Received: 7 May 2019 | Accepted: 10 June 2019

DOI: 10.1002/cia2.12070

ORIGINAL ARTICLE

Journal of Cutaneous Immunology and Allergy

WILEY

Quantitatively immunological characterization of mogamulizumab skin disorders in ATL patients

Asahi Ito MD, PhD[1] | Yui Suzuki DVM, PhD[2] | Ayako Masaki MD, PhD[1,3] | Shinichiro Yoshida MD, PhD[4] | Hitoshi Suzushima MD, PhD[5] | Shigeki Takemoto MD, PhD[6] | Atae Utsunomiya MD, PhD[7] | Toshihiko Ishii Ms[2] | Masanori Hiura Ms[2] | Takeshi Takahashi PhD[8] | Satoshi Yurimoto PhD[8] | Hiroshi Inagaki MD, PhD[3] | Akimichi Morita MD, PhD[9] | Shinsuke Iida MD, PhD[1] | Takashi Ishida MD, PhD[1]

[1]Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, Aichi, Japan

[2]R&D Division, Kyowa Hakko Kirin Co. Ltd., Shizuoka, Japan

[3]Department of Pathology and Molecular Diagnostics, Nagoya City University Graduate School of Medical Sciences, Aichi, Japan

[4]Department of Hematology, National Hospital Organization Nagasaki Medical Center, Nagasaki, Japan

[5]Department of Hematology, Kumamoto Shinto General Hospital, Kumamoto, Japan

[6]Department of Hematology and Institute for Clinical Research, National Hospital Organization Kumamoto Medical Center, Kumamoto, Japan

[7]Department of Hematology, Imamura General Hospital, Kagoshima, Japan

[8]Medical Affairs, Kyowa Hakko Kirin Co. Ltd., Tokyo, Japan

[9]Department of Geriatric and Environmental Dermatology, Nagoya City University Graduate School of Medical Sciences, Aichi, Japan

Correspondence
Asahi Ito, Department of Hematology and Oncology, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8601, Japan.
Email: asahi-i@ga2.so-net.ne.jp

Present address
Shigeki Takemoto, JURAKU Internal Medicine Clinic Hematology/Oncology, Kumamoto, Japan

Takashi Ishida, Division of Hematology and Oncology, Department of Internal Medicine, School of Medicine, Iwate Medical University, Iwate, Japan

Funding information
Kyowa Hakko Kirin

Abstract

Purpose: Skin disorders demonstrate highly variable phenotypical and histopathological features. Mogamulizumab, a humanized anti-CC chemokine receptor 4 monoclonal antibody indicated for the treatment of adult T-cell leukemia-lymphoma, has been shown to induce skin-related adverse events in some patients, including rare cases of Stevens-Johnson syndrome. Hence, we aimed to elucidate immunological primary reactions in skins of mogamulizumab by quantitatively comparing any patterns of other skin disorders.

Methods: We quantitatively analyzed Foxp3$^+$, CD8$^+$, CD4$^+$, granzyme B$^+$, CD56$^+$, and macrophage-derived chemokine-positive cells, and compared the results with trends observed in other inflammatory skin disorders such as psoriasis vulgaris, atopic dermatitis, and lichen planus. The analysis was separately performed in dermis, basement membrane, or epidermis of skins.

Results: Foxp3$^+$/CD8$^+$ cell ratio in dermis and basement membrane of mogamulizumab-emergent skin disorders was significantly lower compared with those of the other skin disorders. In inflammatory skins, the more the number of CD8$^+$ cells were infiltrated, the more the number of Foxp3$^+$ cells were prone to be infiltrated, but not

in mogamulizumab-emergent skin disorders. No significant difference between all of the other skin disorders was observed in other immunological markers.

Conclusion: The low Foxp3$^+$/CD8$^+$ cell ratio of skins is the underlying reason for mogamulizumab-emergent skin disorders.

KEYWORDS
CD8, FoxP3, mogamulizumab

1 | INTRODUCTION

Mogamulizumab, a humanized monoclonal antibody (mAb) targeting the CC chemokine receptor 4 (CCR4), has demonstrated efficacy in patients with CCR4-expressing adult T-cell leukemia-lymphoma (ATL). However, in a phase 2 study of mogamulizumab in Japan, approximately 67% of patients with ATL reported skin disorders during or soon after mogamulizumab therapy,[1] including Stevens-Johnson syndrome (SJS) and toxic epidermal necrosis in some patients.[2,3] This could be due to autoimmune reactions induced by the loss of or reduction in regulatory T cells (Treg) known to express CCR4.[4] Indeed, some case reports have indicated reductions in Treg and increases in CD8$^+$ cells with mogamulizumab treatment; albeit, the assessments were qualitative in nature.[2,3,5] Furthermore, mogamulizumab-induced immune response is thought to differ from responses observed in other inflammatory diseases, including autoimmune diseases; however, no reports directly comparing these responses have been published as yet.

In this study, we quantitatively analyzed the expression of Foxp3$^+$ (a marker of Treg), CD8$^+$, CD4$^+$, granzyme B$^+$ (a marker of cytoplasmic granules of cytotoxic T lymphocytes or natural killer [NK] cells), CD56$^+$ (a marker of NK cells), and macrophage-derived chemokine (MDC)/CCL22$^+$ cells (a CCR4 ligand) in dermis, basement membrane, or epidermis of mogamulizumab-emergent and other inflammatory skin disorders, in order to elucidate the immunological primary reactions of mogamulizumab in skins.

2 | METHODS

This analysis was performed retrospectively using 27 archival skin samples. Of the 27 samples, seven were from patients with ATL in a phase 2 study of mogamulizumab.[1] The patients received intravenous infusions of mogamulizumab once per week for at maximum 8 weeks at a dose of 1.0 mg/kg. Twenty samples from 10 patients with psoriasis vulgaris, five patients with atopic dermatitis, and five patients with lichen planus were included for comparison in the study.

The study was compliant with the Ethical Guidelines for Medical and Health Research Involving Human Subjects, and was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee at each participating site, and written informed consent was obtained from each patient or, if obtaining written consent was not possible, an opt-out consent process was used.

Formalin-fixed skin samples were used for immunohistochemical staining with the following mAbs: anti-Foxp3 (Abcam), anti-CD8 (DAKO), anti-CD4 (Leica), anti-granzyme B (Monosan), anti-CD56 (Leica), and anti-MDC mAb (PeproTech), according to the manufacturers' instructions.

Positively stained cells for each mAb were counted in five or 20 selected epidermis (40 × 40 μm or 20 × 20 μm, respectively), 10 selected basement membrane (50 × 50 μm), and 10 selected dermis (100 × 100 μm) regions (Figure S1). The positively stained cells from each region were summed up and represented as cell number per 0.005 mm^2 of the epidermis, dermis, or basement membrane. Further, the total cell numbers from all three regions were summed up and represented as cell numbers per 0.015 mm^2 of the skin disorders. The ratio of Foxp3$^+$/CD8$^+$ cells was also calculated. Cell counting for Foxp3, CD8, and CD4 was performed independently by three pathologists, and the mean value was reported, whereas positively stained cells for granzyme B, CD56, and MDC were quantified by one pathologist.

The results are shown as a box and whisker plot, indicating quartiles, minimum, and maximum values. Steel's test was performed using SAS (release 9.4, SAS Institute) to compare the results between skin disorders caused by mogamulizumab and other skin disorders, with a statistical significance level of $P < 0.05$.

3 | RESULTS

3.1 | Clinical characteristics of ATL patients with mogamulizumab-emergent skin disorders

The quantitative analysis was retrospectively performed using 27 archival skin samples. Of the 27 samples, seven were from ATL patients who experienced grade 2 or 3 rash (n = 5), SJS (n = 1), or nummular dermatitis (n = 1) in a phase 2 study of mogamulizumab.[1] Clinical characteristics of these seven patients are summarized in Table 1. Patients included all sub-types of aggressive ATL, and most of them had >5 doses of mogamulizumab before the skin biopsy, although the number of patients was small. Of the 7 patients, only patient No. 4 had ATL involvement in skin at the biopsy.

TABLE 1 Clinical characteristics of ATL patients with mogamulizumab-emergent skin disorders

Patient No.	Age	ATL subtype	ATL skin involvement before mogamulizumab doses	No. of mogamulizumab doses before skin biopsy	Skin disorder[a]	Worst grade[b]	Periods (Days) from the 1st mogamulizumab dose to skin biopsy	ATL skin involvement at the biopsy	Best overall response[c]
1	70s	Acute	Negative	8	SJS	3	76	Negative	CR
2	50s	Acute	Positive	8	Rash	2	58	Negative	PR
3	60s	Chronic, unfavorable	Positive	5	Rash	3	33	Negative	PR
4	60s	Chronic, unfavorable	Positive	7	Rash	2	49	Positive	PR
5	70s	Acute	Negative	8	Rash	2	52	Negative	CR
6	80s	Lymphoma	Negative	6	Rash	2	36	Negative	SD
7	60s	Lymphoma	Negative	6	Nummular dermatitis	2	43	Negative	PR

Abbreviations: ATL, adult T-cell leukemia-lymphoma; CR, complete response; PR, partial response; SD, stable disease; SJS, Stevens-Johnson syndrome.

[a]According to the Medical Dictionary for Regulatory Activities/Japanese version terminology.

[b]It was graded according to the National Cancer Institute Common Terminology Criteria for AEs, version 3.0.

[c]Objective responses were assessed after the fourth and eighth infusions of mogamulizumab according to the modified response criteria for ATL.

3.2 | Immunohistochemical staining of infiltrated Foxp3$^+$ and CD8$^+$ cells in skin

Representative immunohistochemical staining of infiltrated Foxp3+ and CD8+ cells in skin is shown in Figure S2. Mogamulizumab-emergent skin lesions (upper panel) showed a paucity of Foxp3$^+$ cells, and a predominant infiltration of CD8$^+$ cells was observed, while psoriasis vulgaris skins (lower panel) showed similar number of Foxp3$^+$ and CD8$^+$ cell infiltration (data not shown for atopic dermatitis and lichen planus).

3.3 | Quantitative analysis of infiltrated Foxp3$^+$, CD8$^+$, CD4$^+$, granzyme B$^+$, CD56$^+$, and MDC$^+$ cells in skin

Positively stained cells for each mAb were counted in epidermis, dermis, and basement membrane regions according to the Method. The cell count was summed up from all the selected regions. Mogamulizumab-emergent skin disorder had a lower number of infiltrated Foxp3$^+$ cells compared with psoriasis vulgaris and lichen planus skins, while significant CD8$^+$ cell infiltration was observed in mogamulizumab-emergent skin disorder compared with psoriasis vulgaris and atopic dermatitis skins (Figure 1A,B). However, the ratio of Foxp3$^+$/CD8$^+$ cells in mogamulizumab-emergent skin disorder was significantly lower than all of them (Figure 1C). In inflammatory skins, the more the number of CD8$^+$ cells were infiltrated, the more the number of Foxp3$^+$ cells were prone to be infiltrated, but not in skin disorders induced by mogamulizumab (Figure 1H).

Mogamulizumab-emergent skin disorder showed a significantly higher number of granzyme B$^+$ cells and CD56$^+$ cells compared with psoriasis vulgaris and atopic dermatitis skins, respectively (Figure 1E,F). No statistically significant difference was observed in the number of CD4$^+$ cells or MDC$^+$ cells between mogamulizumab-emergent and other inflammatory skin disorders (Figure 1D,G).

3.4 | Skin compartment analysis of infiltrated Foxp3$^+$ and CD8$^+$ cells

Infiltrated Foxp3$^+$ and CD8$^+$ cells were separately analyzed from each selected regions of epidermis, dermis, and basement membrane regions. Mogamulizumab-emergent skin disorders had a lower number of Foxp3$^+$ cells compared with those of psoriasis vulgaris, atopic dermatitis, and lichen planus skins. Especially, the decrease was statistically significant in dermis (v.s. psoriasis vulgaris, atopic dermatitis, and lichen planus) and basement membrane (v.s. psoriasis vulgaris and lichen planus) (Figure 2A). On the other hand, the increase in CD8$^+$ cells in mogamulizumab-emergent skin disorders compared with psoriasis vulgaris and atopic dermatitis skins was statistically significant in basement membrane (Figure 2B). Accordingly, the ratio of Foxp3$^+$/CD8$^+$ cells in mogamulizumab-emergent skin disorder of dermis and basement membrane was significantly lower than that observed in psoriasis vulgaris, atopic dermatitis, and lichen planus skins (Figure 2C). Although the Foxp3/CD8 ratio of epidermis was also low, the differences were not statistically significant.

4 | DISCUSSION

The paucity of Foxp3$^+$ cells and the predominant infiltration of CD8$^+$ cells resulted in a significantly lower Foxp3$^+$/CD8$^+$ cell ratio

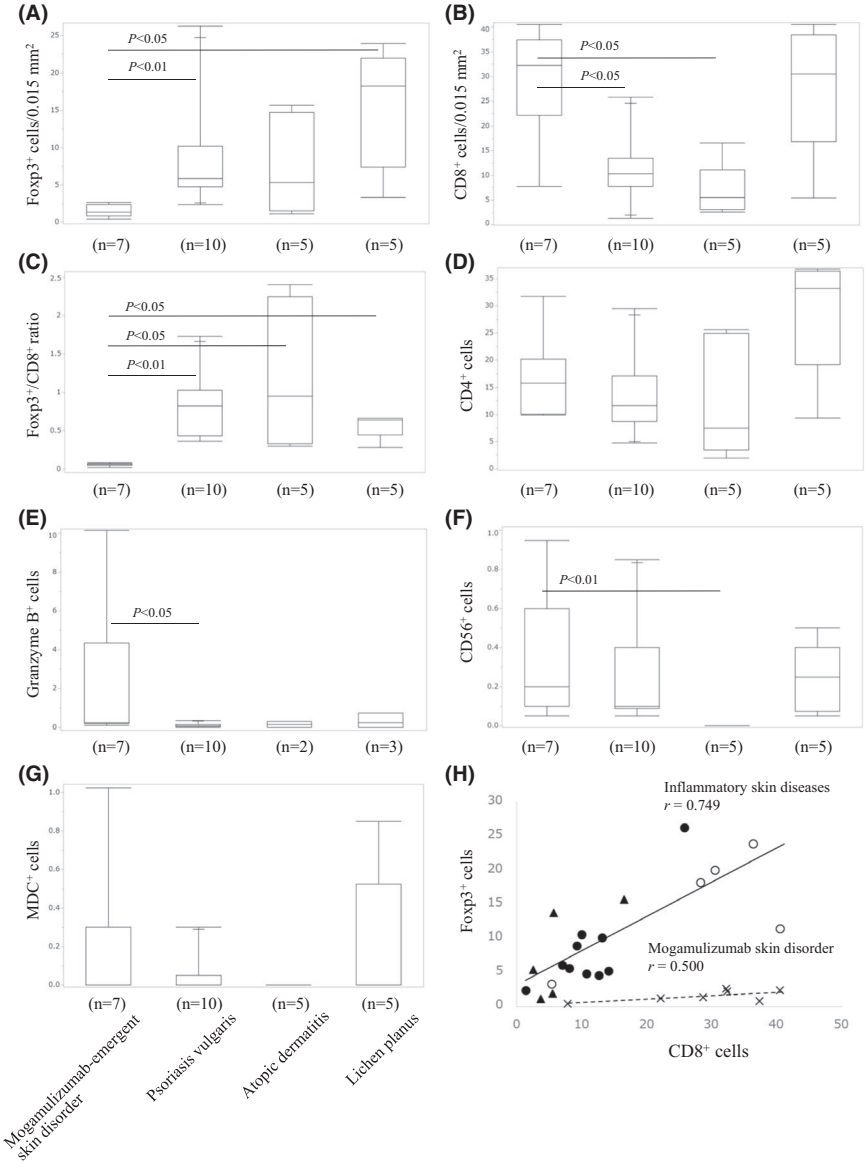

FIGURE 1 Quantitative analysis of infiltrated Foxp3$^+$, CD8$^+$, CD4$^+$, granzyme B$^+$, CD56$^+$, and MDC$^+$ cells. The number of (A) Foxp3$^+$, (B) CD8$^+$, (D) CD4$^+$, (E) granzyme B$^+$, (F) CD56$^+$, (G) MDC$^+$ cells, and (C) Foxp3$^+$/CD8$^+$ cell ratio per 0.015 mm^2 in skins from patients with mogamulizumab-emergent skin disorders, psoriasis vulgaris, atopic dermatitis, and lichen planus was shown, respectively. Box and whisker plot indicated quartiles with minimum and maximum values. P values were calculated by Steel test. (H) Correlation between number of Foxp3$^+$ cells and CD8$^+$ cells in inflammatory skin diseases (psoriasis vulgaris, atopic dermatitis, and lichen planus) and mogamulizumab-emergent skin disorders. Simple linear regression analysis was performed, and regression line was indicated in the graphs. Mogamulizumab-emergent skin disorders; cross. Psoriasis vulgaris; closed triangle. Atopic dermatitis; closed circle. Lichen planus; open circle

in mogamulizumab-emergent skin disorder compared with other inflammatory skin disorders evaluated in this study. This quantitative result was equivalent to previous case reports that qualitatively examined the immune status in mogamulizumab-emergent skin disorders.[2,3,5] No evident difference was observed between one SJS and the other mogamulizumab-emergent skin orders in the study (data not shown). Additionally, we demonstrated that the low Foxp3$^+$/CD8$^+$ cell ratio might be more evident in dermis and basement membrane compared with epidermis. This observation can make us consider that the primary site of mogamulizumab-emergent skin disorders may be dermis and/or basement membrane. However, the less evidence in epidermis may be due to the smaller region of analyzed area in epidermis compared with those in dermis and basement membrane.

While some studies have reported an increased number of Treg in psoriasis skin lesions, other reports are conflicting, and likewise, conflicting results have been observed with regard to the number of Treg in skin lesions from patients with atopic dermatitis.[6] Immune dysregulation polyendocrinopathy enteropathy X-linked syndrome, a rare disease involving Treg dysfunction due to germ line mutations in the *Foxp3* gene, often presents with eczema as one of the characteristic symptoms.[7] Additionally, in Treg-deficient Scurfy mice injected with α-1,3-fucosyltransferase VII-deficient Treg, which show impaired skin migration, Treg accumulation was reduced in the skin selectively, which in turn resulted in severe cutaneous inflammation.[8] These reports indicate that deficiency or dysfunction of Treg in the skin is closely related to onset of skin disorders. In our study, the absolute number of Treg in inflammatory skin diseases varied widely and was different across diseases; however, it had a positive correlation with that of CD8$^+$ cells, suggesting the possibility that more Treg was infiltrated into the lesion, likely to suppress inflammation involving CD8$^+$ cells. Conversely, Treg depletion in the peripheral blood induced by mogamulizumab was durable and profound in patients with ATL[1]; the number of Treg in mogamulizumab-emergent skin lesions was consistently very scarce, although abundant CD8$^+$ cell infiltration was observed,

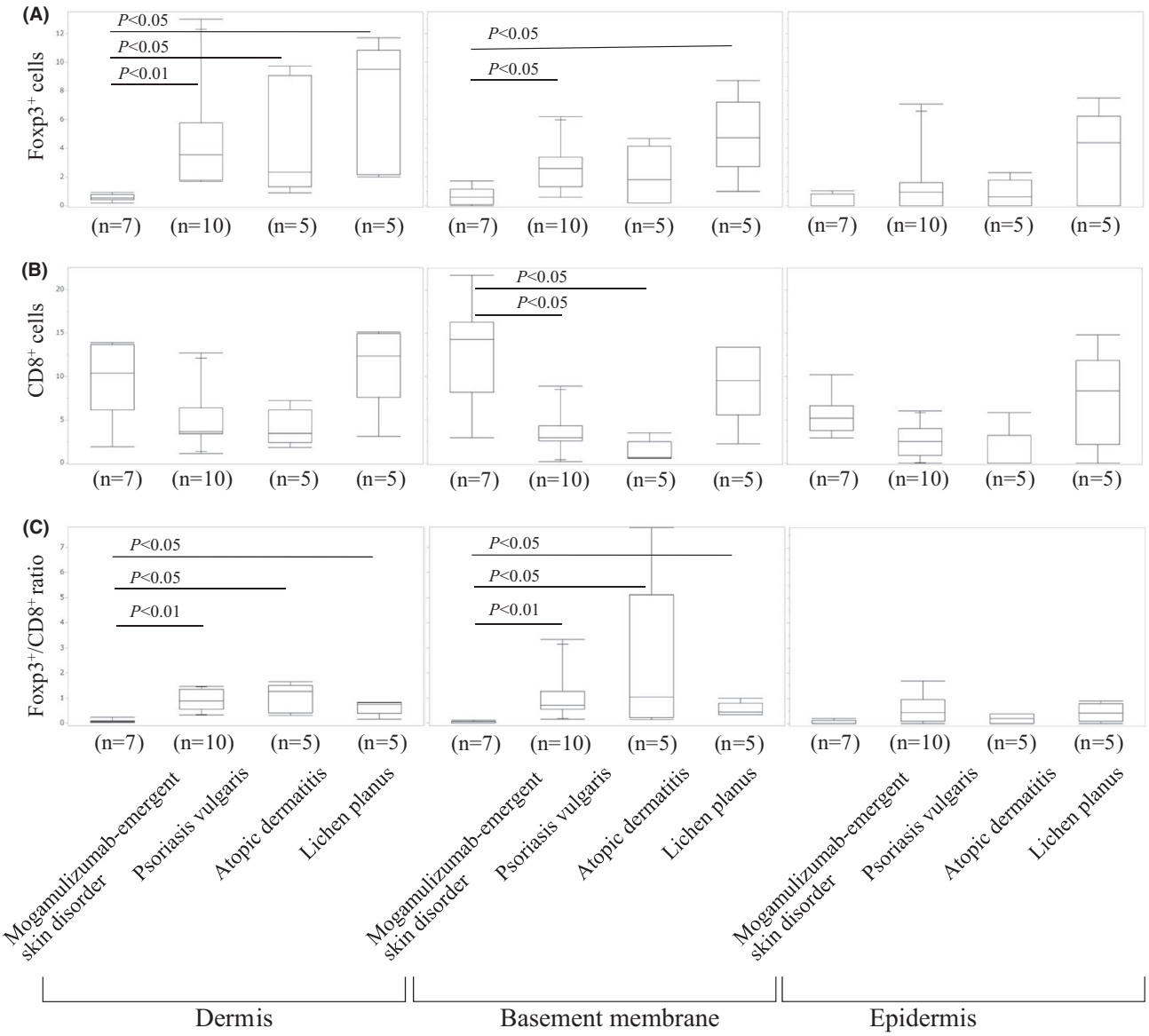

FIGURE 2 Skin compartment analysis of infiltrated Foxp3$^+$ and CD8$^+$ cells. The number of (A) Foxp3$^+$, (B) CD8$^+$, and (C) Foxp3$^+$/CD8$^+$ cell ratio per 0.005 mm^2 in each skin lesion from patients with mogamulizumab-emergent skin disorders, psoriasis vulgaris, atopic dermatitis, and lichen planus was shown, respectively. Box and whisker plot indicated quartiles with minimum and maximum values. *P* values were calculated by Steel test

which implies a loss of immune suppression in ameliorating an exaggerated immune response.

In human T-cell leukemia virus type 1 (HTLV-1) Tax transgenic mice, a significant increase in CD8$^+$ cells and decrease in Treg in the spleen were observed compared with non-transgenic mice,[9] and a majority of HTLV-1 basic leucine zipper factor transgenic mice were reported to develop skin inflammation due to dysfunction of CD4$^+$ Foxp3$^+$ Treg.[10] Furthermore, HTLV-1 infection has been reported to lead to reductions in Treg and increases in CD8$^+$ cells in skin lesions from patients with HTLV-1–associated infective dermatitis.[11] These findings allude that HTLV-1 infection or ATL disease itself may predispose mogamulizumab-treated patients to an exaggerated immune response in the skin. Indeed, the frequency of skin disorders induced by mogamulizumab in the ATL study was higher than that in the peripheral T-cell lymphoma/cutaneous T-cell lymphoma study: 67% vs. 51%, with grade 3/4 of 22% vs. 11%, respectively.[1,12]

In summary, the immune response in mogamulizumab-emergent skin disorders differed from that in other skin disorders. The low Foxp3$^+$/CD8$^+$ cell ratio, possibly in dermis and/or basement membrane, is the underlying reason for mogamulizumab-emergent skin disorder, suggesting that different management strategies may be needed for skin disorders induced by mogamulizumab compared with other inflammatory skin disorders, including autoimmune skin disorders.

ACKNOWLEDGEMENTS

We would like to thank Hisashi Takino for help with the immunohistochemical staining of samples. This study was funded by Kyowa Hakko Kirin Co. Ltd.

CONFLICT OF INTEREST

S. Yoshida received research grants from Kyowa Hakko Kirin during the conduct of the study. A. Utsunomiya received personal fees from Kyowa Hakko Kirin during the conduct of the study, and personal fees from Daiichi Sankyo, Siemens, Bristol-Myers Squibb, Pfizer, Novartis, Nippon Shinyaku, Mundipharma, Chugai Pharma, Ono Pharmaceutical, Eisai, Celgene, Otsuka Pharmaceutical, and JIMRO, outside the submitted work. S. Iida received research grants from Kyowa Hakko Kirin during the conduct of the study; research grants and personal fees from Ono Pharmaceutical, Takeda, Celgene, Janssen, Bristol-Myers Squibb, and Novartis; and research grants from Kyowa Hakko Kirin, Chugai, MSD, Daiichi Sankyo, Gilead, AbbVie, Astellas, and Teijin Pharma, outside the submitted work. T. Ishida received research grants and personal fees from Kyowa Hakko Kirin during the conduct of the study; research grants from Celgene and Bayer AG; and personal fees from Celgene and Mundipharma, outside the submitted work. S. Yurimoto is an employee and stockholder of Kyowa Hakko Kirin. Y. Suzuki, T. Ishii, M. Hiura, and T. Takahashi are employees of Kyowa Hakko Kirin. A. Ito, A. Masaki, H. Suzushima, S. Takemoto, H. Inagaki, and A. Morita have no conflicts to declare.

ORCID

Asahi Ito https://orcid.org/0000-0001-8082-8008

REFERENCES

1. Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, et al. Defucosylated anti-CCR1 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. J Clin Oncol. 2012;30:837–42.
2. Ishida T, Ito A, Sato F, Kusumoto S, Iida S, Inagaki H, et al. Stevens-Johnson syndrome associated with mogamulizumab treatment of adult T-cell leukemia/lymphoma. Cancer Sci. 2013;104:647–50.
3. Honda T, Hishizawa M, Kataoka T, Ohmori K, Takaori-Kondo A, Miyachi Y, et al. Stevens-Johnson syndrome associated with mogamulizumab-induced deficiency of regulatory T cells in an adult T-cell leukaemia patient. Acta Derm Venereol. 2015;95:606–7.
4. Iellem A, Mariani M, Lang R, Recalde H, Panina-Bordignon P, Sinigaglia F, et al. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR4 by CD4(+) CD25(+) regulatory T cells. J Exp Med. 2001;194:847–53.
5. Ureshino H, Shindo T, Nishikawa H, Watanabe N, Watanabe E, Satoh N, et al. Effector regulatory T cells reflect the equilibrium between antitumor immunity and autoimmunity in adult T-cell leukemia. Cancer Immunol Res. 2016;4:644–9.
6. Nedoszytko B, Lange M, Sokołowska-Wojdyło M, Renke J, Trzonkowski P, Sobjanek M, et al. The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part II: the Treg role in skin diseases pathogenesis. Postepy Dermatol Alergol. 2017;34:405–17.
7. Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci. 2018;1417:5–22.
8. Dudda JC, Perdue N, Bachtanian E, Campbell DJ. Foxp3+ regulatory T cells maintain immune homeostasis in the skin. J Exp Med. 2008;205:1559–65.
9. Ohsugi T, Kumasaka T. Low CD4/CD8 T-cell ratio associated with inflammatory arthropathy in human T-cell leukemia virus type I Tax transgenic mice. PLoS ONE. 2011;6:e18518.
10. Satou Y, Yasunaga J-I, Zhao T, Yoshida M, Miyazato P, Takai K, et al. HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation in vivo. PLoS Pathog. 2011;7:e1001274.
11. Torres-Cabala CA, Curry JL, Li Ning Tapia EML, Ramos C, Tetzlaff MT, Prieto VG, et al. HTLV-1-associated infective dermatitis demonstrates low frequency of FOXP3-positive T-regulatory lymphocytes. J Dermatol Sci. 2015;77:150–5.
12. Ogura M, Ishida T, Hatake K, Taniwaki M, Ando K, Tobinai K, et al. Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. J Clin Oncol. 2014;32:1157–63.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Ito A, Suzuki Y, Masaki A, et al. Quantitatively immunological characterization of mogamulizumab skin disorders in ATL patients. *J Cutan Immunol Allergy*. 2019;2:102–107. https://doi.org/10.1002/cia2.12070