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Original article

Relationships among plasma granzyme B level, pruritus and dermatitis in patients with atopic dermatitis

Yayoi Kamata, PhD^a, Utako Kimura, MD, PhD^b, Hironori Matsuda^a,
Suhandy Tenggara, MD, PhD^a, Atsuko Kamo, PhD^a, Yoshie Umehara, PhD^a,
Kyoichi Iizumi, PhD^a, Hiroaki Kawasaki, PhD^a, Yasushi Suga, MD, PhD^b,
Hideoki Ogawa, MD, PhD^a, Mitsutoshi Tominaga, PhD^a, Kenji Takamori, MD, PhD^{a,b,*}

^a Institute for Environmental and Gender-Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan

^b Department of Dermatology, Juntendo University Urayasu Hospital, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan

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ABSTRACT

Background: Atopic dermatitis (AD) is a multifactorial inflammatory skin disease characterized by skin barrier dysfunction, allergic inflammation and intractable pruritus resistant to conventional antipruritic treatments, including H₁-antihistamines. Granzymes (Gzms) are a family of serine proteases expressed by cytotoxic T lymphocytes and natural killer cells that have been shown to modulate inflammation. However, the relationship between Gzms and pathology in AD remains unclear.

Objective: This study assessed the correlation between plasma GzmB levels and severity of pruritus and dermatitis, in AD patients.

Methods: Plasma was collected from 46 patients with AD, 24 patients with psoriasis, and 30 healthy controls. AD severity was assessed with the scoring atopic dermatitis (SCORAD) index, psoriasis severity with the psoriasis area and severity index (PASI), and degree of pruritus by visual analogue scale (VAS) score. GzmA, GzmB and gastrin releasing peptide (GRP) levels were measured by enzyme-linked immunosorbent assays.

Results: Plasma GzmB concentrations were significantly higher in patients with AD and psoriasis than in healthy controls. Correlation analyses showed that plasma GzmB concentrations positively correlated with SCORAD and serum levels of severity markers such as thymus and activation-regulated chemokine, and lactate dehydrogenase in AD patients. Moreover, plasma levels of GRP, an itch-related peptide, were higher in patients with AD, positively correlating with VAS score and plasma GzmB level. In addition, plasma GzmB concentration was significantly lower in the treatment group than the untreated group with AD. Meanwhile, there were no correlations among GzmB levels, VAS score and PASI score in patients with psoriasis. In contrast to the results of plasma GzmB, plasma GzmA levels were unchanged among AD, psoriasis and healthy groups, and showed no correlations with VAS score and SCORAD index in patients with AD.

Conclusion: Plasma GzmB levels may reflect the degree of pruritus and dermatitis in patients with AD.

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Abbreviations: AD, atopic dermatitis; CTLs, cytotoxic T lymphocytes; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GRP, gastrin releasing peptide; Gzm, granzyme; PASI, psoriasis area and severity index; SCORAD, scoring atopic dermatitis; VAS, visual analogue scale.

* Corresponding author at: Institute for Environmental and Gender-Specific Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Tomioka, Urayasu, Chiba 279-0021, Japan.

E-mail addresses: tominaga@juntendo.ac.jp (M. Tominaga),

ktakamor@juntendo.ac.jp (K. Takamori).

1. Introduction

Atopic dermatitis (AD) is a multifactorial inflammatory skin disease characterized by impaired skin barrier and allergic inflammation. Pruritus (or itch) is also a major symptom of AD, but the pruritus is frequently resistant to conventional treatments, such as H₁-antihistamines [1,2]. Currently, such pruritus is a clinical problem that reduces the quality of life in patients with AD worldwide.

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Endogenous proteases (e.g. kallikreins, matriptase and tryptase) and exogenous enzymes (from, e.g., house-dust mite allergens, bacteria, fungi and plants), can activate protease activated receptor (PAR) 2 and induce itch and inflammation in lesional skin of patients with AD [3–6]. Several serine proteases (e.g. tryptase, chymase and kallikreins) have been identified as itch-related mediators *in vivo* [3,7]. Granzymes (Gzms) are a family of serine proteases stored in natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). Gzms A, B, H, K and M have been identified in humans [8]. Gzms A, a trypsin-like enzyme, and B, a chymase-like enzyme, are the most important members of the Gzm family, especially for induction of target cell death [9]. GzmB has the strongest apoptotic activity of all granzymes [10]. In addition to lymphocytes, both ultraviolet A and B irradiation induce GzmB expression in keratinocytes *in vitro* and *in vivo* [11,12], and mouse and human mast cells produce GzmB *in vitro* and *in vivo* [11]. GzmB also functions as processing protease of cytokines, including interleukin (IL)-18 and IL-1 α , and extracellular matrix proteins, including fibronectin and various proteoglycans [8,13]. Higher numbers of GzmB-positive CD4⁺ and CD8⁺ T cells have been observed in the lesional skin of patients with AD and psoriasis than in non-lesional and healthy skin [14,15]. Although GzmB may be involved in pruritus and inflammation of AD, the roles of GzmB in the pathology of AD have not been fully determined.

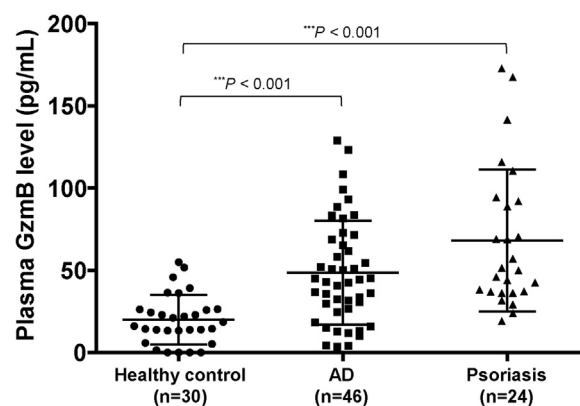


Fig. 1. Quantification of GzmB levels in plasma of patients with AD and psoriasis and healthy controls. Plasma GzmB concentrations in 46 AD patients, 24 psoriatic patients and 30 healthy controls were determined by enzyme-linked immunosorbent assays. Data were evaluated by Dunnett's multiple comparison tests. *** $P < 0.001$.

This study investigated the correlation between plasma GzmB concentrations and the degree of itch and dermatitis, in patients with AD and psoriasis. We here describe that close relationships among plasma GzmB concentrations and severity of pruritus and

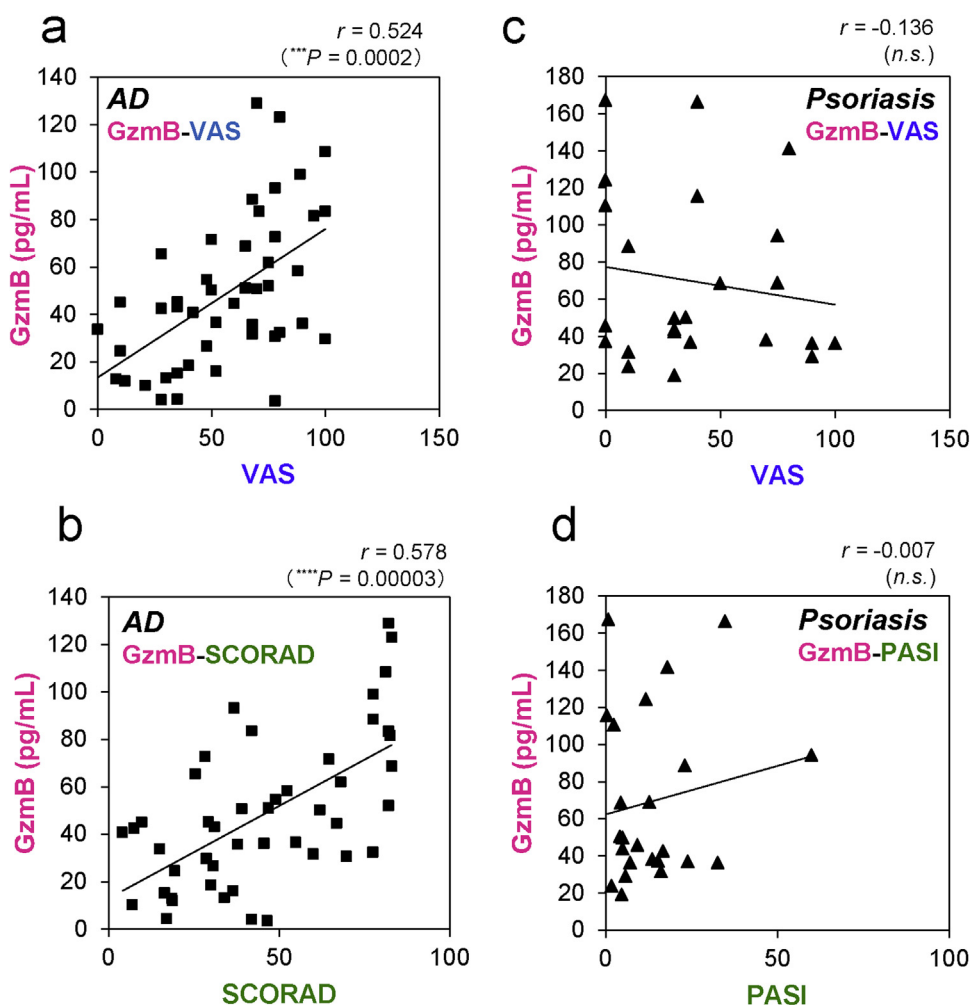


Fig. 2. Scatterplots of correlations among plasma levels of GzmB, VAS score and severity index in patients with AD and psoriasis. Correlations of plasma GzmB level with (a) VAS score and (b) SCORAD in 46 patients with AD; and of plasma GzmB level with (c) VAS score and (d) PASI score in 24 patients with psoriasis. Coefficients (r) were determined by Spearman rank correlation test. $P < 0.05$ indicated statistical significance.

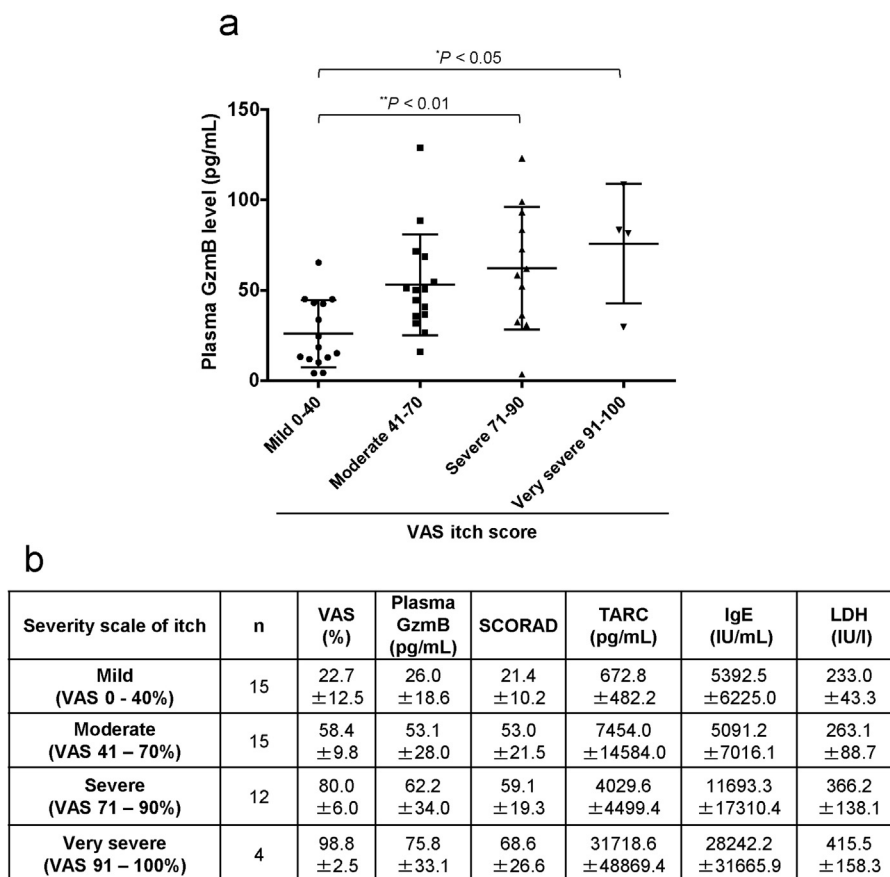


Fig. 3. Relationship between plasma GzmB concentration and severity of itch. VAS score was categorized into 4 grades; 0–40% (mild pruritus, $n = 15$), 41–70% (moderate pruritus, $n = 15$), 71–90% (severe pruritus, $n = 12$) and 91–100% (very severe pruritus, $n = 4$). (a) Plasma GzmB levels in the patients with AD was classified into each grade of VAS score. Data were evaluated by Dunnett's multiple comparison tests. $^{**}P \leq 0.01$ and $^{*}P \leq 0.05$. (b) Plasma GzmB level, SCORAD index, serum TARC, total IgE and LDH levels were classified into each grade of VAS score.

severity of dermatitis, were observed in patients with AD, but not with psoriasis.

2. Materials and methods

2.1. Human samples

Plasma was collected from 46 patients with AD (29 males, 17 females; mean $\pm$ SD age, 37.7 ± 11.0 years), 24 patients with psoriasis (18 males, 6 females; mean $\pm$ SD age, 51.4 ± 20.1 years) and 30 healthy controls (19 males, 11 females; mean $\pm$ SD age, 41.1 ± 12.9 years) using a blood sampling tube containing ethylenediaminetetraacetic acid (EDTA) or heparin (Terumo, Tokyo, Japan). AD was diagnosed clinically based on Hanifin and Rajka criteria [16], and its severity was assessed with the scoring atopic dermatitis index (SCORAD; 0–103 points) [17] and clinical markers (serum total IgE level, serum thymus and activation-regulated chemokine (TARC) level, and lactate dehydrogenase (LDH) level). Serum levels of total IgE, TARC and LDH were obtained from clinical laboratory tests. Psoriasis severity was assessed with the psoriasis area and severity index (PASI; 0–72 points) [18]. The degree of pruritus was evaluated by visual analogue scale (VAS; 0–100%) score [19]. None of the subjects had received oral immunosuppressant (e.g. cyclosporine) or monoclonal antibody therapy (e.g. infliximab and secukinumab) before blood sampling. The study protocol was approved by the medical ethics committee of Juntendo University Urayasu Hospital, and all patients provided written informed consent. This study was conducted according to the principles of the Declaration of Helsinki.

2.2. Enzyme-linked immunosorbent assay (ELISA)

Plasma concentrations of GzmA, GzmB, and gastrin-releasing peptide (GRP) were measured using commercial GzmA, GzmB, and GRP ELISA kits (Usen Life Sciences, Wuhan, China), respectively, according to the manufacturer's instruction. As GRP levels could not be accurately measured in EDTA-plasma, it was measured in heparin-plasma.

2.3. Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Data were evaluated by Dunnett's multiple comparison tests and Student's t -tests and correlation coefficients (r) by Spearman rank correlation test. Correlations were deemed weak for r from 0.2 to 0.4 and moderate for r from above 0.4 to 0.7, with $P < 0.05$ indicating statistical significance.

3. Results

3.1. Relationships among plasma GzmB levels, pruritus and inflammation

In the patients with AD, VAS score was $55.9 \pm 27.8\%$ (mean $\pm$ SD) and SCORAD index was 45.7 ± 24.9 points (mean $\pm$ SD). Serum levels of total IgE, TARC and LDH in the AD patients were 8924.9 ± 14551.2 IU/mL (mean $\pm$ SD), 6526.1 ± 17316.3 pg/mL, 293.4 ± 116.7 IU/L (mean $\pm$ SD), respectively. In the patients with

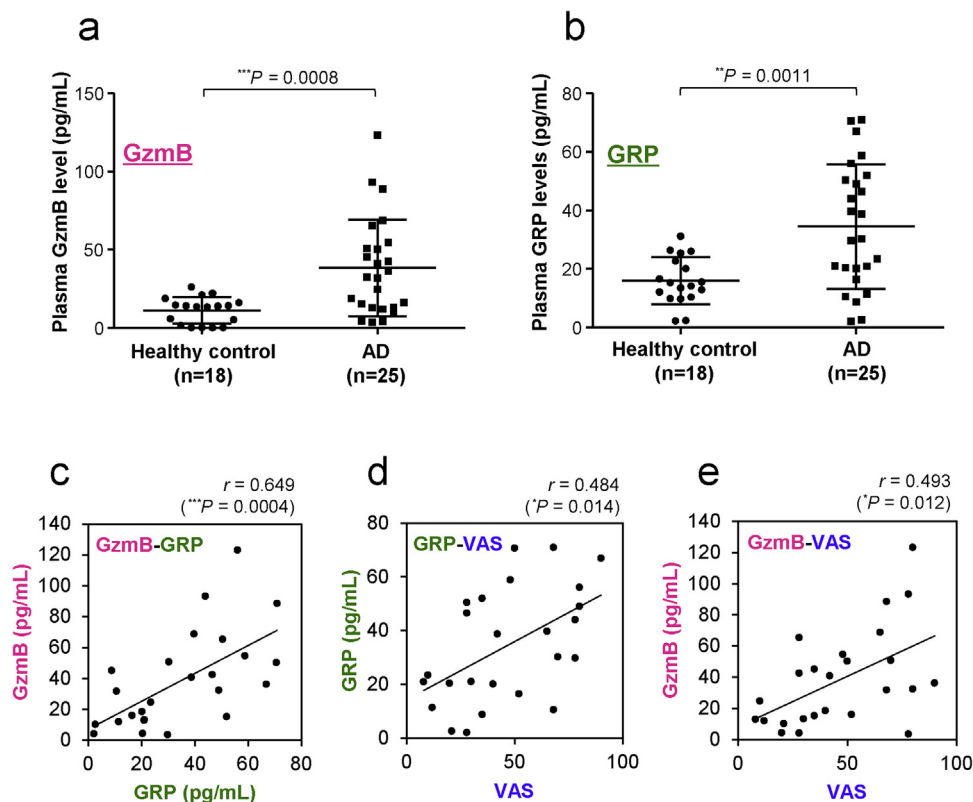


Fig. 4. Correlation of plasma GRP level with GzmB level and VAS score in patients with AD. Plasma GzmB (a) and GRP (b) levels in heparin-treated plasma of 25 AD patients and 18 healthy controls were measured by enzyme-linked immunosorbent assays. Data were evaluated by Student's *t*-tests. Scatterplots of correlations between (c) plasma GzmB and GRP levels; (d) plasma GRP level and VAS itch score; and (e) plasma GzmB level and VAS score. Coefficients (*r*) were determined by Spearman rank correlation tests. *P* < 0.05 indicated statistical significance.

psoriasis, VAS score was $38.8 \pm 32.8\%$ (mean $\pm$ SD) and the PASI score was 13.8 ± 13.7 points (mean $\pm$ SD).

Plasma GzmB concentrations were significantly higher in patients with AD (50.4 ± 33.4 pg/mL, *P* < 0.001) and psoriasis (69.5 ± 45.5 pg/mL, *P* $\leq$ 0.001) than in healthy controls (20.6 ± 15.9 pg/mL) (Fig. 1). In contrast, plasma GzmA concentrations did not differ significantly in patients with AD (114.0 ± 98.3 pg/mL) and psoriasis (90.0 ± 65.0 pg/mL) and in healthy controls (87.4 ± 55.5 pg/mL) (Supplementary Fig. 1).

In AD patients, plasma GzmB concentrations were positively correlated with VAS score (*r* = 0.524, *P* = 0.0002), (Fig. 2a) and were significantly higher in patients with severe (VAS score, 71–90%) and very severe pruritus (VAS score, $\geq 91\%$) than in patients with mild pruritus (VAS score, 0–30%) (Fig. 3). In addition, SCORAD index (*r* = 0.578, *P* = 0.00003), serum TARC level (*r* = 0.364, *P* = 0.018) and serum LDH level (*r* = 0.530, *P* = 0.0002) were also correlated with plasma GzmB concentrations (Fig. 2b and Suppl. Fig. 2). However, there were no correlations among GzmB levels, VAS score (Fig. 2c) and PASI score (Fig. 2d) in patients with psoriasis.

3.2. Correlation of plasma GzmB level with plasma GRP level in AD patients

Heparin-treated plasma for measurement of plasma GRP concentrations were collected from 25 patients with AD (21 males, 4 females; mean $\pm$ SD age, 35.6 ± 9.5 years), with a VAS score of $46.6 \pm 25.0\%$ (mean $\pm$ SD) and a SCORAD index of 38.6 ± 24.0 points (mean $\pm$ SD); and from 18 healthy controls (14 males, 4 females; mean $\pm$ SD age, 40.8 ± 15.0 years). Assays in these patient subsets showed that the GzmB concentration was significantly higher in AD patients than in healthy controls (38.3 ± 31.0 pg/mL vs. 11.6 ± 8.5 pg/mL, *P* = 0.0008) (Fig. 4a).

Similarly, plasma GRP concentration was significantly higher in AD patients than in healthy controls (34.5 ± 21.3 pg/mL vs. 16.0 ± 8.1 pg/mL, *P* = 0.0011) (Fig. 4b). Plasma GRP concentration correlated positively with plasma GzmB concentration (*r* = 0.649, *P* = 0.0004) (Fig. 4c) and VAS score (*r* = 0.484, *P* = 0.014) (Fig. 4d), and plasma GzmB concentration correlated positively with VAS score (*r* = 0.493, *P* = 0.012) (Fig. 4e). In contrast, there was no correlation between plasma GzmA and GRP concentrations (*r* = −0.178) (data not shown).

3.3. Change of plasma Gzm B levels after treatment of AD

Plasma from AD patients was divided into two groups, non-treatment (*n* = 25) and treated groups (*n* = 21). After treatment with oral *H*₁-antihistamines (e.g. levocetirizine, fexofenadine and olopatadine), leukotriene receptor antagonist (montelukast), Th2 cytokine inhibitor (suplatast), betamethasone/d-chlorpheniramine (Celestamine[®]), tacrolimus ointment and/or topical corticosteroids, plasma GzmB levels were significantly decreased in the treated group compared with the non-treatment group (33.8 ± 22.8 pg/mL vs. 66.3 ± 31.9 pg/mL, *P* = 0.0002) (Fig. 5a). Serum LDH level (249.2 ± 74.5 IU/L vs. 346.1 ± 136.5 IU/L, *P* = 0.004; Fig. 5b), TARC level (802.6 ± 661.6 pg/mL vs. 12822.1 ± 24466.5 pg/mL *P* = 0.03; data not shown) and VAS score ($38.8 \pm 24.9\%$ vs. $76.2 \pm 14.0\%$, *P* $\leq$ 0.0001; Fig. 5c) was also significantly decreased in the treated group compared with the non-treatment group.

4. Discussion

Natural sources of Gzms are restricted to peripheral blood CTLs and NK cells [8,9,13]. It has been previously reported that GzmB-

expressing cells are increased in lesions of patients with inflammatory skin diseases, including AD, psoriasis, allergic contact dermatitis and pityriasis rosea compared with healthy skin [12,15]. This study showed that plasma GzmB levels were higher in patients with AD and psoriasis than in healthy controls (Fig. 1). Notably, plasma GzmB levels in patients with AD were also significantly and positively correlated with VAS score (Figs. 2 a and 4 e) and plasma GRP levels (Fig. 4c). The plasma GzmB concentration gradually increased with increasing VAS score (Fig. 3), and both GzmB and VAS score decreased after treatment

of AD (Fig. 5a and c). The serum levels of GRP, an itch-related peptide [20–22], were previously shown to correlate with disease severity and pruritus in patients with AD [23,24]. GRP has been shown to play a key role in mediating itch sensation by interacting with GRP receptor-expressing neurons in the spinal dorsal horn [2,20–22]. These findings suggest that plasma GzmB levels may objectively reflect the degree of pruritus in patients with AD, although it has not been determined whether GzmB evokes scratching behavior *in vivo*.

Of the members of the Gzm family, GzmB exerts the strongest apoptotic activity, inducing caspase-dependent or -independent apoptosis in virus-infected and other potentially harmful cells [8,10]. Generally, an increased level of GzmB in lesional tissue has been linked to the pathogenesis of allergy and inflammatory and/or autoimmune diseases, including AD, allergic asthma and rheumatoid arthritis [14,15,25,26]. Physiologically, GzmB has been reported to promote aging of the skin followed by cleavage of extracellular matrix protein after ultraviolet irradiation [11,13], and to destroy joints by mediating proteoglycan degradation in rheumatoid arthritis [26]. In the patients with AD, GzmB expressing cells are increased in the dermis, dermoepidermal junction area and the site of spongiosis in the epidermis compared with normal skin [14]. In this study, we found a positive correlation among plasma GzmB level, SCORAD index (Fig. 2b) and serum levels of TARC or LDH in the patients with AD (Supplementary Fig. 2), whereas no positive correlation between plasma GzmB and PASI score in patients with psoriasis (Fig. 2d). A recent study has demonstrated that the frequency of CD4⁺CD28^{null} cells expressing cytotoxic granules (e.g. Gzms and perforin) were increased in the peripheral blood of psoriasis patients, although there was no positive correlation between CD4⁺CD28^{null} cells and PASI [27]. In addition, GzmB expressing cells were increased in the dermis of psoriasis, and they were significantly lower as compared with that in lesional skin with AD [14]. These differences may explain the lack of correlation of plasma GzmB concentration with pruritus and disease severity in patients with psoriasis.

GzmB is shown to play a major role in apoptosis [13]. Apoptotic cells have been detected in the lesional epidermis of patients with acute AD and contact dermatitis. Keratinocyte apoptosis is the initiating event in the development of the epidermal pathology in patients with eczematous dermatitis [28]. In fact, both plasma GzmB and clinical markers of AD (e.g. LDH) decreased after treatment of AD (Fig. 5a and b). These findings may explain the correlation between plasma GzmB level and the degree of skin inflammation in patients with AD. In contrast, apoptosis has been reported suppressed in psoriatic lesions, a finding that may be related to the characteristics of psoriatic histopathology [29]. Thus, other pathogenic factors might be related to the severity of psoriasis.

In conclusion, this study has shown that plasma GzmB concentrations correlate with the severity of pruritus and dermatitis in patients with AD, but not those with psoriasis. GzmB may play an important role in the induction of intractable itch and dermatitis in AD, and may reflect the degree of pruritus and dermatitis in patients with AD.

Conflict of interest

The authors have no conflict of interest to declare.

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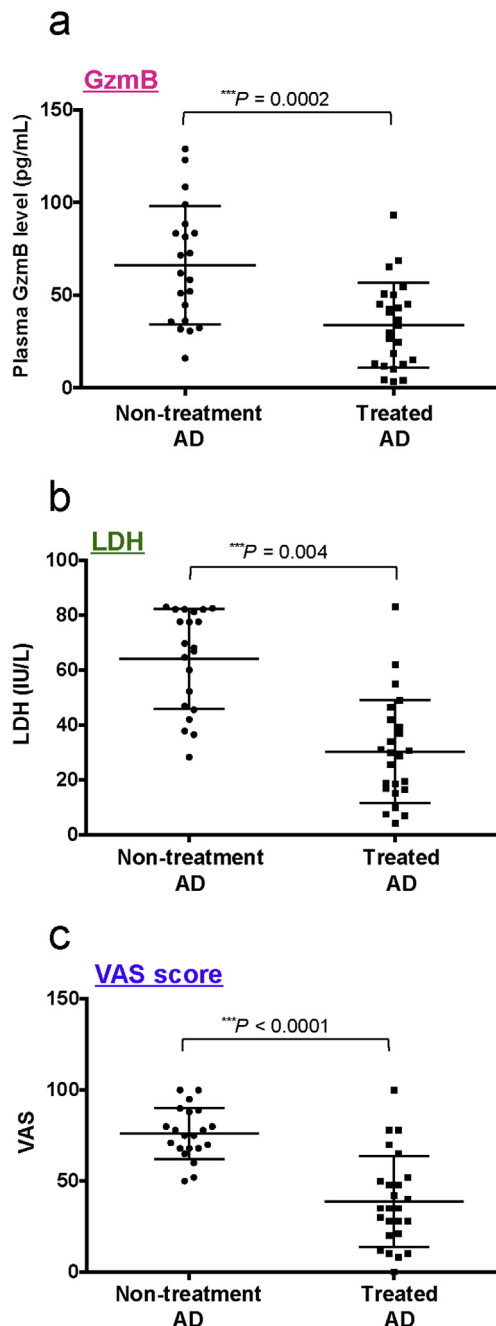


Fig. 5. Comparison of plasma GzmB levels in treated and non-treated patients with AD. Plasma GzmB level (a), serum LDH level (b) and VAS score (c) decreased in treated patients ($n = 25$) compared with non-treatment patients ($n = 21$) with AD. Data were evaluated by Student's *t*-tests. $P \leq 0.05$ indicated statistical significance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2016.09.009>.

References

- [1] A. Ikoma, M. Steinhoff, S. Ständer, G. Yosipovitch, M. Schmelz, The neurobiology of itch, *Nat. Rev. Neurosci.* 7 (2006) 535–547.
- [2] M. Tominaga, K. Takamori, Itch and nerve fibers with special reference to atopic dermatitis: therapeutic implications, *J. Dermatol.* 41 (2014) 205–212.
- [3] C. Kempkes, J. Buddenkotte, F. Cevikbas, T. Buhl, M. Steinhoff, Role of PAR-2 in neuroimmune communication and itch, in: E. Carstens, T. Akiyama (Eds.), *Itch: Mechanisms and Treatment*, CRC Press, 2014, pp. 193–212.
- [4] M.J. Cork, S.G. Danby, Y. Vasilopoulos, J. Hadgraft, M.E. Lane, M. Moustafa, R.H. Guy, A.L. Macgowan, R. Tazi-Ahni, S.J. Ward, Epidermal barrier dysfunction in atopic dermatitis, *J. Invest. Dermatol.* 129 (2009) 1892–1908.
- [5] M. Steinhoff, C.U. Corvera, M.S. Thoma, W. Kong, B.E. McAlpine, G.H. Caughey, J. C. Ansel, N.W. Bunnett, Proteinase-activated receptor-2 in human skin: tissue distribution and activation of keratinocytes by mast cell tryptase, *Exp. Dermatol.* 8 (1999) 282–294.
- [6] S.J. de Veer, L. Furio, J.M. Harris, A. Hovnanian, Proteases and proteomics: cutting to the core of human skin pathologies, *Proteomics Clin. Appl.* 8 (2014) 389–402.
- [7] L. Hansson, A. Bäckman, A. Ny, M. Edlund, E. Ekholm, B. Ekstrand Hammarström, J. Törnell, P. Wallbrandt, H. Wennbo, T. Egelrud, Epidermal overexpression of stratum corneum chymotryptic enzyme in mice: a model for chronic itchy dermatitis, *J. Invest. Dermatol.* 118 (2002) 444–449.
- [8] O. Susanto, J.A. Trapani, D. Brasacchio, Controversies in granzyme biology, *Tissue Antigens* 80 (2012) 477–487.
- [9] C.J. Froelich, B. Granzyme, in: N.D. Rawlings, G. Salvesen (Eds.), *Handbook of Proteolytic Enzymes*, 3rd edn., Elsevier Ltd, 2013, pp. 2718–2721.
- [10] J.A. Trapani, Granzymes: a family of lymphocyte granule serine proteases, *Genome Biol.* 2 (2001) (reviews3014).
- [11] L.G. Parkinson, A. Toro, H. Zhao, K. Brown, S.J. Tebbutt, D.J. Granville, B. Granzyme, mediates both direct and indirect cleavage of extracellular matrix in skin after chronic low-dose ultraviolet light irradiation, *Aging Cell* 14 (2015) 67–77.
- [12] H. Hernandez-Pigeon, C. Jean, A. Charruyer, M.J. Haure, C. Baudouin, M. Charveron, A. Quillet-Mary, G. Laurent, UVA induces granzyme B in human keratinocytes through MIF: implication in extracellular matrix remodeling, *J. Biol. Chem.* 282 (2007) 8157–8164.
- [13] P.R. Hiebert, D.J. Granville, B. Granzyme, in injury, inflammation, and repair, *Trends Mol. Med.* 18 (2012) 732–741.
- [14] N. Yawalkar, S. Schmid, L.R. Braathen, W.J. Pichler, Perforin and granzyme B may contribute to skin inflammation in atopic dermatitis and psoriasis, *Br. J. Dermatol.* 144 (2001) 1133–1139.
- [15] M.R. Hussein, W.M. Abdel-Magid, R. Saleh, E. Nada, Phenotypical characteristics of the immune cells in allergic contact dermatitis, atopic dermatitis and pityriasis rosea, *Pathol. Oncol. Res.* 15 (2009) 73–79.
- [16] J.M. Hanifin, G. Rajka, Diagnostic features of atopic dermatitis, *Acta Derma Venereol.* 92 (1980) 44–47.
- [17] European task force on atopic dermatitis: severity scoring of atopic dermatitis: the SCORAD index, *Dermatology* 186 (1993) 23–31.
- [18] T. Fredriksson, U. Pettersson, Severe psoriasis-oral therapy with a new retinoid, *Dermatologica* 157 (1978) 238–244.
- [19] A. Reich, M. Heisig, N.Q. Phan, K. Taneda, K. Takamori, S. Takeuchi, M. Furue, C. Blome, M. Augustin, S. Ständer, J.C. Szepietowski, Visual analogue scale: evaluation of the instrument for the assessment of pruritus, *Acta Dermatol. Venereol.* 92 (2012) 497–501.
- [20] M. Tominaga, K. Takamori, An update on peripheral mechanisms and treatments of itch, *Biol. Pharm. Bull.* 36 (2013) 1241–1247.
- [21] Y.G. Sun, Z.F. Chen, A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord, *Nature* 448 (2007) 700–703.
- [22] M. Tominaga, H. Ogawa, K. Takamori, Histological characterization of cutaneous nerve fibers containing gastrin-releasing peptide in NC/Nga mice: an atopic dermatitis model, *J. Invest. Dermatol.* 129 (2009) 2901–2905.
- [23] S. Kagami, M. Sugaya, H. Suga, S. Morimura, H. Kai, H. Ohmatsu, H. Fujita, Y. Tsunemi, S. Sato, Serum gastrin-releasing peptide levels correlate with pruritus in patients with atopic dermatitis, *J. Invest. Dermatol.* 133 (2013) 1673–1675.
- [24] A. Tirado-Sánchez, A. Bonifaz, R.M. Ponce-Olivera, Serum gastrin-releasing peptide levels correlate with disease severity and pruritus in patients with atopic dermatitis, *Br. J. Dermatol.* 173 (2015) 298–300.
- [25] K. Bratke, B. Böttcher, K. Leeder, S. Schmidt, M. Küpper, J.C. Virchow Jr., W. Luttmann, Increase in granzyme B⁺ lymphocytes and soluble granzyme B in bronchoalveolar lavage of allergen challenged patients with atopic asthma, *Clin. Exp. Immunol.* 136 (2004) 542–548.
- [26] T.J. Smeets, R.J. Dolhain, F.C. Breedveld, P.P. Tak, Analysis of the cellular infiltrates and expression of cytokines in synovial tissue from patients with rheumatoid arthritis and reactive arthritis, *J. Pathol.* 186 (1998) 75–81.
- [27] X.T. Lima, M.L. Cintra, A.C. Piazza, R.L. Mamoni, R.T. Oliveira, R.F. Magalhães, M. H. Blotta, Frequency and characteristics of circulating CD4(+) CD28(null) T cells in patients with psoriasis, *Br. J. Dermatol.* 173 (October (4)) (2015) 998–1005.
- [28] A. Trautmann, M. Akdis, D. Kleemann, F. Altnauer, H.U. Simon, T. Graeve, M. Noll, E.B. Bröcker, K. Blaser, C.A. Akdis, T cell-mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis, *J. Clin. Invest.* 106 (2000) 25–35.
- [29] H. Takahashi, A. Manabe, A. Ishida-Yamamoto, Y. Hashimoto, H. Iizuka, Aberrant expression of apoptosis-related molecules in psoriatic epidermis, *J. Dermatol. Sci.* 28 (2002) 187–197.