoldering type of ATLL with skin eruptions, especially those of the nodulotumoral type, has a poorer prognosis than ATTL without skin involvement.[19] Another group of investigators reported that the MSTs of the nodulotumoral and maculopapular types were 26 and 80 months, respectively, which are significantly shorter than those in ATLL without cutaneous eruptions.[23] Our findings are in agreement with these observations, and further clarify the relationship between type of skin lesion and prognosis. Skin-targeted therapy using topical steroids, psoralen photochemotherapy, or narrow-band UVB therapy[19] may improve the prognosis of ATLL for patients with skin eruptions.

The purpuric type of ATLL is one of the rarest skin eruptions of ATLL,[24] and has been reported to occur in 1.6% of ATLL patients with

**Table 5. Cox multivariate analyses of clinical factors and OS compared with patients having no eruption**

| | Multivariate | |
|---|---|---|
| **Clinical factor** | **HR (95% CI)** | ***P*** |
| **Clinical subtype** | | |
| Acute type | 1 | |
| Lymphoma type | 2.4 (0.8-7.2) | .110 |
| Chronic type | 0.3 (0.1-0.9) | .036 |
| Smoldering type | 0.4 (0.1-1.1) | .068 |
| **Patient-related factors** | | |
| Sex | | |
| Male | 1 | |
| Female | 0.8 (0.4-1.4) | .440 |
| Age, y | | |
| < 60 | 1 | |
| ≥ 60 | 0.4 (0.2-0.8) | .012 |
| **Complications at diagnosis** | | |
| Absence | 1 | |
| Presence | | |
| Diabetes mellitus | 1.9 (0.7-4.8) | .188 |
| Hypertension | 0.6 (0.2-2.1) | .443 |
| Stroke | 2.6 (0.6-10.5) | .191 |
| Opportunistic infection | 1.1 (0.2-6.1) | .925 |
| **Hematologic factors** | | |
| WBC count, × $10^9$/L | | |
| < 12.0 | 1 | |
| ≥ 12.0 | 1.1 (0.3-3.5) | .864 |
| Total lymphocyte count, × $10^9$/L | | |
| < 6.5 | 1 | |
| ≥ 6.5 | 2.0 (0.7-5.9) | .197 |
| **Laboratory factors** | | |
| LDH | | |
| ≤ NI | 1 | |
| > NI | 1.2 (0.7-2.1) | .596 |
| Calcium | | |
| ≤ NI | 1 | |
| > NI | 1.1 (0.6-1.9) | .826 |
| **T stage** | | |
| T1 | 1 | |
| T2 | 2.4 (0.6-9.7) | .227 |
| T3 | 13.4 (3.3-53.9) | < .001 |
| T4 | 60.8 (10.1-366.4) | < .001 |
| No eruption | 0.9 (0.2-3.6) | .847 |

CI indicates confidence interval; and NI, normal index.

skin lesions.[19] However, its incidence is higher than was previously thought, because we documented a 4.2% frequency in this study. The production of granzyme B by ATLL cells may lead to the destruction of vessels and the development of purpuric eruptions in these patients.[24] The prognosis for the purpuric type of skin lesion has not been investigated because of the rarity of this type. There have been 9 cases of the purpuric type reported in the literature.[24-31] When these are divided into the punctate and macular subtypes, the prognosis of the punctate purpuric subtype might be better than the macular purpuric subtype.[24-31] In our 5 purpuric cases, 2 cases of the punctuate purpuric subtype survived, with a 73.4-month mean survival time (the MST was not estimable), whereas 3 cases of the macular purpuric subtype died with 2.1 months of the MST. This suggests that the punctuate subtype has a good clinical prognosis, and the poor prognosis of the total purpuric type is derived from the macular subtype.

In addition to the purpuric type, the erythrodermic type is a rare skin manifestation in ATLL patients, with a prevalence of 3.5% reported in a previous study[19] and 4.2% in the present study. The majority of ATLL cases associated with the erythrodermic type of skin lesion are aggressive. In our study, all patients with erythrodermic lesions also belonged to the acute type and had the poorest prognosis among all skin eruption types. In patients with cutaneous T-cell lymphoma (CTCL), the erythrodermic type is typically termed SS and also has a poor prognosis.[16] In some erythrodermic CTCL patients, the decreased expression of intercellular adhesion molecule-1 by keratinocytes may lead to an inability of malignant T cells to enter the epidermis and infiltrate the blood and other organs.[32] This pathomechanism in erythrodermic CTCL can also be applied to ATLL, resulting in poor prognosis. Skin biopsy specimens of the erythrodermic type of ATLL revealed scant tumor cell infiltration into the epidermis.[33,34]

We applied MF/SS classification T stages to ATLL assessment, and demonstrated that the OS was worse as the T stage became more advanced. The results shown in Table 3 indicated that the prognosis of T1 stage was better than that for T2, suggesting that the difference in the body surface area of skin lesions is associated with the prognosis of ATLL. Moreover, the prognosis of T3 patients was poorer than those of T1 and T2, indicating that the depth of tumor-cell infiltration is associated with survival rate. T-stage classification accurately reflects the prognosis of ATLL and MF/SS. However, the multipapular and purpuric types are not applicable to T stage. We found that the multipapular type and T2 had similar outcomes, and that the purpuric type had a significantly poorer prognosis than T1. This may provide clinically useful information for patient management and choice of therapy. Moreover, our present study demonstrated that skin eruption is an independent prognostic factor for ATLL patients: the presence of skin eruptions may indicate poorer outcome compared with no eruptions. Therefore, evaluation of skin lesions and treatments targeting the skin may be important for improving clinical outcome.

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## Authorship

Contribution: Y.S. collected and analyzed the data and wrote the manuscript; R.H. analyzed the data; K.H. collected the data; S.O., H.F., S.Y., S.F., M.T., R.K., M.Y., D.N., K.S., R.Y., T.S., T.M., K.I., M.K., and M.N. diagnosed and treated ATLL patients; and Y.T. organized the study.

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Correspondence: Yu Sawada, MD, Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; e-mail: longago@med.uoeh-u.ac.jp; or Ryosuke Hino, MD, PhD, Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan; e-mail: hinoti@med.uoeh-u.ac.jp.

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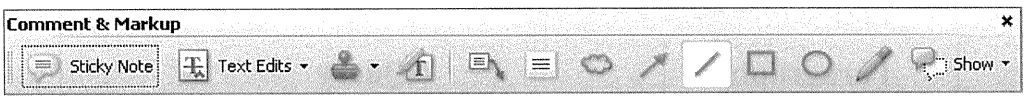


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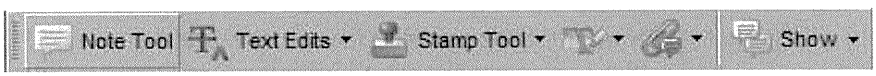


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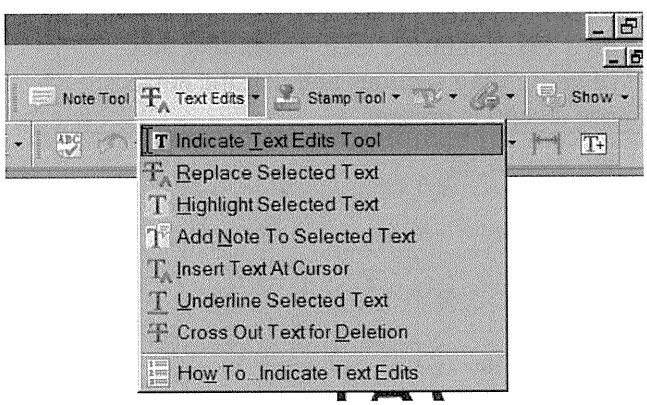


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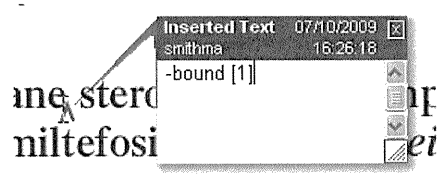


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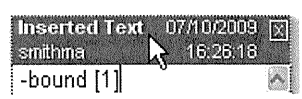


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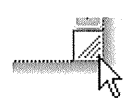

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# Letter to the Editor (case report)

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## Inflammatory cytokine expression in the skin lesions of tumour necrosis factor receptor-associated periodic syndrome

SIR, TNF receptor-associated periodic syndrome (TRAPS) is a rare autosomal-dominant disorder characterized by recurrent episodes of fever, myalgia, abdominal pain, conjunctivitis and skin eruptions, which occur either spontaneously or after minor triggers. This syndrome is associated with missense mutations in *TNFRSF1A*, the gene encoding the 55 kDa TNF receptor [1]. Although the pathogenesis of TRAPS has not been fully elucidated, serum levels of inflammatory cytokines such as TNF-α, IL-6 and IL-1β were elevated in TRAPS, and the patients respond well to the therapies that block these cytokines with respective antibodies [2–4]. These findings suggest that TNF-α, IL-6 or IL-1β may play a crucial role for the development of systemic inflammation in TRAPS patients. Since IL-6 and IL-1β are also known to be essential for the differentiation of Th17 cells producing IL-17 and IL-22 [5], it is an issue whether Th17 cells infiltrate in the skin lesions. Here, we report the expression pattern of inflammatory cytokines, TNF-α, IL-6, IL-1β and IL-17 in the lesional skin of two TRAPS patients, who were reported previously [6, 7].

Case 1 was a 29-year-old Japanese female with a *TNFRSF1A* mutation (N25D) with recurrent episodes of high fever, arthralgia, myalgia, headache and erythema [6]. She had diffuse erythema on her extremities with underlying severe myalgia. Histological examination of an erythematous lesion disclosed a dense inflammatory infiltrate of lymphocytes intermingled with multilobulated cells in the deep dermis and s.c. fat.

Case 2 was a 17-year-old Japanese female with a *TNFRSF1A* mutation (N101K) with recurrent episodes of high fever, arthralgia and erythema [7]. She had diffuse erythema on her cheeks, and confluent maculopapular or mottled erythema on her chest, abdomen and extremities. Histological examination of an erythematous lesion disclosed a mild perivascular inflammatory infiltrate of lymphocytes in the oedematous upper dermis.

We performed immunostaining using deparaffinized 3-μm sections of skin biopsy specimens from the two patients. After quenching non-specific reactions with 0.3% hydrogen peroxide for 15 min, we incubated the sections with anti-TNF-α mAb (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:50 dilution, anti-IL-6 mAb (Rockland Immunochemicals, Gilbertsville, PA, USA) at 1:5000 dilution, anti-IL-1β mAb (Endogen, Woburn, MA, USA) at 1:250 dilution and anti-IL-17 mAb (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100 dilution. A staining kit using biotin-conjugated secondary antibody and peroxidase-conjugated streptavidin in LSAB System HRP (DAKO, Glostrup, Denmark) was applied to the sections sequentially, and positive reaction was visualized with diaminobenzidine.

TNF-α and IL-6-expressing cells were observed in the deep dermis and adipose tissue in Case 1 (Fig. 1A and C), and in the perivascular area of deep dermis in Case 2 (Fig. 1B and D). Intracytoplasmic IL-1β expression was also observed in Case 1 (Fig. 1E), and to a lesser degree in Case 2 (Fig. 1F). However, we could not detect IL-17-expressing cells in Cases 1 and 2 (Fig. 1G and H). These findings suggest that TNF-α, IL-6 and IL-1β may participate in the formation of skin eruptions as well as the systemic inflammation, and IL-17 may not influence the local cutaneous inflammation unlike the above cytokines.

Most of autoinflammatory disorders including TRAPS disclose skin manifestations [8]. Toro *et al.* [9] evaluated the 25 patients with TRAPS and reported that superficial and deep perivascular infiltrates are dominantly composed of lymphocytes and monocytes, suggesting their crucial roles in the pathogenesis of cutaneous lesions of TRAPS. In TRAPS, most of the previous reports focused on the features of circulating monocytes or the serum levels of cytokines; however, local cytokine expression pattern has not been well investigated. Our present findings may indicate that TNF-α, IL-1 and IL-6 are promising therapeutic targets for TRAPS-related cutaneous eruptions, and suggest that etanercept (soluble TNF receptor 2-Fc fusion protein), anakinra (recombinant IL-1 receptor antagonist) and tocilizumab (anti-IL-6 receptor mAb) are potential therapeutic reagents for TRAPS. However, we could not demonstrate the possible efficacy of anti-IL-17 or anti-IL-17 receptor antibodies because two TRAPS patients failed to express IL-17 in the skin.

In another autoinflammatory disorder, cryopyrin-associated periodic syndrome (CAPS), an urticarial rash frequently occurs. The possible pathogenetic roles of mast cells and IL-17-positive cells have been proposed in CAPS on the basis of cutaneous analysis [10]. Our findings suggest that analysis of local cytokine expression in the skin lesions of autoinflammatory disorders may lead to an approach for potential new therapies.

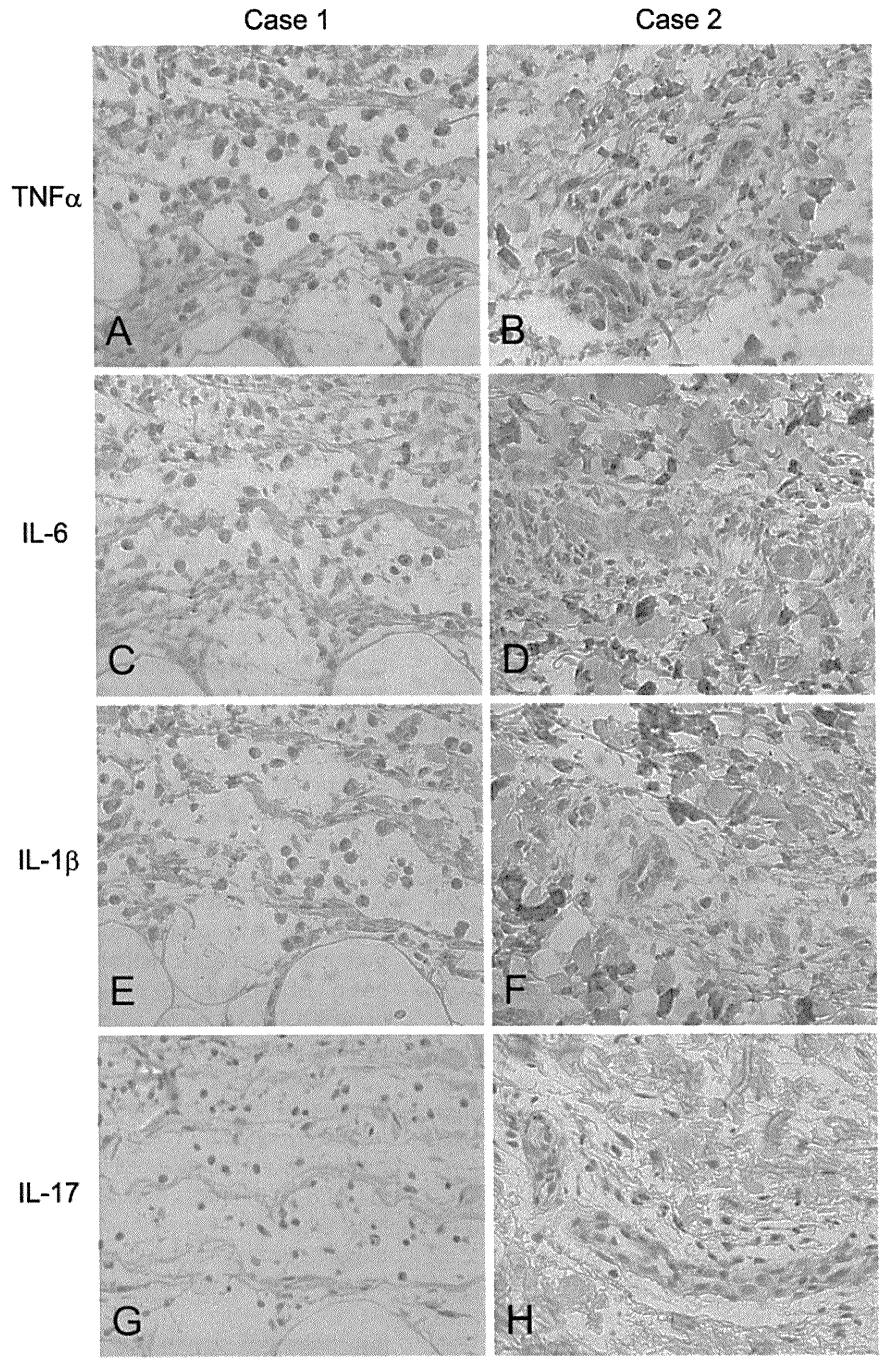
**Rheumatology key message**

- Analysis of cutaneous cytokine expression in TRAPS may lead to an approach for therapeutic targets.

*Disclosure statement*: The authors have declared no conflicts of interest.

LETTER

FIG. 1 Immunohistochemical findings (original magnification, ×400). TNF-α expression in Cases 1 (**A**) and 2 (**B**), IL-6 expression in Cases 1 (**C**) and 2 (**D**), IL-1β expression in Cases 1 (**E**) and 2 (**F**), and lack of IL-17 expression in Cases 1 (**G**) and 2 (**H**).



**Shun Ohmori[1], Ryosuke Hino[1], Miwa Kobayashi[1], Motonobu Nakamura[1] and Yoshiki Tokura[2]**

[1]*Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu and* [2]*Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan.*

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Correspondence to: Shun Ohmori, Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8556, Japan. E-mail: oh-sh@med.uoeh-u.ac.jp

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