Published OnlineFirst May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0292

minutes. Afterwards, the slides were washed with TBS 3 times and incubated with diamino benzidine tetrahydrochloride as substrate and counterstained with hematoxylin (Merk Eurolab). Negative controls without primary antibody were examined simultaneously in each experiment to verify antibody specificity.

To quantify the staining intensity, digitalized specimens were exported to JPG files by NDP view software (Hamamatsu Photonics). The following processes were carried out with Adobe Photoshop CS (J) (Adobe System, Inc.,). Three different areas of the cytoplasm of keratinocytes were selected and expressed as Red channel histograms. In the bar graph, the horizontal and vertical axes represent tone and quantity, respectively. The histogram shows 255 different shades from pitch black (0) to pure white (255), and the number represents the level of brightness of each color. We analyzed the mean intensity of the histogram in the cytoplasm and averaged the value of 3 different areas. To obtain density, we calculated: 255 – "mean" of each color. These values were named as "red density" (RD) and used for further investigation (21).

Statistical analysis

The frequency of Th17 cells and $CD4^+CD25^+$ T cells and the serum concentrations of each cytokine were compared between the ATLL with dermatophytosis patients, ATLL without dermatophytosis patients, dermatophytosis patients without ATLL, and healthy controls. All statistical analyses were carried out using GraphPad Prism 4.0. The Student *t* test was used to calculate statistical differences. All

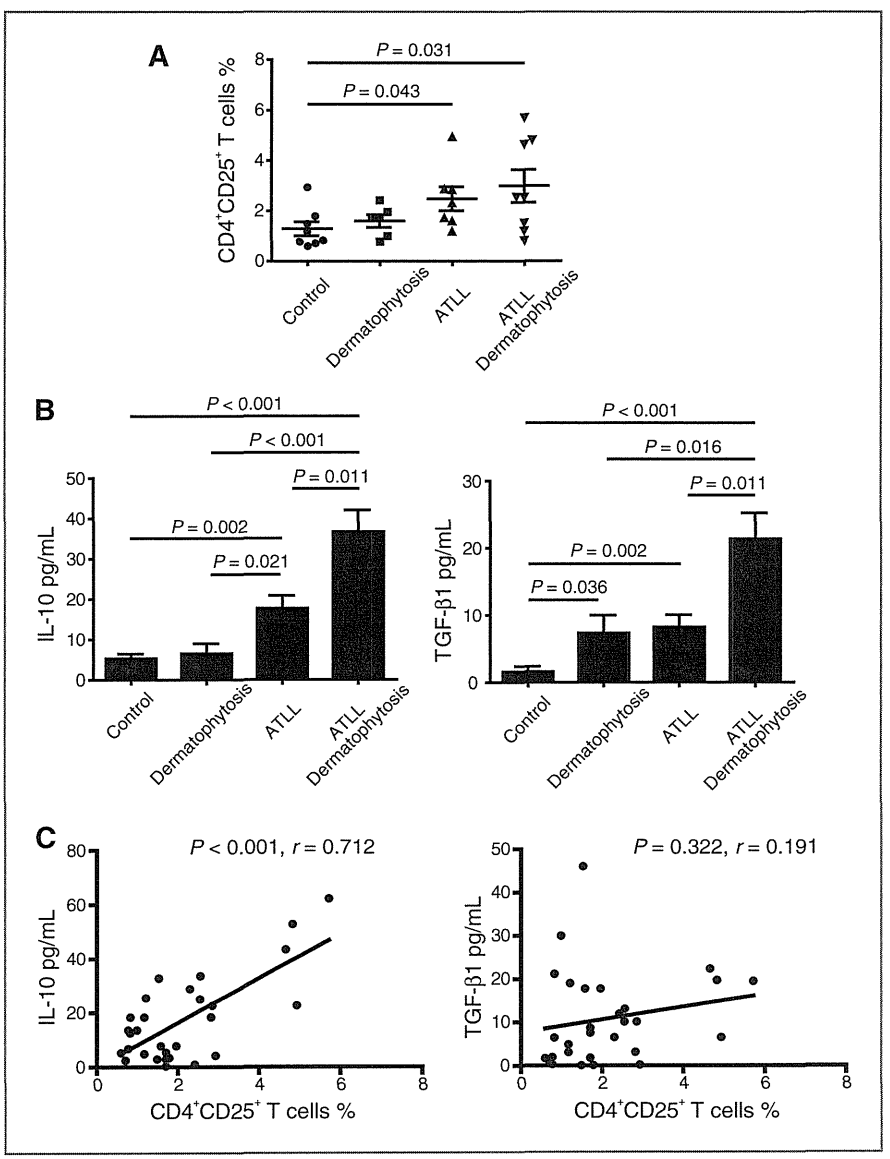


Figure 2. $CD4^+CD25^+$ T-cell frequencies and IL-10 and TGF-β1 levels in peripheral blood. A, the frequencies of $CD4^+CD25^+$ T cells were measured by flow cytometry. B, the serum IL-10 and TGF-β1 levels were measured by ELISA. C, the correlations between the frequency of $CD4^+CD25^+$ T cells and the serum IL-10 (left) and TGF-β1 (right) were analyzed.

Published OnlineFirst May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0292

P values less than 0.05 were considered statistically significant.

Results

Patients' background

The clinical data of all patients are summarized in Table 1. According to the Shimoyama's classification, all ATLL patients belonged to the smoldering type. All dermatophytosis patients with or without ATLL had *tinea pedis et unguium* and/or *tinea corporis*. No dermatophytosis patients regardless of the presence of ATLL had taken any immunosuppressants, oral steroids, or chemotherapy. The χ^2 test and one-way ANOVA revealed that in the individual clinical factors including male/female ratio, age, and counts of lymphocytes, $CD4^+CD25^+$ cells, and $CD4^+CCR4^+$ cells, there were no statistical differences between the groups, except for the percentage of atypical cells and CD4/CD8 ratio.

Absence of dermatophytosis-associated elevations in circulating Th17 cells and serum IL-17 in ATLL patients

Intracelluler cytokine staining of PBMCs revealed that the frequency of circulating Th17 cells of the ATLL patients was significantly lower than that of the healthy controls ($P < 0.001$; Fig. 1A; Supplementary Fig. S1). Whereas the presence of dermatophytosis was associated with elevation of Th17 cell frequency in the non-ATLL patients, the frequency was significantly lower in ATLL patients with dermatophytosis than in those without dermatophytosis ($P = 0.006$). Moreover, the $IL\text{-}17^+$ cell frequencies in the skin biopsies of ATLL with dermatophytosis were lower than those of dermatophytosis (Supplementary Fig. S2A and B). Thus, the results suggested that ATLL patients have a low frequency of circulating Th17 cells, which is further lowered when the patients suffer from dermatophytosis.

In the non-ATLL patients with dermatophytosis, the serum IL-17 level was significantly increased compared with the healthy controls (Fig. 1B). In ATLL patients either with or without dermatophytosis, the IL-17 levels were significantly lower than those in the healthy controls. There was no significant difference in the IL-17 level between ATLL patients with dermatophytosis and those without dermatophytosis. Again, it is suggested that ATLL patients have low levels of IL-17, which cannot be enhanced by the presence of dermatophytosis.

Increased frequency of circulating $CD4^+CD25^+$ T cells and elevated serum IL-10 and TGF-β1 levels in ATLL patients

The number of $CD4^+CD25^+$ T cells reflects both malignant ATLL cells and normal regulatory T cells (Treg) with the former being the majority. The frequencies of circulating $CD4^+CD25^+$ T cells in the ATLL patients, irrespective of having dermatophytosis, were significantly higher than those of the healthy controls and the non-ATLL dermatophytosis patients (Fig. 2A). However, there is no significant difference in the number of $CD25^+$ cells infiltrating in the skin biopsies among the groups (Supplementary Fig. S2C).

In both ATLL patient groups with and without dermatophytosis, the levels of serum IL-10 (Fig. 2B, left) and TGF-β1 (Fig. 2B, right) were significantly increased compared with healthy controls. Moreover, the elevations of the serum IL-10 and TGF-β1 levels were significantly more prominent in the ATLL group with dermatophytosis than in that without dermatophytosis. In the non-ATLL dermatophytosis patients, the serum TGF-β1 level was significantly higher than that of the healthy controls. When the correlations between the frequency of $CD4^+CD25^+$ T cells and the serum IL-10 or TGF-β1 levels were analyzed, the frequency of $CD4^+CD25^+$ T cells correlated with the serum level of IL-10 (Fig. 2C, left) but not TGF-β1 level (Fig. 2C, right). These data suggested that, in association with the increase of $CD4^+CD25^+$ T cell number, regulatory cytokine IL-10 and TGF-β1 are elevated in ATLL patients. It is thought that dermatophytosis is associated more prominently with the increased levels of IL-10 and TGF-β1 in ATLL patients and non-ATLL individuals, respectively.

The correlation between these regulatory cytokines and Th17/IL-17 was also analyzed. The percentage of circulating Th17 cells (Fig. 3, top left) and the serum IL-17 level (Fig. 3, top right) correlated inversely with the serum IL-10 level. On the other hand, there was no significant correlation between TGF-β1 and Th17 cells (Fig. 3, bottom left) or IL-17 (Fig. 3, bottom right), suggesting that IL-10 exerts a more opposite effect on Th17 cells.

No induction of antimicrobial peptides in ATLL patients with dermatophytosis in association with IL-17 reduction

We carried out immunohistochemical staining for HBDs and LL-37 in the dermatophyte-infected and noninfected skin samples of ATLL and non-ATLL individuals. Skin

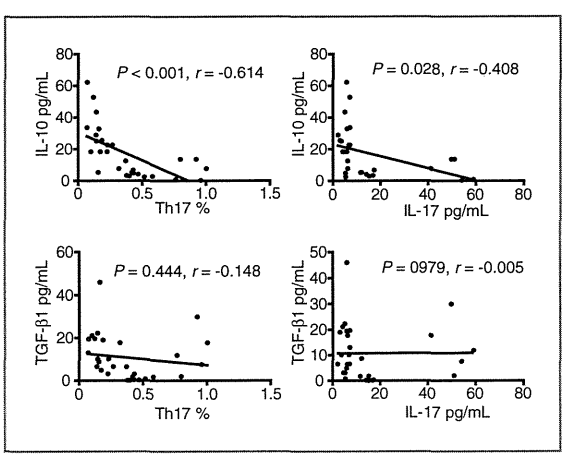


Figure 3. Correlations between $CD4^+CD25^+$ T-cell frequency or serum IL-17 level and serum IL-10 or TGF-β1 level. The data from ATLL patients in Fig. 2 were analyzed.

Published OnlineFirst May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0292

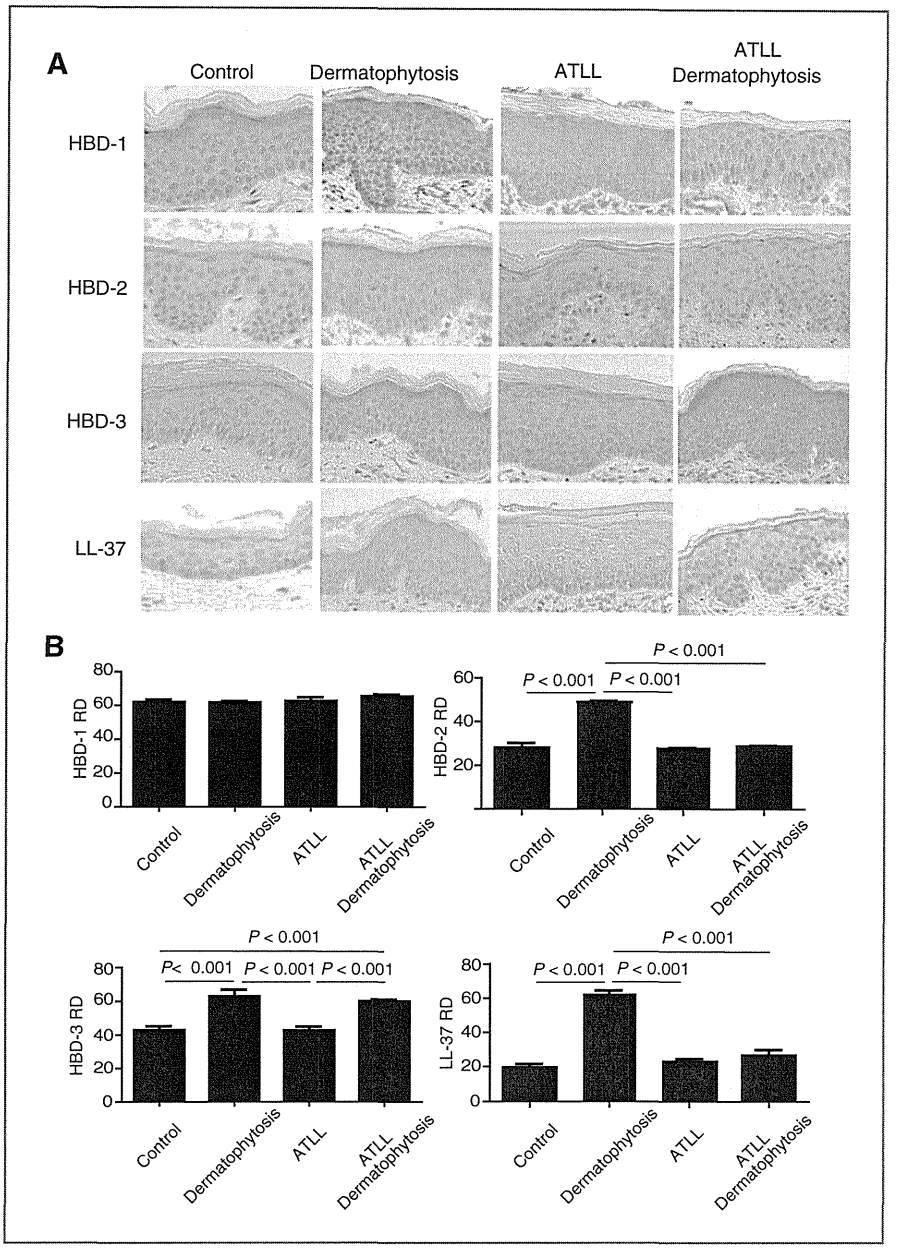


Figure 4. Immunohistochemical staining for HBDs and LL-37. A, representative immunohistochemical staining patterns of HBD-1, HBD-2, HBD-3, and LL-37 in the 4 groups are shown. B, the staining intensities of HBD-1, 2, 3, and LL-37 were expressed as RD as reported previously (21). Bars represent the mean + SD.

biopsy specimens were taken from dermatophytosis lesions of ATLL patients ($n = 8$) and non-ATLL patients ($n = 6$) and from dermatophyte-unaffected sites of ATLL patients ($n = 7$) and non-ATLL patients ($n = 8$). A representative photograph is shown in Fig. 4A, and the mean expression levels in all specimens are in Fig. 4B. In the non-ATLL dermatophytosis lesions, HBD-2 and LL-37, which are IL-17–inducible antimicrobial peptides, were highly expressed in the epidermis. However, in the ATLL patients, these antimicrobial peptides were not induced even in the dermatophytosis lesions. On the other hand, HBD-3 was induced by dermatophyte infection in both non-ATLL and ATLL patients. The HBD-1 expression was unchanged in all groups.

The staining intensity of the antimicropeptides was analyzed as described (21). The relationships between the keratinocyte expression of antimicrobial peptides and the serum IL-17 level were examined. The levels of HBD-2 (Fig. 5, top right) and LL-37 (Fig. 5, bottom right) correlated significantly with the serum IL-17 level. On the other hand, neither HBD-1 (Fig. 5, top left) nor HBD-3 (Fig. 5, bottom left) showed a correlation with the IL-17 level. These results are in agreement with the known notion that the expression

Published OnlineFirst May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0292

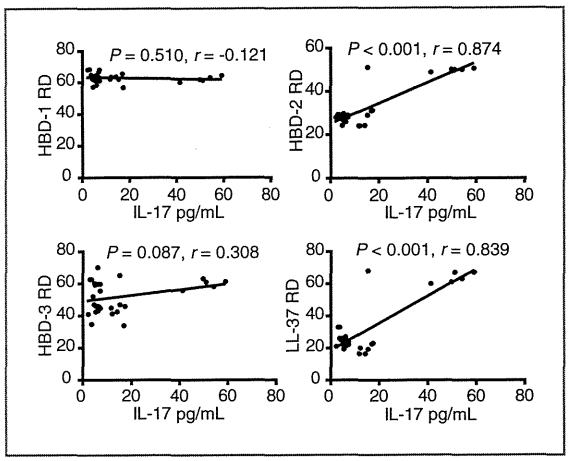


Figure 5. Correlations between serum IL-17 level and serum IL-10 or TGF-β1 level. The data from ATLL patients in Fig. 4 were analyzed.

of HBD-1 is constitutional, and those of HBD-2 and LL-37, but not HBD-3, are promoted by IL-17 (14, 15). It is thus suggested that HBD-2 and LL-37 can be induced by IL-17 only in healthy subjects, and IL-17 might play important role in the epidermal innate immunity against dermatophytes.

Discussion

Patients with ATLL often suffer from various infections, such as pneumocystis carinii, pathogenic fungi, viruses, and parasites, and these infections occasionally result in death (22). They also often exhibit intractable superficial dermatophytosis, and its pathomechanism has been an issue to be clarified. Th17 cell–derived cytokines stimulate keratinocytes to produce antimicrobial peptides (14, 15), and ATLL malignant T cells are assumed to reduce the number and/or function of Th17 cells. Therefore, we addressed the relationship between the abnormality of Th17 cells and the susceptibility to dermatophytosis in ATLL patients. As the circulating $CD4^+CD25^+$ T-cell frequency and the serum IL-10 level were increased, Th17 cells and IL-17 were decreased in ATLL patients, especially when they had dermatophytosis. Therefore, ATLL cells are considered to play a suppressive role for Th17 cells. Both *in vivo* and *in vitro* studies have shown that cellular immune responses are markedly impaired in ATLL patients (23), and ATLL cells secrete various immunosuppressive cytokines such as IL-10 and TGF-β1. In particular, IL-10 produced by ATLL cells suppresses the secretion of Th17-mediated cytokines (24). Although the source of IL-10 is not definitive yet, ATLL cells as well as other Treg or Th2 cells residing in the blood might be activated to produce IL-10 in the patients. Thus, it seems that ATLL cells lead to defective epidermal innate immunity and resultant dermatophytosis by suppressing Th17 cells.

Consistently, we found that the production of antimicrobial peptides by epidermal keratinocytes was depressed in ATLL patients, which presumably attenuated the innate immunity of skin surface environment. IL-17 enhances various antimicrobial peptides such as HBD-2 and LL-37 in keratinocytes (14, 15), but neither hBD-1 nor hBD-3 (25). HBD-2 is active against fungi and effective for *tinea corporis* (26), and LL-37, one of the peptide forms of human cathelicidin, has an activity against fungi (27). Defective IL-17 production has been observed in several fungal infectious disorders such as recurrent vulvovaginal candidiasis, onychomycosis, and chronic mucocutaneous candidiasis (28, 29). Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy had severely reduced IL-17 responses to *Candida albicans* antigens, and it was strongly associated with neutralizing autoantibodies to IL-17 (29). These findings imply that Th17-mediated antimicrobial peptides play an essential role in innate immunity of the skin.

Our study on the ATLL immune condition showed that Th17 cells are deeply involved in the mechanism underlying the keratinocyte production of dermatophyte-eliminating antimicrobial peptides. Other infectious conditions might also be associated with the reduced number of Th17 cells in ATLL patents.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y. Sawada, M. Nakamura, R. Kabashima-Kubo, M. Kobayashi, Y. Tokura
Development of methodology: Y. Sawada, M. Nakamura, R. Kabashima-Kubo, Y. Tokura
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Sawada, M. Nakamura, R. Kabashima-Kubo
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Sawada, M. Nakamura, R. Kabashima-Kubo, Y. Tokura
Writing, review, and/or revision of the manuscript: Y. Sawada, M. Nakamura, Y. Tokura
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Nakamura, Y. Tokura
Study supervision: M. Nakamura, T. Shimauchi, Y. Tokura

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 25, 2012; revised April 15, 2012; accepted May 17, 2012; published OnlineFirst May 30, 2012.

References

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. Blood 1977;50:481–92.
2. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. Proc Natl Acad Sci U S A 1982;79:2031–5.
3. Tsukasaki K, Hermine O, Bazarbachi A, Ratner L, Ramos JC, Harrington W Jr, et al. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a

Published OnlineFirst May 30, 2012; DOI:10.1158/1078-0432.CCR-12-0292

proposal from an international consensus meeting. J Clin Oncol 2009;27:453–9.
4. Iwanaga M, Chiyoda S, Kusaba E, Kamihira S. Trends in the seroprevalence of HTLV-1 in Japanese blood donors in Nagasaki Prefecture, 2000–2006. Int J Hematol 2009;90:186–90.
5. Tajima K. The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. Int J Cancer 1990;45:237–43.
6. Levine PH, Blattner WA, Clark J, Tarone R, Maloney EM, Murphy EM, et al. Geographic distribution of HTLV-I and identification of a new high-risk population. Int J Cancer 1988;42:7–12.
7. Fleming AF, Maharajan R, Abraham M, Kulkami AG, Bhusnurmath SR, Okpara RA, et al. Antibodies to HTLV-I in Nigerian blood-donors, their relatives and patients with leukaemias, lymphomas and other diseases. Int J Cancer 1986;38:809–13.
8. Shimoyama M. Diagnosis criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma: a report from the Lymphoma Study Group. Br J Hematol 1991;79:426–37.
9. Johno M, Kojo Y, Ohishi M. ATLL and eruption. Practical Dermatology 1987;9:206–10. (in Japanese)
10. Inoue S, Tajiri A, Ogata K, Kuroki Y. ATLL with skin eruption. Biomedicine & Therapeutics 1989;22:174–8. (in Japanese)
11. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345–52.
12. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003;52:65–70.
13. Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol 2008;128:2625–30.
14. Eyerich K, Pennino D, Scarponi C, Foerster S, Nasorri F, Behrendt H, et al. IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response. J Allergy Clin Immunol 2009;123:59–66.
15. Peric M, Koglin S, Kim SM, Morizane S, Besch R, Prinz JC, et al. IL-17A enhances vitamin D3-induced expression of cathelicidin antimicrobial peptide in human keratinocytes. J Immunol 2008;181:8504–12.
16. Shimauchi T, Imai S, Hino R, Tokura Y. Production of thymus and activation-regulated chemokine and macrophage-derived chemokine by CCR4+ adult T-cell leukemia cells. Clin Cancer Res 2005;11:2427–35.
17. Mori N, Gill PS, Mougdil T, Murakami S, Eto S, Prager D. Interleukin-10 gene expression in adult T-cell leukemia. Blood 1996;88:1035–45.
18. Gu Y, Yang J, Ouyang X, Liu W, Li H, Yang J, et al. Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. Eur J Immunol 2008;38:1807–13.
19. Yamada Y. Phenotypic and function analysis of leukemic cells from 16 patients with adult T-cell leukemia/lymphoma. Blood 1983;61:192–9.
20. Tsukasaki K, Ikeda S, Murata K, Maeda T, Atogami S, Sohda H, et al. Characteristics of chemotherapy-induced clinical remission in long survivors with aggressive adult T-cell leukemia/Lymphoma. Leuk Res 1993;17:157–66.
21. Hino R, Kabashima K, Kato Y, Yagi H, Nakamura M, Honjo T, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010;116:1757–66.
22. Bunn PA Jr, Schechter GP, Jaffe E, Blayney D, Young RC, Matthews MJ, et al. Clinical course of retrovirus associated T-cell lymphoma in the United States: staging evaluation and management. N Eng J Med 1983;309:257–64.
23. Shaw GM, Broder S, Essex M, Gallo RC. Human T-cell leukemia virus: its discovery and role in leukemogenesis and immunosuppression. Adv Intern Med 1984;30:1–27.
24. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. Immunity 2011;34:566–78.
25. Ghannam S, Dejou C, Pedretti N, Giot JP, Dorgham K, Boukhaddaoui H, et al. CCL20 and β-defensin-2 induce arrest of human Th17 cells on inflamed endothelium *in vitro* under flow conditions. J Immunol 2011;186:1411–20.
26. Jensen JM, Pfeiffer S, Akaki T, Schröder JM, Kleine M, Neumann C, et al. Barrier function, epidermal differentiation, and human beta-defensin 2 expression in tinea corporis. J Invest Dermatol 2007;127:1720–7.
27. Zaiou M, Gallo RL. Cathelicidins, essential gene-encoded mammalian antibiotics. J Mol Med 2002;80:549–61.
28. Ferwerda B, Ferwerda G, Plantinga TS, Willment JA, van Spriel AB, Venselaar H, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med 2009;361:1760–7.
29. Kisand K, Bøe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med 2010;207:299–308.

Our findings indicated that conidia and yeast of *S. schenckii* triggered the inflammatory responses characterized by production of cytokine and chemokine (IL-6 and IL-8) and activation of the TLR2, 4 and NF-κB signaling pathways.

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China (No. 30671893) and Jiangsu Province Natural Science Foundation (No. BK2011128) to M. Li.

References

[1] Kauffmann CA. Sporotrichosis. Clin Infect Dis 1999;29:231–6.
[2] Hinshaw M, Longley JB. Fungal diseases. In: Elder ED, Elenitsas R, Johnson BL, Murphy GF, editors. Lever's histopathology of the skin. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 625.
[3] Sassá MF, Saturi AE, Souza LF, Ribeiro LC, Sgarbi DB, Carlos IZ. Response of macrophage Toll-like receptor 4 to a *Sporothrix schenckii* lipid extract during experimental sporotrichosis. Immunology 2009;128:301–9.
[4] Uenotsuchi T, Takeuchi S, Matsuda T, Urabe K, Koga T, Uchi H, et al. Differential induction of Th1-prone immunity by human dendritic cells activated with *Sporothrix schenckii* of cutaneous and visceral origins to determine their different virulence. Int Immunol 2006;18:1637–46.
[5] Li M, Chen Q, Shen YN, Liu WD. Candida albicans phospholipomannan triggers inflammatory responses of human keratinocytes through Toll-like receptor 2. Exp Dermatol 2009;18:603–10.
[6] Kobayashi M, Yoshiki R, Sakabe J, Kabashima Nakamura M, Tokura Y. Expression of toll-like receptor 2, NOD2 and dectin-1 and stimulatory effects of their ligands and histamine in normal human keratinocytes. Br J Dermol 2009;160:297–304.
[7] Kawai K, Shimura H, Minagawa M, Ito A, Tomiyama K, Ito M. Expression of functional Toll-like receptor 2 on human epidermal keratinocytes. J Dermatol Sci 2002;30:185–94.
[8] Lee HM, Shin DM, Choi DK, Lee ZW, Kim KH, Yuk JM, et al. Innate immune responses to *Mycobacterium ulcerans* via toll-like receptors and dectin-1 in human keratinocytes. Cell Microbiol 2009;11:678–92.
[9] Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol 2008;6:67–78.
[10] Carlos IZ, Sgarbi DB, Placeres MC. Host organism defense by a peptide-polysaccharide extracted from the fungus *Sporothrix schenckii*. Mycopathologia 1999;144:9–14.

Min Li[a,1,*], Qing Chen[b,1], Jun Sun[b], Yongnian Shen[a], Weida Liu[a]
[a]*Department of Medical Mycology, China Medical Fungi Culture Collection Center, Institute of Dermatology, Chinese Academy of Medical Science & Peking Union Medical College, Nanjing 210042, PR China*;
[b]*Jiangsu Province Blood Center, Nanjing 210042, PR China*

*Corresponding author at: Department of Medical Mycology, China Medical Fungi Culture Collection Center, Institute of Dermatology, Chinese Academy of Medical Science & Peking Union Medical College, 12 Jiangwangmiao St., Nanjing 210042, PR China.

Tel.: +86 25 8547 8983
E-mail address: minli08@gmail.com (M. Li)

[1]These two authors contributed equally to this study.

2 October 2011

doi:10.1016/j.jdermsci.2012.01.003

Letter to the Editor

Heparin serves as a natural stimulant of the inflammasome and exacerbates the symptoms of tumor necrosis factor receptor-associated periodic syndrome (TRAPS)

Keywords:
Tumor necrosis factor receptor-associated periodic syndrome (TRAPS); Heparin; Inflammasome

To the editor

Tumor necrosis factor 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 spontaneously or after some triggers. This syndrome is associated with missense mutations in *TNFRSF1A*, the gene encoding the 55 kDa type 1 tumor necrosis factor receptor (TNFR1) [1]. TRAPS-associated mutant TNFR1 accumulates intracellularly and does not act as a conventional TNF receptor [2,3]. Although the pathogenesis of TRAPS has not been fully elucidated, the accumulation of mutant TNFR1 causes spontaneous mitogen-activated protein kinase (MAPK) activation [3] or nuclear factor κB (NF-κB) activation [4,5]. In TRAPS patients, therefore, even minor triggers that stimulate innate immune system can lead to inflammatory cytokine production, such as interleukin-1β (IL-1β), IL-6 or TNFα [3].

It is now generally accepted that activation and release of IL-1β requires the two distinct signals [6,7]. In the first signal, stimulation of innate immune cells with toll-like receptor agonists, such as lipopolysaccharide (LPS) induces the synthesis of pro-IL-1β through NF-κB activation. Subsequently, other reagents, such as adenosine triphosphate (ATP), trigger the second signal and stimulate cytosolic multiprotein complex inflammasome, and inactive pro-caspase-1 is converted into the active caspase-1 that cleaves IL-1β.

Heparin, a highly sulfated glycosaminoglycan, is widely used as an anticoagulant. Although several lines of evidence have shown that the other glycosaminoglycans, such as hyaluronan or biglycan stimulate the inflammasome, it still remains to be known whether heparin also serves as an activator of the inflammasome [8,9]. Here, we describe a TRAPS patient exacerbated by a heparin injection and provide possibility that heparin serves as a natural stimulant of the inflammasome.

The patient was a 16-year-old Japanese woman who was referred to us with a one-year history of episodic fever, myalgia, abdominal pain, dyspnea and localized edema. Laboratory examination showed no remarkable abnormalities including white blood cell counts, C-reactive protein or any of autoantibodies even upon an attack. Her mother and grandfather also had histories of the similar episodic inflammation. We suspected that she had hereditary periodic fever syndrome and performed genomic DNA analysis after obtaining informed consent. We searched for a mutation in the *TNFRSF1A* gene by amplifying the coding exons and flanking regions of the *TNFRSF1A* gene with polymerase chain reaction (PCR) using the specific primers. PCR products were purified and directly sequenced. Genetic evaluation revealed a missense mutation (T61I) in exon 3 of *TNFRSF1A* gene in the patient, her mother and grandfather, leading to the diagnosis of TRAPS. Since her recurrent symptoms could not be successfully

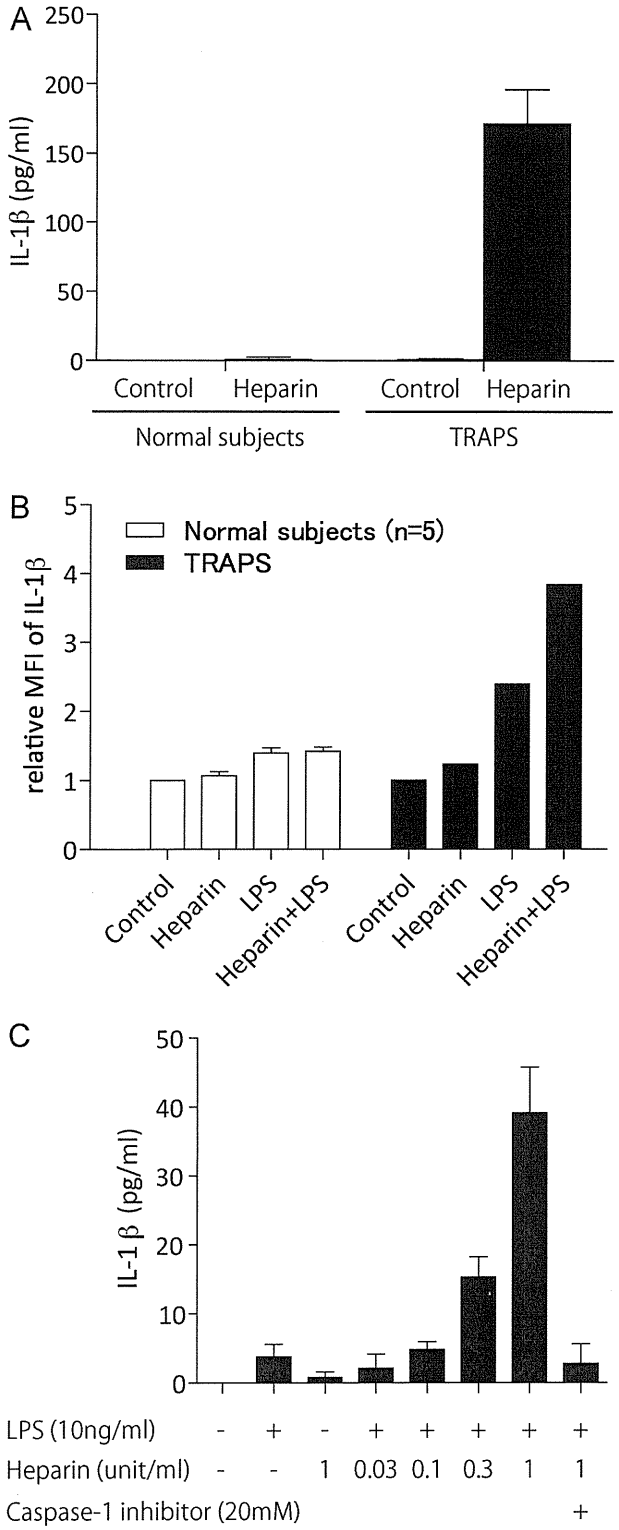


Fig. 1. IL-1β production stimulated by heparin in PBMCs from a TRAPS patient and in THP-1 cells. (A) Peripheral blood mononuclear cells (PBMCs) from TRAPS patient and normal subjects ($n = 5$) were incubated with heparin (1 unit/ml) for 18 h, and IL-1β concentration in the supernatants was measured by enzyme-linked immunosorbent assay (ELISA). (B) PBMCs were incubated with heparin (1 unit/ml), LPS (10 ng/ml), or combination of heparin and LPS, and intracellular cytokine staining of IL-1β was performed. Relative mean fluorescent intensity (MFI) of IL-1β in $CD14^+$ monocytes was examined by flow cytometry. (C) THP-1 cells were incubated with LPS (10 ng/ml) and varying doses of heparin (0.03, 0.1, 0.3, 1.0 unit/ml) in the presence or absence of caspase-1 inhibitor (20 mM), and IL-1β concentration in the supernatants was measured by ELISA. Values are means ± SD.

resolved with oral prednisolone (20 mg/day), anti-TNF antibody, infliximab, was administered and her periodic inflammation entirely subsided.

Interestingly, she had an episode of systemic inflammation including fever, myalgia, abdominal pain and dyspnea when injected with even a small amount of heparin for venous lock. Furthermore, topical skin application of heparinoid ointment evoked her painful erythema. These symptoms urged us to investigate whether heparin is one of the stimulants for the inflammasome, because, in TRAPS patient, NF-κB might be activated by the abnormal TNFR1 signaling resulting in the accumulation of cytoplasmic pro-IL-1β.

To decipher the hyperresponse to heparin in TRAPS patient, we focused on IL-1β production. After informed consent was obtained, peripheral blood mononuclear cells (PBMCs) from the patient and normal subjects were incubated for 18 h with heparin at 1 unit/ml. IL-1β concentration in the supernatants was measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D System, Minneapolis, MN, USA). Heparin augmented the IL-1β concentration in the patient's PBMCs, but not in normal PBMCs (Fig. 1A).

We also performed intracellular cytokine staining and investigated IL-1β synthesis by flow cytometry. PBMCs were incubated with heparin (1 unit/ml), LPS (10 ng/ml), or combination of heparin and LPS. Relative mean fluorescent intensity of IL-1β in $CD14^+$ monocytes was elevated by adding heparin and LPS or LPS alone in the TRAPS patient, but not in healthy controls (Fig. 1B). The additive effects of LPS and heparin raised a possibility that LPS induces pro-IL-1β production and heparin activates inflammasome in TRAPS.

To determine whether IL-1β secretion by heparin was dependent on the inflammasome, we evaluated IL-1β production in the presence or absence of caspase-1 inhibitor. THP-1 cells, a human monocytic cell line, were incubated with LPS (10 ng/ml) and varying doses of heparin (0.03, 0.1, 0.3, 1.0 unit/ml), and IL-1β concentration in the supernatants was measured by ELISA. Heparin stimulated IL-1β secretion in a dose dependent manner in concert with LPS, and the production of IL-1β was inhibited by the administration of caspase-1 inhibitor (Fig. 1C). These results indicate a capability of heparin for the natural stimulants to activate the inflammasome.

TRAPS is a systemic autoinflammatory disease associated with enhanced innate immune responsiveness and abnormal intracellular trafficking of TNFR1 owing to missense mutations in *TNFRSF1A*. Recently, Simon et al. showed that the enhanced inflammation in TRAPS was linked to increased activation of the MAPKs p38 and JUN N-terminal kinase (JNK), and that PBMCs from TRAPS patients overproduce inflammatory cytokine in response to low-dose LPS [3]. Other investigators reported that different mutations in *TNFRSF1A* stimulated distinct NF-κB subunits, such as p65 or c-Rel, and this enhanced varying cytokine secretion profiles [5]. In this study, we demonstrated that heparin exacerbated inflammatory symptoms and stimulated monocytes to produce IL-1β in T61I TRAPS patient. These results fit with the clinical features of TRAPS, in which slight stimuli can provoke clinical episodes of inflammation, and the clinical manifestations differ between individual cases. In summary, we consider that heparin is one of the natural stimulants of the inflammasome. It is possible that other inflammasome activators act as triggers of inflammatory attacks in TRAPS patients.

Financial support

None.

References

[1] McDermott MF, Assentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell 1999;97:133–44.
[2] Lobito AA, Kimberley FC, Muppidi JR, Komarow H, Jackson AJ, Hull KM, et al. Abnormal disulfide-linked oligomerization results in ER retention and altered signaling by TNFR1 mutants in TNFR1-associated periodic fever syndrome (TRAPS). Blood 2006;108:1320–7.
[3] Simon A, Park H, Maddipati R, Lobito AA, Bulua AC, Jackson AJ, et al. Concerted action of wild-type and mutant TNF receptors enhances inflammation in TNF receptor 1-associated periodic syndrome. Proc Natl Acad Sci USA 2010; 107:9801–6.
[4] Nedjai B, Hitman GA, Yousaf N, Chernajovsky Y, Stjernberg S, Pettersson T, et al. Abnormal tumor necrosis factor receptor 1 cell surface expression and NF-κB activation in tumor necrosis factor receptor-associated periodic syndrome. Arthritis Rheum 2008;58:273–83.
[5] Nedjai B, Hitman GA, Church LD, Minden K, Whiteford ML, McKee S, et al. Differential cytokine secretion results from p65 and c-Rel NF-κB subunit signaling in peripheral blood mononuclear cells of TNF receptor-associated periodic syndrome patients. Cell Immunol 2011;268:55–9.
[6] Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat Rev Immunol 2007;7:31–40.
[7] Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821–32.
[8] Yamasaki K, Muto J, Taylor KR, Cogen AL, Audish D, Bertin J, et al. NLRP3/cryopyrin is necessary for interleukin-1β (IL-1β) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury. J Biol Chem 2009;284:12762–71.
[9] Babelova A, Moreth K, Tsalastra-Greul W, Zeng-Brouwers J, Eickelberg O, Young MF, et al. Biglycan, a danger signal that activates the NLRP3 inflammasome via toll-like and P2X receptors. J Biol Chem 2009;284:24035–48.

Shun Ohmori[a,*], Ryosuke Hino[a], Motonobu Nakamura[a], Yoshiki Tokura[b]
[a]*Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan*
[b]*Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan*

*Corresponding author at: Department of Dermatology, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8556, Japan.

Tel.: +81 93 691 7445; fax: +81 93 691 0907
E-mail address: oh-sh@med.uoeh-u.ac.jp (S. Ohmori)

23 August 2011
9 November 2011
10 November 2011

doi:10.1016/j.jdermsci.2011.11.006

Acta Derm Venereol 2013; 93: 181–215

LETTERS TO THE EDITOR

Clearance Efficacy of Autoantibodies in Double Filtration Plasmapheresis for Pemphigus Foliaceus

Akira Kasuya, Mutsumi Moriki, Kazuki Tatsuno, Satoshi Hirakawa and Yoshiki Tokura

Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-Ku, Hamamatsu 431-3192, Japan. E-mail: casuaki@hama-med.ac.jp

Accepted May 22, 2012; Epub ahead of print Sep 5, 2012

Plasma exchange is a therapeutic option in severe cases of pemphigus. Both centrifugal plasmapheresis and double filtration plasmapheresis (DFPP) are available, but the latter, newer, procedure currently prevails because of its safety advantage (1, 2). In DFPP, immunoglobulins (Igs) are selectively removed, while minimizing the loss of albumin (3). In several studies, the removal rates (RRs) of anti-desmoglein (Dsg) 1 and Dsg3 autoantibodies have been estimated by using serum antibody titres immediately before and after plasmapheresis (3). This simple estimation is designated RR1 in the present study. Given the data for anti-Dsg titres and Ig amounts in the exchanged effluents, however, we can calculate the corrected or compensated RR, named RR2 in this study, which reflects the elimination efficacy of antibodies more accurately than RR1. RR2 of pemphigus autoantibodies has been reported only in centrifugal plasmapheresis (4), but not in DFPP. This study examined RR2 of anti-Dsg1 autoantibody with reference to total immunoglobulins (Igs) in 4 cycles of DFPP performed in 2 patients with pemphigus foliaceus (PF).

PATIENTS AND METHODS

Two patients with PF were enrolled in this study.

Case 1. Case 1 was an 85-year-old Japanese man with erosions on his trunk and extremities. There was no involvement of the oral mucous membrane. He had had chronic myelomonocytic leukaemia for 6 months without treatment. Immediately before the start of DFPP, the enzyme-linked immunosorbent assay (ELISA) titres for Dsg1 and Dsg3 were 2,031 and < 5 indices, respectively. Following administration of prednisolone (60 mg daily) and mizoribine (100 mg daily), DFPP was implemented twice in total on 2 consecutive days. Immediately after the second DFPP, anti-Dsg1 antibody was decreased dramatically to 220 index, with therapeutic success. However, the patient had severe sepsis, with serum IgG lowered from 1,320 to 407 mg/dl. Blood culture and culture of the tip of the central venous catheter was positive for *Staphylococcus aureus*. Serum albumin and fibrinogen levels were decreased from 3.5 to 2.5 g/dl, and 87 to >50 mg/dl, respectively. Over a period of 2 months after DFPP, prednisolone was tapered to 30 mg/day without clinical recurrence.

Case 2. Case 2 was a 48-year-old Japanese man who had had PF for 2 years, which was controlled with oral prednisolone (20–60 mg daily) plus cyclosporine (150 mg daily). However, flaccid vesicles and erosions continued to recur. Because of the adverse effects of corticosteroids (pulmonary embolism and avascular necrosis of the femoral bone), and of intravenous high-dose Ig (thrombocytopaenia, platelet count <50,000 μl), 2 courses of DFPP were implemented on 2 consecutive days. The ELISA titre for anti-Dsg1 autoantibody was decreased from 88 to 18 and anti-Dsg3 antibody to < 5, with therapeutic success. Serum albumin and fibrinogen levels were decreased from 4.0 to 3.7 g/dl, and 274 to 220 mg/dl, respectively. For 3 months after DFPP, no recurrence of symptoms was observed with prednisolone (20 mg/day) plus cyclosporine (150 mg daily).

For DFPP, an apheresis device (Plasauto iQ21) was used, with a primary membrane (Plasmaflo OP-05W) and secondary membrane (Cascadeflo EC-20W), all of which were from Asahi Kasei Kuraray Medical Co. Ltd, Tokyo, Japan. The serum levels of anti-Dsg1, anti-Dsg3, IgG, IgA, and IgM were monitored just before and immediately after each DFPP treatment. The exchanged effluents were also subjected to measurement of anti-Dsg1 antibodies and Igs. RR was calculated in two ways, as reported previously (4):

RR1 = {(Index[pre]–Index[post])}/Index[pre] × 100 (%), where Index[pre] and Index[post] are the ELISA index values before and after a cycle of plasmapheresis, respectively.

RR2 = {Index[e]×V[e]}/{Index[pre]×V[body]}×100 (%), where V[body] = V[plasma]/0.45 (L), V[plasma] = V[blood] × (1–hematocrit), and V[blood] = weight [kg]/13.

Note that Index[e] and V[e] are the ELISA index value and the volume of effluent, respectively, and V[body] is the total volume of body fluid containing autoantibodies and Igs.

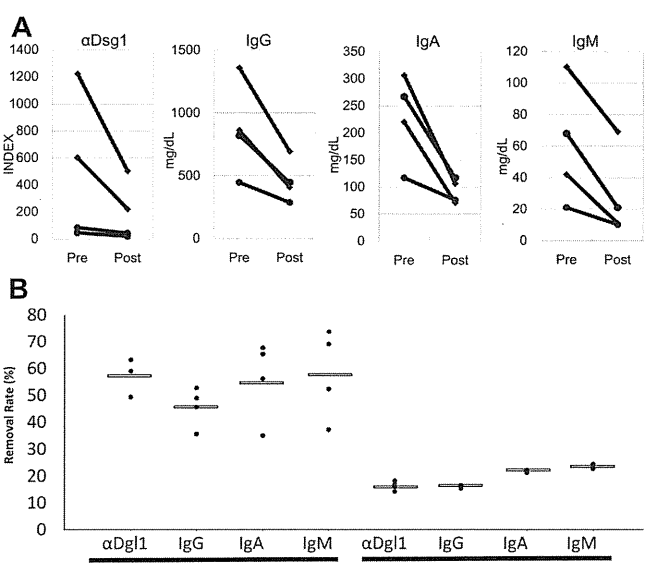


Fig. 1. Serum levels of anti-desmoglein (Dsg) 1 antibody and each class of immunoglobulins, and their removal rates by double filtration plasmapheresis (DFPP). (A) Serum levels of Dsg1 antibody, immunoglobulin (Ig)G, IgA and IgM were measured before (Pre) and after (Post) each cycle of plasmapheresis. (B) Removal rates (RRs) of anti-Dsg1 antibody, IgG, IgA and IgM. αDsg1: anti-Dsg1 antibody. Open bars indicate mean removal rates.

V[plasma] and V[blood] are the volumes of plasma and blood, respectively. V[body] was estimated from V[plasma], based on the fact that approximately 45% of the total body pool of Igs resides in the intravascular space (4). V[blood] was calculated based on the fact that 7.7% of body weight is approximately equal to V[blood].

RESULTS

All serum levels of anti-Dsg1 antibody and Igs were decreased after DFPP (Fig. 1A). RR1, representing the percentage of Igs eliminated from sera, and RR2, representing the corrected value of RR in consideration of the amount of removed autoantibodies in the effluents, were calculated. In the 4 cycles of DFPP, the mean values of RR1 and RR2 of anti-Dsg1 antibody were 57.7% and 16.4%, respectively (Fig. 1B). The RR2 of IgG tended to be lower than that of IgA or IgM, suggesting that IgA and IgM were eliminated more efficiently than IgG. The removal of anti-Dsg1 antibody was comparable to that of IgG. The total volume of plasma desorbed by each treatment was 0.51 and 0.55 l in Case 1 and 0.81 and 0.83 l in Case 2, respectively.

DISCUSSION

The merits of DFPP, including avoidance of albumin loss, and no need for fresh frozen plasma, have encouraged us to choose DFPP over centrifugal plasmapheresis. However, the clearance efficacy of DFPP has not been evaluated in a comparison with centrifugal plasmapheresis. Our study demonstrated that the mean values of RR1 (57.7%) and RR2 (16.4%) of anti-Dsg1 antibody are comparable to the reported values of centrifugal plasmapheresis (RR1, 48.4%; RR2, 16.4%) (4), although the number of patients is limited ($n=2$). By RR2 analysis, we also found that IgM and IgA, pentamer and dimer Igs, respectively, are eliminated to a greater degree than IgG. As anti-Dsg1 antibody belongs to IgG class, the autoantibody was removed at a similar rate to that of IgG.

Our results also highlight the need to avoid infection after DFPP. Patients with IgG levels <100 mg/dl or IgM levels <20 mg/dl for prolonged periods have an increased risk of recurrent and occasional life-threatening infectious episodes (5). Some reports suggest that transient depletions of IgG and/or IgM by plasmapheresis or immunosuppressive drug are not generally associated with an increased risk of infection. However, when DFPP is combined with long-term use of immunosuppressants and prednisolone and underlying leukaemia, DFPP could be the cause of sepsis, as observed in Case 1. While DFPP is effective for autoantibody removal, it should be stressed that IgM, IgA and IgG are eliminated in parallel with, or more profoundly than, autoantibodies.

The authors declare no conflicts of interest.

REFERENCES

1. Aoyama Y, Nagasawa C, Nagai M, Kitajima Y. Severe pemphigus vulgaris: successful combination therapy of plasmapheresis followed by intravenous high-dose immunoglobulin to prevent rebound increase in pathogenic IgG. Eur J Dermatol 2008; 18: 557–560.
2. Hatano Y, Katagiri K, Arakawa S, Umeki T, Takayasu S, Fujiwara S. Successful treatment by double-filtration plasmapheresis of a patient with bullous pemphigoid: effects in vivo on transcripts of several genes for chemokines and cytokines in peripheral blood mononuclear cells. Br J Dermatol 2003; 148: 573–579.
3. Yeh JH, Chen WH, Chiu HC, Bay CH. Clearance studies during subsequent sessions of double filtration plasmapheresis. Artif Organs 2006; 30: 111–114.
4. Nagasaka T, Fujii Y, Ishida A, Handa M, Tanikawa A, Amagai M, et al. Evaluating efficacy of plasmapheresis for patients with pemphigus using desmoglein enzyme-linked immunosorbent assay. Br J Dermatol 2008; 158: 685–690.
5. Furst DE. Serum immunoglobulins and risk of infection: how low can you go? Semin Arthritis Rheum 2009; 39: 18–29.

doi: 10.1111/1346-8138.12128 *Journal of Dermatology* 2013; **40**: 1–3

CONCISE COMMUNICATION

Alopecia areata possibly induced by autoimmune reaction in a patient with human T-cell lymphotropic virus-1-associated myelopathy

Taisuke ITO,[1] Shinichiro SHIMADA,[2] Tatsuyoshi MORI,[3] Yoshiki TOKURA[1]

[1]Department of Dermatology, Hamamatsu University School of Medicine, [2]Shimada Dermatoclinic, Hamamatsu, and [3]Department of Dermatology, Fujinomiya City General Hospital, Fujinomiya, Japan

ABSTRACT

A 38-year-old female patient suffered from alopecia areata totalis followed by human T-cell lymphotropic virus-1-associated myelopathy (HAM). These two diseases have recently been considered to be related to cell-mediated autoimmune reactions. Immunohistochemistry revealed accumulation of CXCR3$^+$ CD8$^+$ T cells around hair bulbs in alopecic lesions. Furthermore, flow cytometric analysis showed the elevated frequency of CD8$^+$ human leukocyte antigen DR$^+$-activated T cells at the initial time and declined at the hair regrowth phase with HAM. CD4$^+$ CD25$^+$ adult T-cell leukemia/lymphoma cells were elevated at hair loss phase and decreased after improvement of hair loss. These results suggest that autoreactive and cytotoxic CD8$^+$ T cells induce not only alopecia areata but also HAM in ATL patients. This case highlights that the autoimmune reactions may play an important role in the pathogenesis of alopecia areata and HAM.

Key words: alopecia areata, autoimmunity, CXCR3, human T-cell lymphotropic virus-1-associated myelopathy, human T-cell lymphotropic virus-1.

INTRODUCTION

Alopecia areata (AA) has recently been considered as a tissue-specific autoimmune disease, although the autoantigen has not been fully determined.[1–4] An accumulation of CD4$^+$ and CD8$^+$ T cells in and around hair follicles is the characteristic feature of AA and histologically called "swarm of bees".[5] Melanogenesis-related proteins are strong candidate targets of CD8$^+$ T cells (cytotoxic T cells, Tc1) that react with autoantigens after the collapse of hair follicle immune privilege.[6,7] Recent studies also indicate that class I major histocompatibility complex-restricted CD8$^+$ T cells can independently mediate the pathological response in AA.[8] It is known that human T-cell lymphotropic virus-1 (HTLV-1) causes adult T-cell leukemia/lymphoma (ATLL), and may induce HTLV-1-associated myelopathy (HAM) if the patient's immunity against HTLV-1 highly operates. Here, we describe the development of AA in a patient with HAM.

CASE REPORT

A 38-year-old female patient felt itchy on her scalp 1 month prior to the scalp hair loss, and consequently suffered total hair loss. Therefore, she was referred to our outpatient clinic due to a 1-month history of total scalp hair loss (Fig. 1a). Dermoscopic observation revealed exclamation mark hairs and black dots in the hair-loss lesion. A biopsied specimen showed an accumulation of lymphocytes around the hair bulb ("swarm of bees"; Fig. 1b). There were some eosinophils in the dermis but not around hair bulbs. Given these results, her hair loss was diagnosed as alopecia areata totalis. She was topically treated with betamethasone butyrate propionate. One month after the treatment, she felt mild myalgia and walking disturbance, and was referred to the Department of Neurology at another hospital. She showed spastic paraplegia, bladder and rectal disturbance, and extensive deep tendon reflex. Bone marrow aspiration was performed, and HTLV-1 genome was detected by Southern blot analysis. Anti-HTLV-1 antibody was positive in her serum. Her mother was also positive for anti-HTLV-1 antibody. The patient was subsequently diagnosed as having HAM. She was treated with non-steroidal anti-inflammatory drugs and rehabilitation. Fortunately, her symptoms were not so developed, and stable for 6 months after the treatments.

Immunohistochemical analysis of the alopecia lesion revealed that both CD4$^+$ (Fig. 1c) and CD8$^+$ T cells (Fig. 1d) infiltrated around hair follicles. These T cells expressed CXCR3 (Fig. 1e), representing T-helper (Th)1 and Tc1 cells. CCR4$^+$ cells (Th2 cells and tumor cells) accumulated to a lesser extent.

The patient's peripheral blood mononuclear cells (PBMC) were examined by flow cytometry at the time of diagnosis of AA

Correspondence: Taisuke Ito, M.D., Ph.D., Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashiku, Hamamatsu 431-1192, Japan. Email: itoutai@hama-med.ac.jp
Received 23 January 2013; accepted 29 January 2013.

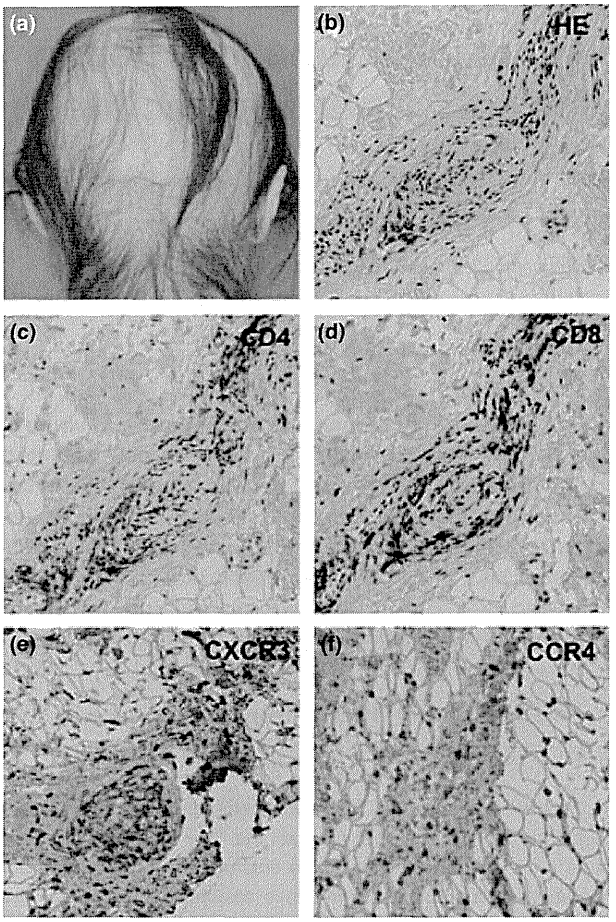


Figure 1. (a) Clinical appearance of hair loss on the scalp. (b) Hematoxylin–eosin staining (original magnification ×100), showing lymphocytic infiltration around the atrophic hair bulb with melanin deposition in the alopecia areata lesion. Immunohistochemical staining of (c) $CD4^+$, (d) $CD8^+$, (e) $CXCR3^+$ and (f) $CCR4^+$ cells.

and HAM and at the time of resolution (3 months after the topical treatment with betamethasone butyrate propionate). The frequency of T cells positive for CD4 and CD25, a combination marker of HTLV-1-infected tumor cells,[3] was increased in the patient's PBMC at the time of diagnosis (5.2%; Fig. 2a), and decreased 3 months later (0.2%; Fig. 2b). $CD4^+$ $CCR4^+$ T cells, another T-cell population including the tumor cells, were also reduced in percentage (data not shown). During this clinical course, the percentage of $CD8^+$ human leukocyte antigen-DR^+ T cells (largely activated Tc1 cells) was elevated at the initial time (3.4%; Fig. 2c) and declined at the hair regrowth phase (0.3%; Fig. 2d). Thus, as the alopecia was improved, activated Tc1 cells as well as the tumor cells were decreased in the blood.

DISCUSSION

To our knowledge, this is the first reported case of AA with HAM. The pathogenesis of HAM remains incompletely understood, but the autoimmunity theory has been put forward.[9,10]

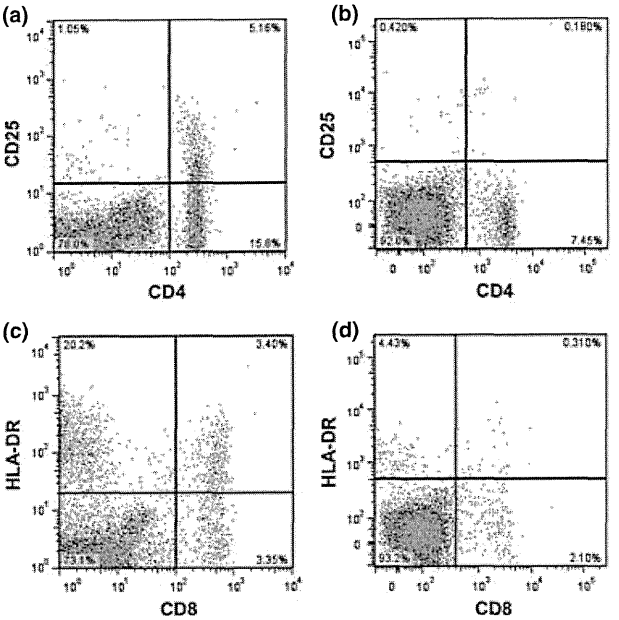


Figure 2. Flow cytometric analysis of peripheral blood mononuclear cells from the patient at the hair loss and recovery phases. CD4 (PerCP) and CD25 (PE) at the (a) hair loss and (b) recovery phases. CD8 (PerCP) and human leukocyte antigen-DR (PE) at the (c) hair loss and (d) recovery phases.

HTLV-1-infected cells infiltrate the spinal cord and reside in the neural tissues. HTLV-1-specific Tc1 cells recognize these infected cells and induce apoptosis of the infected cells and production of Bcl-3 protein. Consequently, inflammatory cytokines are produced in neural tissues, causing severe damage.[9] While a relationship between HAM and autoimmunity has been well discussed,[10] the association of HTLV-1 with a variety of autoimmune disorders including T-cell alveolitis, myopathy, uveitis, arthritis and Sjögren's syndrome has been reported.[11–14] There are significantly more HTLV-1-specific $CD8^+$ T cells in patients with HAM and the other autoimmune disorders than in asymptomatic HTLV-1 carriers.[10] Thus, the diversity, frequency and repertoire of HTLV-1-specific Tc1 clones may be related to the hyperimmune response in patients with HAM.

In our case, $CXCR3^+$ $CD4^+$ T cells, but not $CCR4^+$ $CD4^+$ T cells, were predominantly observed around the hair bulb. Therefore, it seems that the hair follicles were not attacked by ATLL tumor cells per se, but autoreactive Tc1 cells served as effector cells. It is tempting to speculate that activated Tc1 cells developed as a result of HTLV-1-infected tumor cell elimination and attacked hair follicle autoantigens.

REFERENCES

1 Gilhar A, Etzioni A, Paus R. Alopecia areata. *N Engl J Med* 2012; **366**: 1515–1525.
2 Ito T, Hashizume H, Shimauchi T *et al.* CXCL10 produced from hair follicles induces Th1 and Tc1 cell infiltration in the acute phase of alopecia areata followed by sustained Tc1 accumulation in the chronic phase. *J Dermatol Sci* 2012; **69**: 140–147.

3 McDonagh AJ, Tazi-Ahnini R. Epidemiology and genetics of alopecia areata. *Clin Exp Dermatol* 2002; **27**: 405–409.
4 Lu W, Shapiro J, Yu M *et al.* Alopecia areata: pathogenesis and potential for therapy. *Expert Rev Mol Med* 2006; **8**: 1–19.
5 Todes-Taylor N, Turner R, Wood GS *et al.* T cell subpopulations in alopecia areata. *J Am Acad Dermatol* 1984; **11**: 216–223.
6 Paus R, Slominski A, Czarnetzki BM. Is alopecia areata an autoimmune-response against melanogenesis-related proteins, exposed by abnormal MHC class I expression in the anagen hair bulb? *Yale J Biol Med* 1993; **66**: 541–554.
7 Gilhar A, Landau M, Assay B *et al.* Melanocyte associated T-cell epitopes can function as autoantigens for transfer of alopecia areata to human scalp explants on Prkdcscid mice. *J Invest Dermatol* 2001; **117**: 1357–1362.
8 Alli R, Nguyen P, Boyd K *et al.* A mouse model of clonal $CD8^+$ T lymphocyte-mediated alopecia areata progressing to alopecia universalis. *J Immunol* 2012; **188**: 477–486.
9 Nishioka K, Sumida T, Hasunuma T. Human T lymphotropic virus type I in arthropathy and autoimmune disorders. *Arthritis Rheum* 1996; **39**: 1410–1418.
10 Kozako T, Akimoto M, Toji S *et al.* Target epitopes of HTLV-1 recognized by class I MHC-restricted cytotoxic T lymphocytes in patients with myelopathy and spastic paraparesis and infected patients with autoimmune disorders. *J Med Virol* 2011; **83**: 501–509.
11 Sugimoto M, Nakashima H, Watanabe S *et al.* T-Lymphocyte alveolitis in HTLV-lassociated myelopathy. *Lancet* 1987; **2**: 1220.
12 Vernant JC, Buisson G, Magdeleine J *et al.* T-Lymphocyte alveolitis, tropical spastic paresis, and Sjogren syndrome. *Lancet* 1988; **1**: 177.
13 Nishioka K, Maruyama I, Sato K *et al.* Chronic inflammatory arthropathy associated with HTLV-I. *Lancet* 1989; **1**: 441.
14 Terada K, Katamine S, Eguchi K *et al.* Prevalence of serum and salivary antibodies to HTLV-1 in Sjogren's syndrome. *Lancet* 1994; **344**: 1116–1119.

Granulocyte-colony Stimulating Factor-producing Primary Cutaneous Anaplastic Large Cell Lymphoma with Cerebral Metastasis

Akira Kasuya, Satoshi Hirakawa, Hideo Hashizume and Yoshiki Tokura

Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-Ku, Hamamatsu 431-3192, Japan. E-mail: casuaki@hama-med.ac.jp

Accepted February 15, 2012.

Tumours producing granulocyte-colony stimulating factor (G-CSF) have been documented in various tissues, with remarkable neutrophilia as an unexplained laboratory finding before the diagnosis (1). G-CSF-producing malignant lymphomas are rare; there have been only 2 documented cases of Hodgkin's lymphoma and 1 of gastric anaplastic large cell lymphoma (ALCL) (2, 3). We report here a case of G-CSF-producing primary cutaneous ALCL with cerebral metastasis. To the best of our knowledge, this is the first reported case of G-CSF-producing primary cutaneous lymphoma.

CASE REPORT

A 78-year-old Japanese man was admitted to our hospital for further evaluation and treatment of a 2-year history of lobulated tumours on his forehead. Two months before admission, the tumours enlarged with ulceration and necrosis on the surface (Fig. 1). No swollen superficial lymph node was palpable. Concomitantly with the tumour growth, the patient had intermittent high fever and leukocytosis (38,300/μl) with prominent neutrophilia (87%). The following values were elevated: C-reactive protein, 5.0 mg/dl (normal, 0.0–0.5 mg/dl); serum soluble interleukin-2 receptor, 4,270 U/ml (145–519 U/ml); and serum G-CSF 847 pg/ml (<39.0 pg/ml). The patient's serum was negative for anti-human T-lymphotropic virus type 1 (HTLV-1) antibodies. Peripheral blood smear showed no atypical cell. Repeated blood culture was negative for any bacteria. No visceral lesions were detected by 18F-positron emission tomography (PET) or computed tomography (CT) scan.

Skin biopsy revealed massive infiltration of atypical large cells in the dermis and subcutaneous fat (Fig. 2A). The atypical cells were positive for CD30 (Fig. 2B) and negative for CD3, CD20, CD79a, and anaplastic lymphoma kinase. PCR analysis confirmed clonal gene rearrangement of the T-cell antigen receptor Cβ1 chain. When deparaffinized sections were immunohistochemically stained with anti-G-CSF rabbit polyclonal antibody (Abcam, Cambridge, UK), positive immunoreactivity was found in neoplastic cells and intercellular spaces (Fig. 2C). On the other hand, tumour cells in 2 cases of typical primary cutaneous ALCL as control were negative (Fig. 2D). There was no influx of granulocytes in the tumour of our case. This may be explained by the fact that G-CSF does not have chemotactic effect (4).

A biopsy specimen from the tumour was measured for the concentration of G-CSF (Special Reference Laboratories, Tokyo, Japan). The G-CSF level was 20,600 pg/g tissue weight, which was much higher than the serum level (847 pg/ml). These findings suggested that the neoplastic cells produced G-CSF. We diagnosed the tumour as G-CSF-producing primary cutaneous ALCL.

After radiation therapy (45 Gy/15 Fr), the tumour disappeared, and leukocyte count and serum G-CSF level decreased to 5,400/μl and 107 pg/ml, respectively. One month later, however, he complained of visual field defect. Brain magnetic resonance image revealed a hypophysal tumour, suggesting metastasis of ALCL. Additional radiation therapy to the tumour, 30 Gy/15 Fr, resulted in a partial reduction in its size.

DISCUSSION

G-CSF-producing tumours are rare, but clinically important, because its symptoms, neutrophilia and intermittent fever, mimic bacterial sepsis. In the case described here, negative results in repeated blood culture

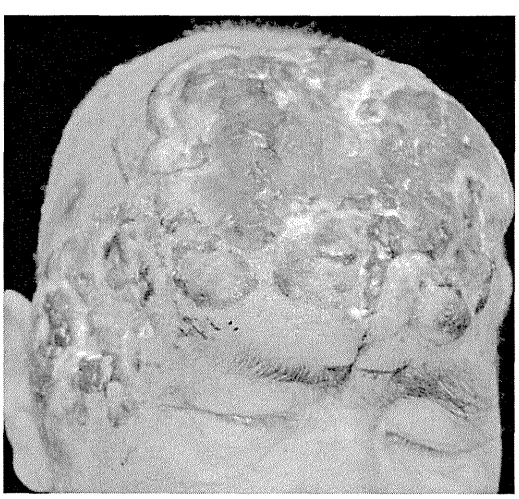


Fig. 1. Clinical appearance of the tumour on the forehead and frontal scalp.

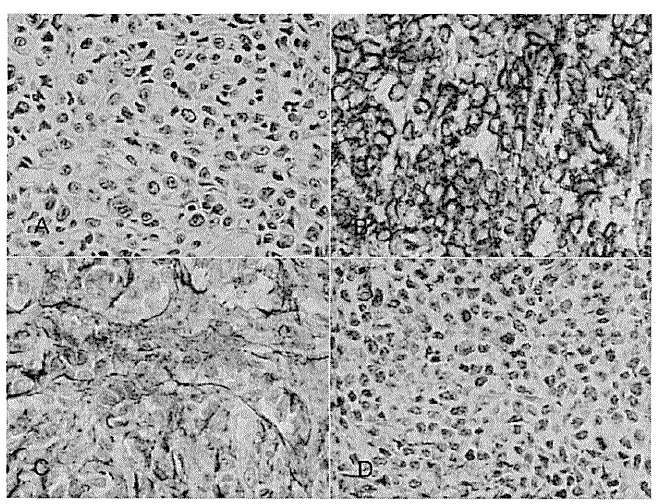


Fig. 2. Histopathology and immunohistochemistry of the tumour (original magnification ×400). (A) Massive infiltration of atypical cells in dermis (haematoxylin-eosin stain). (B) Tumour cells positive for CD30. (C) Tumour cells and interstitial tissue positive for granulocyte-colony stimulating factor. (D) Representative primary cutaneous anaplastic large cell lymphoma as control.

and failures to find visceral abscess in imaging studies made us think of G-CSF-producing tumour.

The diagnostic criteria for G-CSF-producing tumours were proposed (5). In our case, the diagnosis was made based on the following findings: (*i*) leukocytosis predominant with granulocytosis; (*ii*) elevated serum G-CSF; (*iii*) reductions in blood neutrophil number and serum G-CSF level after successful treatment; and (*iv*) demonstration of intratumour G-CSF production by immunohistochemistry and tissue G-CSF measurement.

Primary cutaneous ALCL usually has a good prognosis, and cases with a fatal outcome have rarely been reported (6). However, aggressive cerebral metastasis was found in our case. Rapid progression and multiple metastasis are frequently documented (7). In a case of G-CSF-producing squamous cell carcinoma, the presence of G-CSF receptors on the tumour cell was demonstrated by immunohistochemistry (1), suggesting that autocrine expansion of the tumour cell with G-CSF contributes to the poor prognosis. Overexpressed G-CSF might activate the Janus kinase–signal transducer and activator of transcription (JAK-STAT) pathway through the receptor, leading to unusual enlargement and cerebral metastasis, as in the present case.

The authors declare no conflicts of interest.

REFERENCES

1. Toyoda M, Chikamatsu K, Sakakura K, Fukuda Y, Takahashi K, Miyashita M, et al. A case of squamous cell carcinoma of the head and neck producing granulocyte-colony stimulating factor with marked leukocytosis. Auris Nasus Larynx 2007; 34: 267–271.
2. Shimizu K, Iida M, Takahashi S. Hodgkin's disease producing granulocyte colony stimulating factor. Am J Hematol 2001; 68: 134.
3. Sugimoto M, Kajimura M, Hanai H, Shirai N, Tanioka F, Kaneko E. G-CSF-producing gastric anaplastic large cell lymphoma complicating esophageal cancer. Dig Dis Sci 1999; 44: 2035–2038.
4. Wengner A, Pitchford S, Furze R, Rankin S. The coordinated action of G-CSF and ELR CXC chemokines in neutrophil mobilization during acute inflammation. Blood 2008; 111: 42–49.
5. Asano S, Urabe A, Okabe T, Sato N, Kondo Y. Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. Blood 1977; 49: 845–852.
6. Tokura Y, Sugita K, Yagi H, Shimauchi T, Kabashima K, Takigawa M. Primary cutaneous anaplastic large cell lymphoma with fatal leukemic outcome in association with CLA and CCR4-negative conversion. J Am Acad Dermatol 2007; 57: S92–96.
7. Takahashi H, Yasuda A, Ochi N, Sakamoto M, Takayama S, Wakasugi T, et al. Granulocyte-colony stimulating factor producing rectal cancer. World J Surg Oncol 2008, 6: 70.

Paediatric Acute Generalized Exanthematous Pustulosis Induced by Paracetamol with High Serum Levels of Interleukin-8 and -22: A Case Report

Takatsune Umayahara, Takatoshi Shimauchi, Toshiharu Fujiyama, Taisuke Ito, Satoshi Hirakawa and Yoshiki Tokura*

*Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan. *E-mail: tokura@hama-med.ac.jp*

Accepted July 4, 2012.

Acute generalized exanthematous pustulosis (AGEP) is a rare skin disorder, characterized by acute development of numerous, pin-head sized, non-follicular, sterile pustules that usually begin in intertriginous folds with high fever and neutrophilia (1–3). The condition is frequently induced by hypersensitivity reaction to drugs (1). AGEP usually affects adults; paediatric cases have rarely been reported (4–7). We describe here a case of paediatric AGEP induced by paracetamol. High levels of serum interleukin (IL)-8 and IL-22 observed in our patient suggest a role of these cytokines/chemokines in the pathogenesis of AGEP.

CASE REPORT

A 7-year-old boy was referred to us with a generalized eruption. Five days prior to our initial examination, the patient had developed upper respiratory symptoms, diagnosed as influenza A, and he had received oral paracetamol and oseltamivir. Two days after the start of treatment, he developed an itchy exanthema on the trunk with a high fever. On examination, the patient had an erythematous eruption on his trunk (Fig. 1a) and the proximal parts of his arms and thighs. Numerous small pustules, less than 1 mm in diameter, were present, especially on the inner aspects of the thighs (Fig. 1b), axillae, and lumbar region. Slightly swollen cervical lymph nodes were palpable. Laboratory investigations showed a normal leukocyte count, but C-reactive protein was elevated (1.4 mg/dl; normal < 0.1 mg/dl).

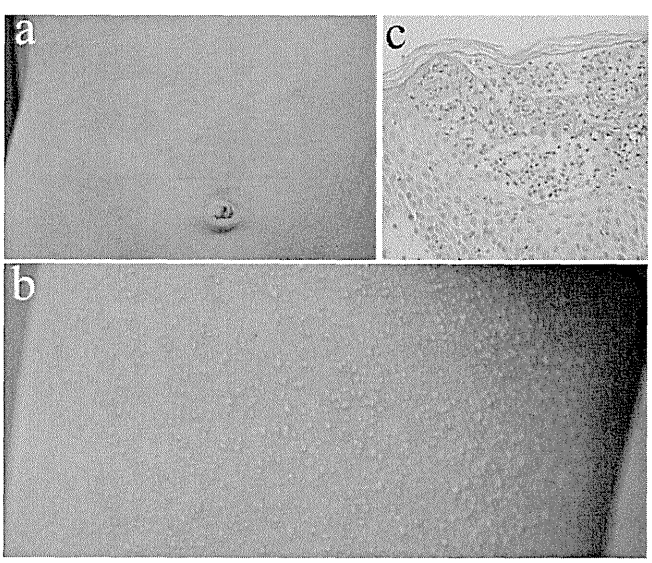


Fig. 1. Clinical and histopathological appearances. (a) Clinical appearance, showing an erythematous eruption present on the trunk. (b) Close-up view, showing multiple small pustules on the thigh. (c) Histopathology, exhibiting subcorneal collection of many neutrophils in the epidermis (haematoxylin and eosin (HE), original magnification ×200).

Histopathologically, there were subcorneal neutrophilic pustules and a dermal lymphocytic infiltrate (Fig. 1c). We determined the likelihood of AGEP by using the reported scoring system, which can be used to identify cases of AGEP based on morphology, course and histology of the skin reaction (8). Our patient had a score of 11, indicating a definite diagnosis of AGEP. The culprit drugs and results of lymphocyte transformation test performed 19 days after disease onset were as follows (stimulation index [SI] ≥ 1.8 is considered positive): paracetamol, 1,516 cpm (SI 2.75); oseltamivir, 450 cpm (SI 0.81); and no-addition control, 551 cpm. We thus diagnosed the eruption as AGEP induced by paracetamol. Discontinuation of paracetamol and oral administration of prednisolone (5 mg daily for 7 days) improved the patient's skin lesions within 2 weeks.

The serum level of IL-8 was measured with Cytometric Bead Array (BD Biosciences, San Diego, CA, USA), serum levels of IL-17A, IL-22 and TNF-α with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), and that of chemerin with an ELISA kit (Millipore, St Charles, MO, USA) in the patient and 4 healthy individuals. These cytokines and chemokines may be involved in the accumulation of neutrophils in the epidermis (9, 10). A blood sample was taken from the patient 5 days after the onset of eruption. It is noted that IL-8 and IL-22 were markedly elevated in the patient, compared with the normal individuals (Table I). IL-17 and tumour necrosis factor-α (TNF-α) were below the level of detection in both patient and normal healthy controls. Chemerin, a chemoattractant for plasmacytoid dendritic cells (pDC) (11, 12), was not increased in the patient compared with the normal subjects.

DISCUSSION

AGEP should be differentiated from pustular psoriasis (von-Zumbusch type). In our patient, the skin eruption and high fever were improved by discontinuation of paracetamol and 7-day administration of prednisolone (5 mg daily), and there was no recurrence thereafter. This shorter duration supports the diagnosis of AGEP. The SI of 2.75 in a lymphocyte stimulation test was significantly high (13). The possibility of the causative role of infection could not be completely ruled out, but at least paracetamol contributed to the eruption,

Table I. *Serum levels of cytokines/chemokines*

	Serum levels	
Cytokines/chemokines	Patient	Normal subjects (n=4, mean ± SD)
IL-8	274.6 pg/ml	1.29 ± 1.49 pg/ml
IL-17A	UDL	UDL (all)
IL-22	25.3 pg/ml	UDL (all)
TNF-α	UDL	UDL (all)
Chemerin	176.8 pg/ml	229.0 ± 31.0 pg/ml

UDL: under the detection level; SD: standard deviation.

and infection may participate in the occurrence of the eruption. Although AGEP is generally considered to be an adult disease (1–3), recent reports suggest that it occasionally occurs in paediatric individuals (4–7). Thus, children may develop AGEP upon administration of antibiotics or non-steroidal anti-inflammatory drugs, as seen in our patient.

As the lymphocyte transformation test with a causative drug usually shows a high SI, drug-specific T cells are thought to mediate AGEP (2, 3). Drug-specific $CD4^+$ and $CD8^+$ T cells play an important role by producing neutrophil chemo-attractant IL-8. To explain the mechanism of subcorneal accumulation of neutrophils, however, a certain population of drug-specific T cells are thought to stimulate keratinocytes to produce IL-8, and the keratinocyte-derived IL-8 may contribute to the accumulation of neutrophils in the lesional epidermis. In fact, the elevated expression of IL-8 was observed in keratinocytes as well as infiltrating mononuclear cells (3).

Th17 cell is a $CD4^+$ T helper cell subset capable of producing IL-17 and IL-22, and dysregulated Th17 responses mediate a variety of skin inflammatory conditions, such as psoriasis (9) and atopic dermatitis (14). IL-17 and IL-22 exert a strong synergistic effect on the production of IL-8 by keratinocytes (14). Increased frequencies of Th17 cells and high levels of IL-22 have been reported in AGEP (15, 16).

Our study showed an increase in serum IL-8 and IL-22 in a paediatric patient with AGEP. Since the amount of IL-17A was below the limit of detection, the involvement of Th17 cells remains unclear in this single case report. In order to maintain Th17 cells, IL-23 released from dendritic cells (DCs) is important (10), and DCs are activated by TNF-α in an autocrine manner (9). Alternatively, type I interferon derived from pDCs may indirectly lead to Th17 cell stimulation with the help of chemerin serving as a pDC-chemo-attracting factor (11). While patients with psoriasis have higher levels of chemerin (12), our AGEP patient did not have an increased level of chemerin in the peripheral blood, suggesting that pDC are not substantially involved in the pathogenesis. Although IL-17 and/or IL-22 may be involved in the pathogenesis of AGEP, the exact role of Th17 cells in this drug eruption, and their stimulation mechanism, are as yet unknown.

The authors declare no conflicts of interest.

REFERENCES

1. Sidoroff A, Dunant A, Viboud C, Halevy S, Bouwes Bavinck JN, Naldi L, et al. Risk factors for acute generalized exanthematous pustulosis (AGEP): results of a multinational case-control study (EuroSCAR). Br J Dermatol 2007; 157: 989–996.
2. Schaerli P, Britschgi M, Keller M, Steiner UC, Steinmann LS, Moser B, et al. Characterization of human T cells that regulate neutrophilic skin inflammation. J Immunol 2004; 173: 2151–2158.
3. Britschgi M, Steiner UC, Schmid S, Depta JP, Senti G, Bircher A, et al. T-cell involvement in drug-induced acute generalized exanthematous pustulosis. J Clin Invest 2001; 107: 1433–1441.
4. Miteva L, Kadurina M, Schwartz RA. Childhood acute generalized exanthematous pustulosis induced by oral ketoconazole. Acta Dermatovenerol Croat 2010; 18: 267–270.
5. Poliak N, Elias M, Cianferoni A, Treat J. Acute generalized exanthematous pustulosis: the first pediatric case caused by a contrast agent. Ann Allergy Asthma Immunol 2010; 105: 242–243.
6. Ozmen S, Misirlioglu ED, Gurkan A, Arda N, Bostanci I. Is acute generalized exanthematous pustulosis an uncommon condition in childhood? Allergy 2010; 65: 1490–1492.
7. Riten K, Shahina Q, Jeannette J, Palma-Diaz MF. A severe case of acute generalized exanthematous pustulosis (AGEP) in a child after the administration of amoxicillin-clavulanic acid: brief report. Pediatr Dermatol 2009; 26: 623–625.
8. Sidoroff A, Halevy S, Bavinck JN, Vaillant L, Roujeau JC. Acute generalized exanthematous pustulosis – a clinical reaction pattern. J Cutan Pathol 2001; 28: 113–119.
9. Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Johnson-Huang LM, Nograles KE, White TR, et al. Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol 2009; 129: 79–88.
10. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a Th17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007; 445: 648–651.
11. Albanesi C, Scarponi C, Pallotta S, Daniele R, Bosisio D, Madonna S, et al. Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. J Exp Med 2009; 206: 249–258.
12. Nakajima H, Nakajima K, Nagano Y, Yamamoto M, Tarutani M, Takahashi M, et al. Circulating level of chemerin is upregulated in psoriasis. J Dermatol Sci 2010; 60: 45–47.
13. Sawada Y, Nakamura M, Tokura Y. Generalized fixed drug eruption caused by pazufloxacin. Acta Derm Venereol 2011; 91: 600–601.
14. Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y. Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol 2008; 128: 2625–2630.
15. Nakamizo S, Kobayashi S, Usui T, Miyachi Y, Kabashima K. Clopidogrel-induced acute generalized exanthematous pustulosis with elevated Th17 cytokines levels as determined by a drug lymphocyte stimulation test. Br J Dermatol 2010; 162: 1402–1403.
16. Kabashima R, Sugita K, Sawada Y, Hino R, Nakamura M, Tokura Y. Increased circulating Th17 frequencies and serum IL-22 levels in patients with acute generalized exanthematous pustulosis. J Eur Acad Dermatol Venereol 2011; 25: 485–488.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/1346-8138.12068
Journal of Dermatology 2013; **40**: 1–2

LETTER TO THE EDITOR

Identification of a novel heterozygous mutation in the first Japanese case of Marie Unna hereditary hypotrichosis

Dear Editor,

Marie Unna hereditary hypotrichosis (MUHH; Online Mendelian Inheritance in Man [OMIM] no. 146550) is a rare type of autosomal dominant hereditary hypotrichosis that has been mainly reported in European and US patients.[1] Recently, the *U2HR* gene located within the 5′-untranslated region of the human hairless (*HR*) gene was identified as a causative gene responsible for MUHH.[2] Here, we report a novel heterozygous mutation in the *U2HR* gene in a Japanese patient with MUHH.

A 3-year-old girl was referred to our outpatient clinic because of short and sparse hair. When the patient was 1 year old, her parents noticed kinky hair on her scalp. At the age of 2 years, she developed hair loss on the scalp. On examination, the hair on the vertex and front scalp was especially short and wiry as compared to the hair on the back of her scalp, and thus, the baldness resembled androgenetic alopecia (AGA) (Fig. 1a,b). Her father has shown a similar hypotrichosis phenotype, suggesting an autosomal dominant inheritance trait. The patient's teeth, mucous membrane, nails and bones, as well as the general physical and mental development were normal. Light microscopy revealed that the vertical hairs were twisted and the diameter was slightly irregular (Fig. 1c). Scanning electron microscopy showed that the cuticle was undefined and rough (Fig. 1d). Based on these findings, we diagnosed the patient as having MUHH.

Using the patient's genomic DNA, mutation analysis for the *U2HR* gene was performed as described previously[2] after obtaining the informed consent of her parents. However, both parents intensely rejected the gene analysis. Institutional approval for the study was obtained, and all procedures adhered to the Declaration of Helsinki principles. Direct sequencing analysis identified a heterozygous missense mutation c.38G > T (p.Arg13Leu) in the *U2HR* gene of the patient (Fig. 2a), which was not detected in 100 healthy Japanese control individuals (Fig. 2b; data not shown). This mutation has not previously been reported.

To the best of our knowledge, this is the first report of a Japanese patient with MUHH. In 1925, Marie Unna first described this rare autosomal dominant hypotrichosis in a seven-generation family from northern Germany.[3] Since then, cases have been reported from several European countries, the USA and China.[1] MUHH is characterized by coarse, wiry and twisted hair development at an early age that progresses to alopecia during puberty. The hair loss mainly starts from the vertex area so that it appears to be AGA.[3,4]

Herein, we identified a novel heterozygous mutation c.38G > T (p.Arg13Leu) in the *U2HR* gene in the first reported Japanese patient with MUHH. Wen *et al.*[2] recently reported that *U2HR* is responsible for MUHH. The human *HR* gene on chromosome 8p21 was previously reported as a causative gene for autosomal recessive hair loss disorder, known as atrichia with papular lesions (OMIM no. 209500).[5] Later on, it has

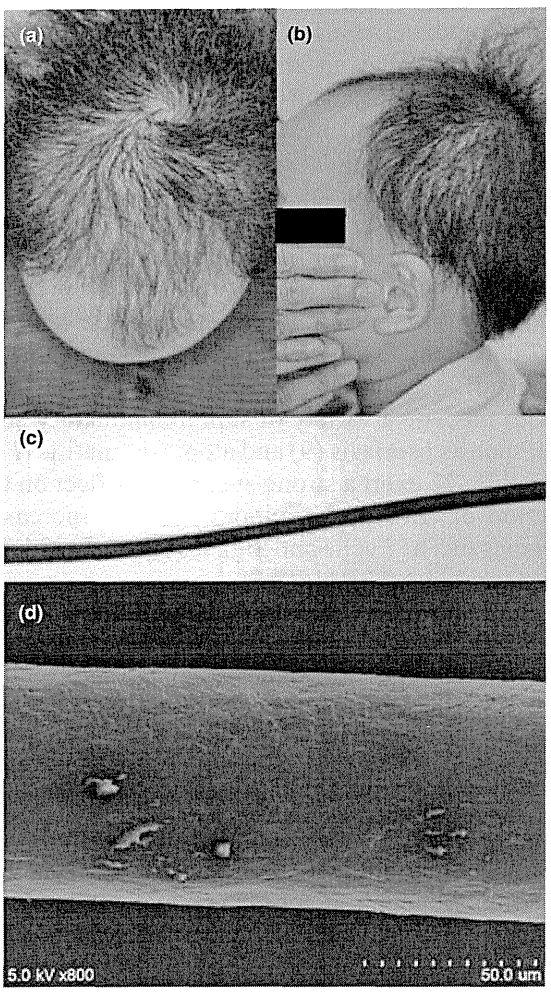


Figure 1. Clinical and microscopic features of an affected individual with Marie Unna hereditary hypotrichosis. (a) Sparse and wiry hair in the vertex and frontal zone of the scalp. (b) Increased density of scalp hair on the occipital area. (c) Light microscopy showing that the vertical hairs are twisted and the diameter is slightly irregular. (d) Scanning electron microscopy showing undefined and rough cuticle.

Correspondence: Taisuke Ito, Ph.D., M.D., Department of Dermatology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-1192, Japan. Email: itoutai@hama-med.ac.jp

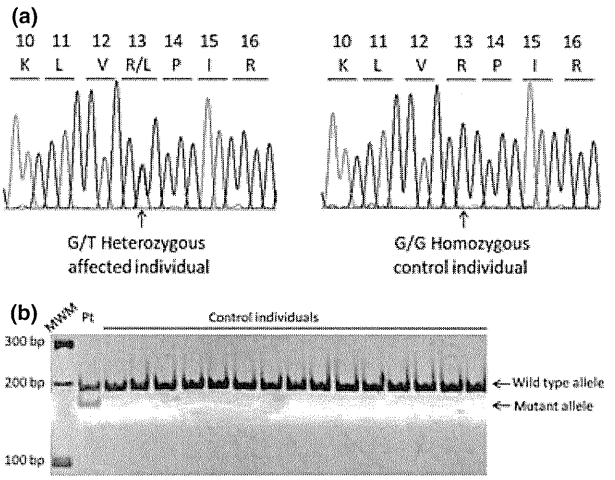


Figure 2. Identification of a novel mutation in the *U2HR* gene. (a) Identification of a heterozygous mutation c.38G > T (p. Arg13Leu) in the *U2HR* gene of the affected individual with Marie Unna hereditary hypotrichosis. Position of the mutation is indicated by arrows. (b) To screen for the mutation c.38G > T (p.Arg13Leu) in the *U2HR* gene, polymerase chain reaction (PCR) was performed with a forward primer (5′-CA ACTCGGCCATCTCCGAC-3′) and a mismatched reverse primer (5′-TGCAGGATGCGGCACACGGCGCGGATCTGC-3′). Note that the G > T substitution was introduced into the reverse primer to generate a restriction enzyme site for *Pst*I only in the PCR product amplified from the mutant allele (shown in bold). The PCR products, 196 bp in size, were digested by *Pst*I at 37°C for 1 h, and analyzed on 8% polyacrylamide gels. Only the PCR product from the mutant allele was digested into 169 and 27 bp fragments. The 27 bp fragment is not shown. MWM, molecular weight markers; Pt, patient.

been reported that the 5′-untranslated region of the *HR* gene contains four potential open reading frames (ORF): *U1HR*, *U2HR*, *U3HR* and *U4HR*.[2] Of these, *U2HR* encodes a functional inhibitory peptide composed of 34 amino acid residues.[2] Notably, all the mutations responsible for MUHH have been found in the *U2HR* region. Mutations in the *U2HR* have been shown to cause increased translation of the main physiological ORF of the *HR* gene that links inhibition of Wnt inhibitor, such as SFRP2. Excessive induction of Wnt signaling induces the proliferation of epithelial cells and the terminal differentiation of epidermal keratinocytes resulting in epidermal hyperplasia and the formation of abnormally proliferating follicular cysts.[1,2]

In conclusion, we report the first Japanese case of MUHH with a novel heterozygous mutation in the *U2HR* gene, characterized by AGA-like hair loss. Although only several cases of MUHH have been reported in the Asian populations, it should be kept in mind that MUHH is a differential diagnosis for AGA-like hypotrichosis in young patients.

Taisuke ITO,[1] Yutaka SHIMOMURA,[2] Masaaki OGAI,[3] Jun-Ichi SAKABE,[1] Yoshiki TOKURA,[1]

[1]*Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu,* [2]*Laboratory of Genetic Skin Diseases, Niigata University Graduate School of Medical and Dental Sciences, Niigata, and* [3]*Department of Dermatology, Seirei Hamamatsu General Hospital, Hamamatsu, Japan*

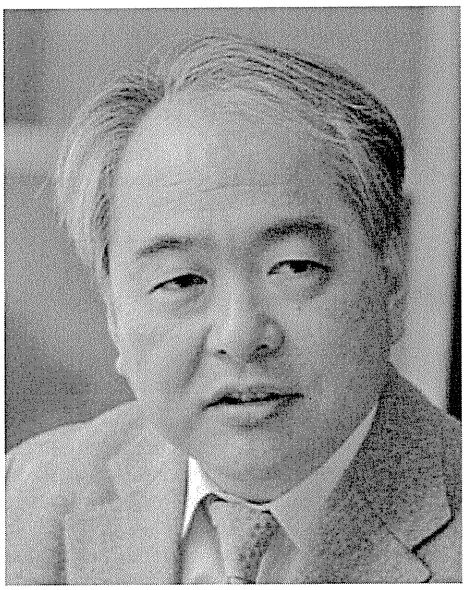
REFERENCES

1 Kim JK, Kim E, Baek IC *et al.* Overexpression of Hr links excessive induction of Wnt signaling to Marie Unna hereditary hypotrichosis. *Hum Mol Genet* 2012; **19**: 3445–3453.
2 Wen Y, Liu Y, Xu Y *et al.* Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis. *Nat Genet* 2009; **41**: 228–233.
3 Unna M. Uber hypotrichosis congenita hereditaria. *Dermatol Wochenschr* 1925; **81**: 1167–1178.
4 Cai LQ, Wang PG, Gao M *et al.* A novel U2HR non-synonymous mutation in a Chinese patient with Marie Unna hereditary hypotrichosis. *J Dermatol Sci* 2009; **55**: 125–127.
5 Ahmad W, Faiyaz ul Haque M, Brancolini V *et al.* Alopecia universalis associated with a mutation in the human hairless gene. *Science* 1998; **279**: 720–724.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY
doi: 10.1111/1346-8138.12086
Journal of Dermatology 2013; **40**: 1

EDITORIAL

New Year's Greetings



We are happy to inform you that the Journal of Dermatology (JD) is firmly establishing a status in the arena of international dermatology journals. This success has been accomplished mainly by 32 Section Editors on the editorial team. Since their continuous efforts are extraordinary, I would like to express my gratitude to all the Section Editors and the Associate Editor Dr. Takafumi Kadono for their contribution to the successful review process of the JD.

The number of submitted manuscripts continues to grow, as it was 258 in 2008 but increased to more than 800 in 2012. The JD is the official journal of the Japanese Dermatological Association (JDA). Therefore, the JD office is based in Asia, but it provides an important avenue through which the JDA can become more visible to dermatologists over the world. In this context, many papers have recently been submitted not only by domestic dermatologists and skin researchers but also by oversea dermatologists from Asian countries, European countries, and the US. In 2011, 38% of the submitted papers were from Japan, 31% from other Asian countries, and the rest from European countries and the US. As such, the acceptance rate has declined to approximately 30%, but I plan to advance the quality of work in this journal so that we have a great impact as a reliable source of reference.

The current Impact Factor of JD is 1.493 in 2011 (published in 2012), which has been elevated from 0.694 in 2007, 1.175 in 2008, 1.008 in 2009, and 1.355 in 2010. Currently, the JD ranks 29 out of 58 international dermatological journals. Our goal is to bring the Impact Factor rating up to a higher value. To this end, we are keen to accept good papers seemingly contributing to the Impact Factor, but we do not constructively take the self-citation strategy to elevate the Impact Factor.

We have decided to publish more papers of Original Articles to reduce the number of Letter-to-the-Editor. Upon this alteration, we also encourage authors to submit papers with a new format, which is applied to the Concise Communication, a short but substantially long format of 1500 words. We believe that this category is beneficial for authors to report their studies in greater depth than the Letter-to-the-Editor format.

The success of the journal depends on your contribution, so I encourage the qualified dermatologists and skin researchers to submit your excellent works. Your active participation is greatly appreciated.

Sincerely,

Yoshiki TOKURA, M.D, Ph.D
The Journal of Dermatology
Editor-in-Chief