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Sweat allergy: extrinsic or intrinsic?

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Abstract

Sweat has been recognized as an exacerbation factor in all age groups of atopic dermatitis (AD). Elevation of body core temperature with sweating is a trigger of cholinergic urticaria (CholU). Recently, we reported the improvement of AD symptoms by spray containing tannic acid, which suppresses basophil histamine release by semi-purified sweat antigen *in vitro*, and by showering that removes antigens in sweat from the skin surface. Sweat contains small amount of proteins including proteases,

protease inhibitors, and anti-microbial peptides. We finally identified MGL_1304 secreted by *Malassezia (M.) globosa* as a major histamine releasing antigen in human sweat. MGL_1304 is detected as a 17-kDa protein in sweat and exhibits almost the highest histamine release ability from basophils of patients with AD and CholU among antigens derived from *Malassezia* species. Moreover, serum levels of anti-MGL_1304 IgE of patients with AD and CholU were significantly higher than those of normal controls. The recombinant protein produced by *Pichia pastoris* possessed comparable allergenicity to native MGL_1304. We found a monoclonal IgE antibody against MGL_1304 which did not elicit histamine release from sensitized mast cells. Desensitization therapy using autologous sweat, or MGL_1304 purified from culture of *M. globosa* or its cognates might be beneficial for patients with intractable CholU due to sweat allergy. (207<250 words)

Abbreviations

AD: atopic dermatitis; CholU: cholinergic urticaria

Key words

sweat, atopic dermatitis, cholinergic urticaria, *Malassezia globosa*, MGL_1304

Contents

1. Introduction

2. Background of sweat allergy

3. Contents of eccrine sweat

4. Identification of MGL_1304 in sweat

5. Clinical applications of MGL_1304

5-1. ELISA system measuring MGL_1304-specific immunoglobulins

5-2. Generation of recombinant MGL_1304 by *Pichia pastoris*

5-3. A monoclonal IgE antibody against MGL_1304

6. Coping techniques with sweat allergy

6-1. Sweat allergy in AD

6-2. Sweat allergy in CholU

7. Concluding remarks

Acknowledgments

References

1. Introduction

Atopic dermatitis (AD) is a chronic relapsing eczematous skin disease characterized by pruritus and inflammation, accompanied by cutaneous physiological dysfunction (dry and barrier-disrupted skin) [1]. Sweat is considered as one of the major exacerbation factors for atopic dermatitis across all age groups [2]. It might account for characteristic distribution of eczematous lesions in sweat-susceptible areas (such as cubital and popliteal fossa). On the other hand, sweat constitutes natural moisturizing factors and maintain the physiological hydration state of stratum corneum [3]. Cholinergic urticaria (CholU) is a subtype of chronic inducible urticarias, symptoms of which are evoked by sweating due to the elevation of body core temperature [4, 5]. Although the pathogenesis of CholU has not been fully clarified, approximately half of the patients with CholU are known to possess atopic diathesis [6].

2. Background of sweat allergy

Sweat allergy, hypersensitivity against one's own sweat, was first described in the literature written in English, by Adachi et al. in 1989. They demonstrated hypersensitivity against sweat in patients with AD [7] and subsequently CholU [8] by performing intradermal skin tests with autologous sweat. A representative positive reaction of an intradermal skin test with autologous sweat is shown in Fig. 1A. In 2002,

we reported that patients with AD possessed hypersensitivity against crude and semi-purified sweat by performing intradermal skin tests and basophil histamine release tests (HRTs) [9]. We further purified human sweat from healthy volunteers by histamine-releasing activity on basophils obtained from patients with AD, and demonstrated that histamine release induced by the semi-purified sweat antigen is dependent on specific IgE in the sera of patients with AD [10]. These observations proved sweat allergy as a type I hypersensitivity against crude sweat or ingredients in sweat. The positive rates of sweat allergy in AD and CholU were 77-96% and 66-100%, respectively, as shown in Table 1. The antigen lost its histamine releasing activity following treatment with proteinase K or trypsin [10], suggesting it was protein. The sera of patients with positive HRTs against semi-purified sweat antigen also possessed histamine release neutralization activity due to either IgE and/or IgG antibody against sweat antigen [11]. However, the substance which induces histamine release from basophils of patients with AD and CholU had not been identified for more than two decades since the first report by Adachi et al. [7].

3. Contents of eccrine sweat

Human eccrine sweat consists of water, sodium, potassium, bicarbonate, chloride, lactate, urea, ammonia, small quantities of various amino acids, and proteins [12]. Proteins in eccrine sweat consist of proteases (e.g. kallikreins), protease inhibitors (e.g. cystatins), and anti-microbial peptides (e.g. dermocidin). Besides substances that induce the immediate hypersensitivity reaction, proinflammatory cytokines (IL-1 α , IL-1 β , and IL-31) are contained in human sweat [13]. It was also reported that the concentration of nickel in sweat [14] and serum [15] was higher in patients with intrinsic AD than that of patients with extrinsic AD. Moreover, the positive rate of patch testing to nickel and cobalt was high in intrinsic AD. Thus, nickel excreted in sweat might be an exacerbation factor for intrinsic AD. Recently, Ilves et al. reported that the immediate reaction to autologous sweat was associated with clinical severity, serum total and specific IgE and sweat tryptase activity [16].

4. Identification of MGL_1304 in sweat

A certain population of patients with AD show IgE reactivity to a variety of human protein antigens, namely, autoallergy [17]. Although the pathological roles of such autoantigens in AD are still controversial, some of them have cross antigenicities to environmental allergens derived from pollen or fungi [18, 19]. Among them, human

manganese superoxide dismutase and thioredoxin were highly cross reactive to the counterparts of *M. sympodialis*, Mala s 11 and Mala s 13, respectively, suggesting pathogenic roles of the autoallergy in AD [20, 21]. On the other hand, a recent report showed that the intensity of IgE autoreactivity, but not IgE against exogenous allergens, seemed to follow skin inflammation, suggesting that autoallergy was a mirror of tissue damage [22]. We hypothesized that sweat antigens are of human origin and generated a cDNA library from mRNA of human skin using pME18S mammalian expression vector (Fig. 1B). We initially attempted the expression cloning of sweat antigens by transfecting COS7 cells and examined 120,000 cDNA clones by HRTs. However, no cDNA clone that induced histamine release from basophils of patients with AD was detected (unpublished), suggesting that extrinsic factors might be involved in sweat allergy.

Meanwhile, we further purified the semi-purified sweat antigen [10] with two additional reverse phase chromatography columns. Mass spectrometric analysis of a peak fraction of UV absorption, which corresponded to the peak fraction of histamine releasing activity revealed a sequence identical to a part of the hypothetical protein secreted by *M. globosa*, MGL_1304 [23]. We then generated recombinant MGL_1304 by *Escherichia (E.) coli*, and confirmed that it induces histamine release from basophils

of patients with AD. The recombinant MGL_1304 was immunologically identical with previously reported [10] “the semi-purified sweat antigen” by neutralization assays using immunoblotting and degranulation assays with RBL-48 cells, rat basophilic leukemia cell line expressing human high affinity IgE receptor. Immunoblots with monoclonal antibody against MGL_1304 revealed that the size of MGL_1304 was 17 kDa and 29 kDa, respectively, in sweat and in *M. globosa* (Fig. 2A). These observations indicated that MGL_1304 secreted by *M. globosa* was processed by unidentified proteases and converted into the mature form in sweat. MGL_1304 was identified by HRTs, thus a major histamine-releasing antigen in sweat. However, it is a minor allergen in lysates of *M. globosa* detected by immunoblotting with sera of patients with AD (Fig. 2B). Our observation was consistent with previous reports; Koyama et al. found that 45-50 kDa proteins of *M. globosa* were most recognized components by IgE of patients with AD [24]; Ishibashi et al. found that a 42-kDa protein of (MGp42) was the major allergen of *M. globosa* determined by immunoblotting [25].

5. Clinical application of MGL_1304

5-1. ELISA system measuring MGL_1304-specific immunoglobulins

HRTs require fresh blood cells from patients, and their results are qualitative rather than quantitative. Moreover, peripheral blood basophils from 10% to 20% of donors fail to degranulate in response to anti-IgE [26] (nonresponder/nonreleaser basophils). Thus, we established the ELISA system to measure human serum immunoglobulins (IgE, IgG, and IgG4) specific against MGL_1304 [27]. The levels of MGL_1304-specific IgE of patients with AD and CholU were significantly higher than those of normal controls (Fig. 2C). Unlike MGL_1304-specific IgE, the levels of MGL_1304-specific IgG and IgG4 of patients with AD were not significantly higher than that of normal controls. These results indicated that the sensitization of MGL_1304 occurred even in healthy individuals, and the disturbance of skin barrier function did not affect the sensitization of MGL_1304 itself. The difference might be partially due to atopic diathesis which tends to generate high amounts of IgE as compared with healthy individuals. The levels of IgE against MGL_1304 correlated with disease severity of AD (Fig. 2D). However, the mechanism underlying the development of MGL_1304-specific IgE in patients with CholU remained uncertain. Using human IgE monoclonal antibody that specifically binds to MGL_1304 (ABS-IgE), 1 unit of anti-MGL_1304 IgE shown in Fig. 5 was determined as 32 pg [28]. The amount of

MGL_1304 in human sweat can also be measured by sandwich ELISA using ABS-IgE [28].

5-2. Generation of recombinant MGL_1304 by *Pichia pastoris*

To facilitate the estimation of hypersensitivity against sweat, HRTs by purified antigen from sweat have become commercially available in Japan (Allerport® HRT, Kyowa Medics, Tokyo, Japan). However, purifying antigen from crude sweat is costly and time-consuming. Recombinant MGL_1304 produced by *E. coli* had a large chaperon protein to be solubilized and lacked fungal-type glycosylation. Therefore, we generated recombinant MGL_1304 by using *Pichia pastoris* as a substitution of native MGL_1304 [29]. MGL_1304 by *Pichia pastoris* has a 100-fold higher histamine release ability than that by *E. coli* in HRTs. Moreover, in degranulation assays using RBL48 cells sensitized with sera of patients, recombinant MGL_1304 by *Pichia pastoris*, but not that produced by *E. coli*, induced release of β -hexosaminidase as native antigen (Fig. 3). Thus, the recombinant protein generated by *Pichia pastoris* may be a superior surrogate of native MGL_1304 to estimate sweat allergy.

5-3. A monoclonal IgE against MGL_1304

Recently we identified a human monoclonal IgE (ABS-IgE) that binds to the high affinity IgE receptor (FcεRI) and MGL_1304 with high affinity, but does not release histamine from basophils and mast cells [28]. ABS-IgE prevented histamine release from mast cells and basophils by neutralizing MGL_1304 not only in a free form in solution, but also on FcεRI expressed on the cell surface without cell activation. ABS-IgE may be used both to quantify the amount of MGL_1304 and anti-MGL_1304 IgE, and possibly for the treatment of diseases caused/aggravated by type I allergy to MGL_1304.

6. Coping techniques with sweat allergy

6-1. Sweat allergy in AD

Removal of antigen from skin surface by showering or bathing is an easy, convenient way to deal with sweat allergy in patients with AD. Kameyoshi [30] and Mochizuki [31] reported previously that elementary school children with AD who showered at the school during the summer season improved skin symptoms of AD. We further explored natural substances that were harmless to human skin and neutralize the histamine release ability of semi-purified sweat allergen. We selected tannic acid (TA), a natural polyphenolic compound and protein denaturing agent contained in grapes and

green tea. Previous reports showed that TA removed peanut antigens [32], reduced mite allergens, but not cat allergens [33]. TA suppressed histamine release by semi-purified sweat antigen in a dose-dependent manner *in vitro* [34]. Spray, after bathing water, and aerosol spray containing TA all improved the symptoms of patients with AD [34]. The mechanism of the antigen-inactivating effect of TA might be degenerating and/or transferring antigens from soluble fractions into insoluble fractions [32]. Indeed, lower concentrations of TA inhibited IgE binding to recombinant MGL_1304 produced by *Pichia Pastoris* and higher concentration of TA transferred the antigen into insoluble fraction (Fig. 4A). In addition, TA ameliorated the activation of keratinocytes due to upregulation of peroxisome proliferator-activated receptor (PPAR) γ [35]. Therefore, the clinical effects of TA might also be due to the effect on keratinocytes.

6-2. Sweat allergy in CholU

Nakamizo et al. proposed that CholU should be categorized into four subtypes based on its pathogenesis: (1) CholU with poral occlusion, (2) CholU with acquired generalized hypohidrosis, (3) CholU with sweat allergy, and (4) idiopathic CholU [36]. Sawada et al. reported that eight out of nine patients with CholU with acquired hypohidrosis and anhidrosis were negative in autologous sweat skin test [37]. We

performed HRTs using basophils of 4 patients with CholU who were aware of hypohidrosis and severe prickling sensation, and all basophils did not release histamine against sweat extracts (Fig. 4B). Thus, CholU with acquired generalized hypohidrosis and CholU with sweat allergy may be classified into two distinct disease entities based on different pathogenesis. Systemic steroid therapy might improve sweating and symptoms of CholU with anhidrosis/hypohidrosis [38]. On the other hand, desensitization therapy might be beneficial for patients with CholU with sweat allergy. Kozaru [39] and Tanaka [40] previously reported the effect of desensitization therapy with crude sweat and semi-purified sweat antigen, respectively. Metz et al. reported that omalizumab (anti-IgE) therapy was effective for chronic spontaneous urticaria (83% complete response), but less effective for cholinergic urticaria (62% complete response) [41].

7. Conclusions

MGL_1304 was found as a main antigen in human sweat, which induced histamine release from basophils of patients with AD. However, several questions remain unresolved (Fig. 5): How does MGL_1304 penetrate the epidermis and reach dermal mast cells bearing specific IgE? What are the roles of other substances in human

sweat which induce histamine release? Are there any autoantigens homologous to MGL_1304 in sweat? Further investigations are needed to understand sweat allergy and to innovate better therapies for allergic diseases associated with sweat, such as AD and CholU.

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References

- [1] H. Saeki, M. Furue, F. Furukawa, M. Hide, M. Ohtsuki, I. Katayama, R. Sasaki, H. Suto, K. Takehara, C.f.G.f.t.M.o.A.D.o.J.D. ASSOCIATION, Guidelines for management of atopic dermatitis, *J Dermatol* 36(10) (2009) 563-77.
- [2] I. Katayama, Y. Kohno, K. Akiyama, M. Aihara, N. Kondo, H. Saeki, S. Shoji, H. Yamada, K. Nakamura, J.S.o. Allergology, Japanese Guideline for Atopic Dermatitis 2014, *Allergol Int* 63(3) (2014) 377-98.

- [3] A. Watabe, T. Sugawara, K. Kikuchi, K. Yamasaki, S. Sakai, S. Aiba, Sweat constitutes several natural moisturizing factors, lactate, urea, sodium, and potassium, *J Dermatol Sci* 72(2) (2013) 177-82.
- [4] S. Altrichter, J. Salow, E. Ardelean, M.K. Church, A. Werner, M. Maurer, Development of a standardized pulse-controlled ergometry test for diagnosing and investigating cholinergic urticaria, *J Dermatol Sci* 75(2) (2014) 88-93.
- [5] M. Magerl, S. Altrichter, E. Borzova, A. Giménez-Arnau, C.E. Grattan, F. Lawlor, P. Mathelier-Fusade, R.Y. Meshkova, T. Zuberbier, M. Metz, M. Maurer, The definition, diagnostic testing, and management of chronic inducible urticarias - The EAACI/GA(2) LEN/EDF/UNEV consensus recommendations 2016 update and revision, *Allergy* 71(6) (2016) 780-802.
- [6] S. Takahagi, T. Tanaka, K. Ishii, H. Suzuki, Y. Kameyoshi, H. Shindo, M. Hide, Sweat antigen induces histamine release from basophils of patients with cholinergic urticaria associated with atopic diathesis, *Br J Dermatol* 160(2) (2009) 426-8.
- [7] K. Adachi, T. Aoki, IgE antibody to sweat in atopic dermatitis, *Acta Derm Venereol Suppl (Stockh)* 144 (1989) 83-7.
- [8] J. Adachi, T. Aoki, A. Yamatodani, Demonstration of sweat allergy in cholinergic urticaria., *J Dermatol Sci* 7(2) (1994) 142-9.

- [9] M. Hide, T. Tanaka, Y. Yamamura, O. Koro, S. Yamamoto, IgE-mediated hypersensitivity against human sweat antigen in patients with atopic dermatitis, *Acta Derm Venereol* 82(5) (2002) 335-40.
- [10] A. Tanaka, T. Tanaka, H. Suzuki, K. Ishii, Y. Kameyoshi, M. Hide, Semi-purification of the immunoglobulin E-sweat antigen acting on mast cells and basophils in atopic dermatitis, *Exp Dermatol* 15(4) (2006) 283-90.
- [11] H. Shindo, K. Ishii, Y. Yanase, H. Suzuki, M. Hide, Histamine release-neutralization assay for sera of patients with atopic dermatitis and/or cholinergic urticaria is useful to screen type I hypersensitivity against sweat antigens, *Arch Dermatol Res* 304(8) (2012) 647-54.
- [12] K. Sato, W.H. Kang, K. Saga, K.T. Sato, Biology of sweat glands and their disorders. I. Normal sweat gland function, *J Am Acad Dermatol* 20(4) (1989) 537-63.
- [13] X. Dai, H. Okazaki, Y. Hanakawa, M. Murakami, M. Tohyama, Y. Shirakata, K. Sayama, Eccrine sweat contains IL-1 α , IL-1 β and IL-31 and activates epidermal keratinocytes as a danger signal, *PLoS One* 8(7) (2013) e67666.
- [14] H. Yamaguchi, R. Kabashima-Kubo, T. Bito, J. Sakabe, T. Shimauchi, T. Ito, S. Hirakawa, N. Hirasawa, K. Ogasawara, Y. Tokura, High frequencies of positive

- nickel/cobalt patch tests and high sweat nickel concentration in patients with intrinsic atopic dermatitis, *J Dermatol Sci* 72(3) (2013) 240-5.
- [15] H. Yamaguchi, N. Hirasawa, S. Asakawa, K. Okita, Y. Tokura, Intrinsic atopic dermatitis shows high serum nickel concentration, *Allergol Int* 64(3) (2015) 282-4.
- [16] T. Ilves, A. Virolainen, I.T. Harvima, Immediate Wheal Reactivity to Autologous Sweat in Atopic Dermatitis Is Associated with Clinical Severity, Serum Total and Specific IgE and Sweat Tryptase Activity, *Int Arch Allergy Immunol* 170(2) (2016) 84-91.
- [17] R. Valenta, D. Maurer, R. Steiner, S. Seiberler, W.R. Sperr, P. Valent, S. Spitzauer, S. Kapiotis, J. Smolen, G. Stingl, Immunoglobulin E response to human proteins in atopic patients., *J Invest Dermatol* 107(2) (1996) 203-8.
- [18] F. Cipriani, G. Ricci, M.C. Leoni, L. Capra, G. Baviera, G. Longo, N. Maiello, E. Galli, Autoimmunity in atopic dermatitis: biomarker or simply epiphenomenon?, *J Dermatol* 41(7) (2014) 569-76.
- [19] T.S. Tang, T. Bieber, H.C. Williams, Does "autoreactivity" play a role in atopic dermatitis?, *J Allergy Clin Immunol* 129(5) (2012) 1209-1215 e2.
- [20] M. Vilhelmsson, C. Johansson, G. Jacobsson-Ekman, R. Cramer, A. Zargari, A. Scheynius, The *Malassezia sympodialis* allergen Mala s 11 induces human dendritic

cell maturation, in contrast to its human homologue manganese superoxide dismutase.,

Int Arch Allergy Immunol 143(2) (2007) 155-62.

[21] H. Balaji, A. Heratizadeh, K. Wichmann, M. Niebuhr, R. Cramer, A. Scheynius, T.

Werfel, *Malassezia sympodialis* thioredoxin-specific T cells are highly cross-reactive to human thioredoxin in atopic dermatitis, J Allergy Clin Immunol 128(1) (2011) 92-99 e4.

[22] S. Lucae, P. Schmid-Grendelmeier, B. Wüthrich, D. Kraft, R. Valenta, B. Linhart,

IgE responses to exogenous and endogenous allergens in atopic dermatitis patients under long-term systemic cyclosporine A treatment, Allergy 71(1) (2016) 115-8.

[23] T. Hiragun, K. Ishii, M. Hiragun, H. Suzuki, T. Kan, S. Mihara, Y. Yanase, J.

Bartels, J.M. Schröder, M. Hide, Fungal protein MGL_1304 in sweat is an allergen for atopic dermatitis patients, J Allergy Clin Immunol 132(3) (2013) 608-615.e4.

[24] T. Koyama, T. Kanbe, A. Ishiguro, A. Kikuchi, Y. Tomita, Antigenic components of

Malassezia species for immunoglobulin E antibodies in sera of patients with atopic dermatitis, J Dermatol Sci 26(3) (2001) 201-8.

[25] Y. Ishibashi, H. Kato, Y. Asahi, T. Sugita, A. Nishikawa, Identification of the major

allergen of *Malassezia globosa* relevant for atopic dermatitis, J Dermatol Sci 55(3) (2009) 185-92.

- [26] C.L. Kepley, L. Youssef, R.P. Andrews, B.S. Wilson, J.M. Oliver, Syk deficiency in nonreleaser basophils, *J Allergy Clin Immunol* 104(2 Pt 1) (1999) 279-84.
- [27] M. Hiragun, T. Hiragun, K. Ishii, H. Suzuki, A. Tanaka, Y. Yanase, S. Mihara, Y. Haruta, N. Kohno, M. Hide, Elevated serum IgE against MGL_1304 in patients with atopic dermatitis and cholinergic urticaria, *Allergol Int* 63(1) (2014) 83-93.
- [28] K. Ishii, M. Hiragun, T. Hiragun, T. Kan, T. Kawaguchi, Y. Yanase, A. Tanaka, S. Takahagi, M. Hide, A human monoclonal IgE antibody that binds to MGL_1304, a major allergen in human sweat, without activation of mast cells and basophils, *Biochem Biophys Res Commun* 468(1-2) (2015) 99-104.
- [29] T. Kan, T. Hiragun, K. Ishii, M. Hiragun, Y. Yanase, A. Tanaka, M. Hide, Evaluation of recombinant MGL_1304 produced by *Pichia pastoris* for clinical application to sweat allergy, *Allergol Int* 64(3) (2015) 266-71.
- [30] Y. Kameyoshi, T. Tanaka, M. Mochizuki, O. Koro, S. Mihara, T. Hiragun, M. Tanaka, M. Hide, [Taking showers at school is beneficial for children with severer atopic dermatitis], *Arerugi* 57(2) (2008) 130-7.
- [31] H. Mochizuki, R. Muramatsu, H. Tadaki, T. Mizuno, H. Arakawa, A. Morikawa, Effects of skin care with shower therapy on children with atopic dermatitis in elementary schools., *Pediatr Dermatol* 26(2) (2009) 223-5.

- [32] S.Y. Chung, S. Reed, Removing peanut allergens by tannic acid, *Food Chem* 134(3) (2012) 1468-73.
- [33] J.A. Woodfolk, M.L. Hayden, N. Couture, T.A. Platts-Mills, Chemical treatment of carpets to reduce allergen: comparison of the effects of tannic acid and other treatments on proteins derived from dust mites and cats, *J Allergy Clin Immunol* 96(3) (1995) 325-33.
- [34] H. Shindo, S. Takahagi, S. Mihara, T. Tanaka, K. Ishii, M. Hide, S. Suzuki, H. Kanatani, S. Yano, [Efficacy of sweat-antigen-inactivating skin care products on itching of patients with atopic dermatitis], *Arerugi* 60(1) (2011) 33-42.
- [35] V. Karuppagounder, S. Arumugam, R.A. Thandavarayan, V. Pitchaimani, R. Sreedhar, R. Afrin, M. Harima, H. Suzuki, M. Nomoto, S. Miyashita, K. Suzuki, M. Nakamura, K. Ueno, K. Watanabe, Tannic acid modulates NF κ B signaling pathway and skin inflammation in NC/Nga mice through PPAR γ expression, *Cytokine* 76(2) (2015) 206-13.
- [36] S. Nakamizo, G. Egawa, Y. Miyachi, K. Kabashima, Cholinergic urticaria: pathogenesis-based categorization and its treatment options, *J Eur Acad Dermatol Venereol* 26(1) (2012) 114-6.

- [37] Y. Sawada, M. Nakamura, T. Bito, J. Sakabe, R. Kabashima-Kubo, R. Hino, M. Kobayashi, Y. Tokura, Decreased expression of acetylcholine esterase in cholinergic urticaria with hypohidrosis or anhidrosis, *J Invest Dermatol* 134(1) (2014) 276-9.
- [38] T. Munetsugu, T. Fujimoto, Y. Oshima, K. Sano, H. Murota, T. Satoh, S. Iwase, M. Asahina, Y. Nakazato, H. Yokozeki, Revised guideline for the diagnosis and treatment of acquired idiopathic generalized anhidrosis in Japan, *J Dermatol* (2016).
- [39] T. Kozaru, A. Fukunaga, K. Taguchi, K. Ogura, T. Nagano, M. Oka, T. Horikawa, C. Nishigori, Rapid desensitization with autologous sweat in cholinergic urticaria., *Allergol Int* 60(3) (2011) 277-81.
- [40] T. Tanaka, K. Ishii, H. Suzuki, Y. Kameyoshi, M. Hide, [Cholinergic urticaria successfully treated by immunotherapy with partially purified sweat antigen]. *Arerugi* 56(1) (2007) 54-7.
- [41] M. Metz, T. Ohanyan, M.K. Church, M. Maurer, Omalizumab is an effective and rapidly acting therapy in difficult-to-treat chronic urticaria: a retrospective clinical analysis, *J Dermatol Sci* 73(1) (2014) 57-62.

Biography



Takaaki Hiragun received his MD and PhD degree in 1995 and 1998, respectively. From 2001 to 2004, he was a visiting fellow at Laboratory of Molecular Immunology, NHLBI, NIH. From 2004 to 2007, he was an assistant professor at Department of Dermatology, Hiroshima University. From 2007 to 2008, he was the section chief of Dermatology at Chuden Hospital. From 2008 to 2013, he was an assistant Professor at Natural Science Center for Basic Research and Development, Hiroshima University. From 2013 to 2014, he was a lecturer at Department of Dermatology, Hiroshima University Hospital. Since 2014, he has been an associate professor at Department of Dermatology, Hiroshima University.

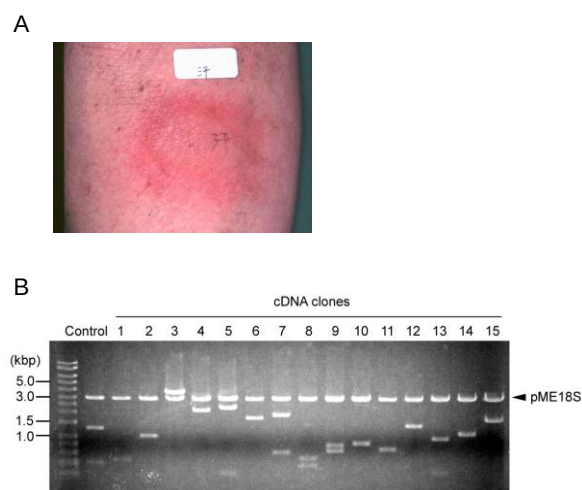


Fig. 1

Fig. 1 (A) Positive reaction of an intradermal skin test using autologous sweat in a patient with atopic dermatitis. (From clinical data of Hiroshima University.) (B) A skin cDNA library constructed and used for the expression cloning of intrinsic sweat antigens. A cDNA library was generated by pME18S mammalian expression vector and human skin mRNA, and possessed 7.9×10^6 CFUs with average insert size at 1.6 kbps.

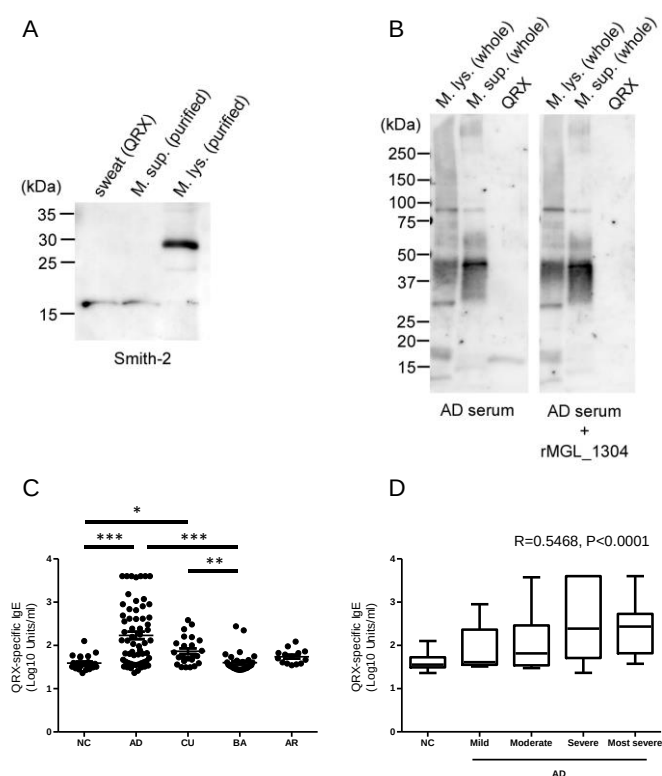


Fig. 2

Fig. 2 (A) An immunoblot of MGL_1304. MGL_1304 was cleaved and secreted into sweat from *M. globosa*. Histamine releasing activity was purified from sweat (QRX), culture supernatants (M. sup. (purified)), and lysates (M. lys. (purified)) of *M. globosa*. The purified proteins were blotted with Smith-2. (B) The whole lysate (M. lys. (whole)) and whole culture supernatant (M. sup. (whole)) of *M. globosa*, and QRX were blotted by sera of patients with AD or sera pretreated with rMGL_1304. (From ref. 23 with permission.) (C and D) Comparisons of serum levels of QRX-specific IgE among diseases and correlations with severities of AD. (C) Serum levels of QRX-specific IgE in patients with each disease were plotted and analyzed by the Kruskal-Wallis test. (D) The serum levels of QRX-specific IgE in patients showing various severities of atopic dermatitis were plotted and analyzed by Spearman rank correlation. NC: normal controls, AD: atopic dermatitis, CU: cholinergic urticaria, BA: bronchial asthma, AR: allergic rhinitis. *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$. (From ref. 27 with permission.)

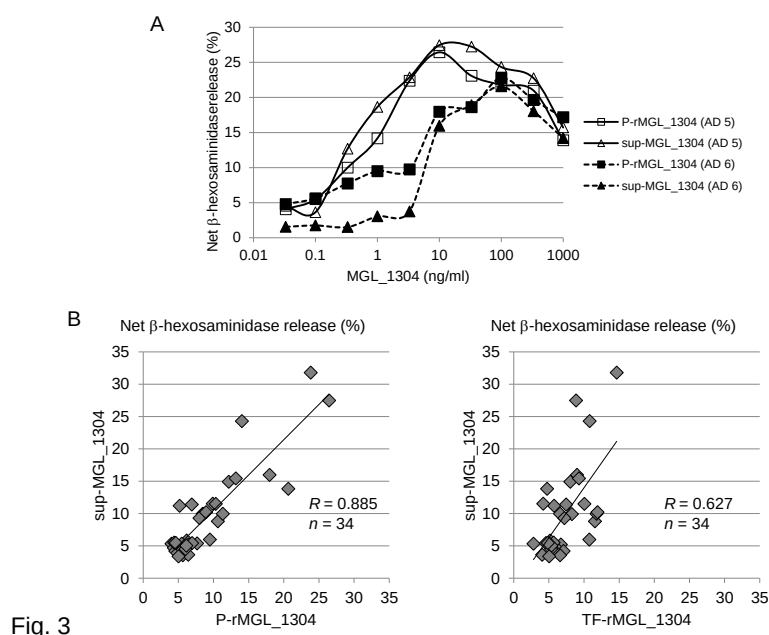


Fig. 3 Comparison of native- and recombinant MGL_1304. (A) RBL-48 cells were sensitized with two patients with AD, stimulated with series of concentrations of *Pichia pastoris*-derived rMGL_1304 (P-rMGL_1304) and native MGL_1304 (sup-MGL_1304), and net β -hexosaminidase release (%) were measured. The concentrations of MGL_1304 were normalized by the sandwich ELISA. (B) RBL-48 cells were sensitized with sera of patients with AD, stimulated with 10 ng/ml of P-rMGL_1304, 10 ng/ml of sup-MGL_1304, or 1 μ g/ml of *E. coli*-derived rMGL_1304 (TF-rMGL_1304), and release of β -hexosaminidase were measured. The correlation coefficient of net β -hexosaminidase release (%) between sup-MGL_1304 and P-rMGL_1304, or sup-MGL_1304 and TF-rMGL1304 were shown. (From ref. 29 with permission.)

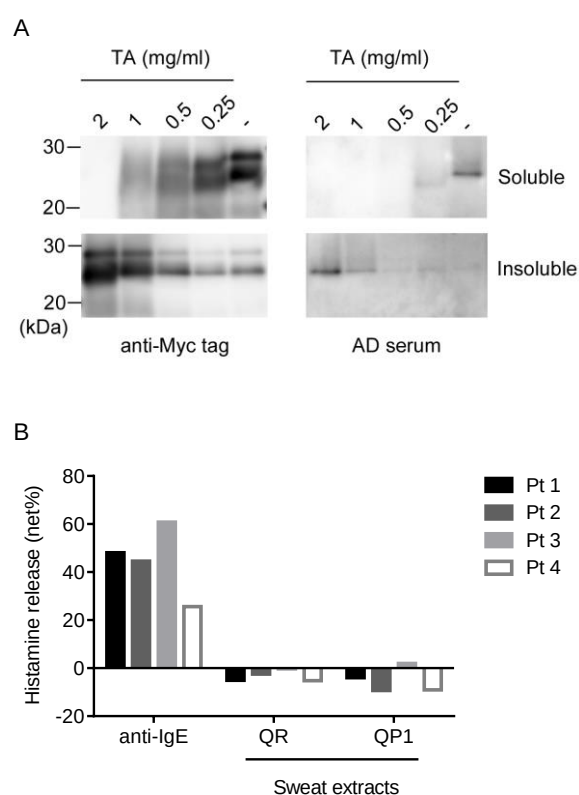


Fig. 4

Fig. 4 (A) Treatment with MGL_1304 with tannic acid. Recombinant MGL_1304 by *Pichia pastoris* were incubated with indicated concentration of tannic acid for 20 min. Proteins were centrifuged and supernatants (soluble) and pellets (insoluble) were subjected for immunoblotting with anti-Myc tag antibody and AD serum. (B) HRTs of patients with ChoU who were aware of hypohidrosis and severe prickling sensation. HRTs were performed using peripheral blood basophils of 4 patients. QR: sweat extract including MGL_1304; QP1: sweat extract other than MGL_1304.

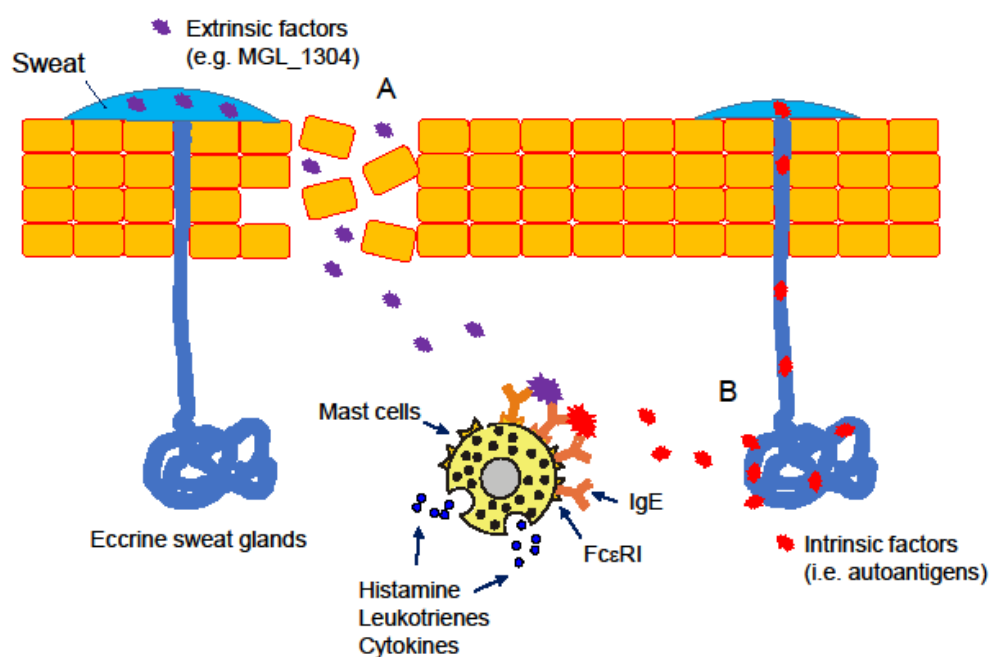


Fig. 5

Fig. 5 A schematic figure of sweat allergy. (A) Extrinsic factors (e.g. MGL_1304) in sweat might penetrate damaged epidermis and reach dermal mast cells bearing specific IgE. (B) Intrinsic factors (i.e. autoantigens) might leak from eccrine sweat glands and reach dermal mast cells. In response to these antigens, dermal mast cells release histamine, leukotrienes, and cytokines.

Table 1. Positive rates of sweat allergy in patients with atopic dermatitis and cholinergic urticaria

Authors [ref.]	Atopic Dermatitis	Cholinergic Urticaria	Healthy Controls
Adachi K [7]	96% (43/45)		-18% (4/22)
Adachi J [8]	-	100% (20/20)	0% (0/20)
Hide M[9]	85% (56/66)		11% (3/27)
Tanaka A [10]	77% (47/61)		-9% (4/46)
Takahagi S [6]	-	66% (23/35)	0% (0/14)