Journal of Dermatological Science 94 (2019) 354–357

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Letter to the Editor

Stratum corneum Toll-like receptor 3 expressions correlate with the severity of atopic dermatitis lesions

To the Editor

TLR3 recognizes viral double-stranded RNA and polyinosinic: polycytidylic acid (poly(I:C)) and induces the production of type I interferons and the expression of proinflammatory cytokines [1]. We and others have found that TLR3 signaling is involved in allergic skin inflammation and barrier dysfunction [2–4], which are related to the features of AD. However, TLR3 expression in the lesions of AD patients and its involvement in the pathogenesis of AD have not been examined in previous studies.

We examined TLR3 expressions in the skin of AD patients and healthy controls and investigated whether the degree of TLR3 expressions in the skin is related to the severity of AD; eruption type; or epidermal barrier function in AD patients. Thirty patients (17 males and 13 females; median age: 42 years, range: 21–62) who had been diagnosed with AD and 29 healthy controls (11 males and 18 females; median age: 34 years, range: 22–44) were included in this study (see Supplementary Methods).

We first examined the epidermal expression of TLR3 in the skin of AD patients and healthy controls using immunohistochemical staining. TLR3 was strongly expressed in the epidermal cells and the sweat glands of both the healthy controls and AD patients (Fig. 1a). We next evaluated TLR3 expressions in the stratum corneum using immunostaining (Supplementary Fig. S1). The mean fluorescence intensity was 81.77 ± 64.82 ($n = 24$) in the stratum corneum obtained from the representative affected trunk skin in AD patients, 22.48 ± 22.17 ($n = 6$) in the stratum corneum tissue obtained from the unaffected skin of the trunk in AD patients, and 27.09 ± 13.13 ($n = 29$) in the stratum corneum tissue obtained from of the trunk skin in healthy controls (Fig. 1b). The scTLR3 expression level of the trunk was significantly higher in the affected skin of AD patients than that in the unaffected skin of AD patients or the skin of healthy controls.

The correlations between the scTLR3 expression level and skin lesion severity scores, and the SCORAD, objective SCORAD were assessed in the areas of affected skin on the trunk subjected to tape-stripping in AD patients. The scTLR3 expression level of the trunk was significantly correlated with the total intensity scores, the erythema score, oozing/crusting score, edema/papule score, excoriation score, lichenification score, and xerosis score (Fig. 1c). Although the correlation between the scTLR3 expression level of the trunk and the itching score was not significant, the scTLR3 expression level of the trunk tended to be correlated with the degree of itching. Furthermore, the scTLR3 expression level of the trunk was significantly correlated with the scoring AD (SCORAD) and objective SCORAD (Fig. 1c). There was no correlation between the scTLR3 expression level and the chronicity of AD (Supplementary Fig. S2). Conductance was correlated inversely with that in the affected trunk skin in AD patients. Although TEWL tended to be correlated with scTLR3 expression in the affected trunk skin in the patients with AD, but there was no statistically significant correlation (Fig. 1c).

The relationships between the scTLR3 expression level in the affected trunk skin and laboratory markers were examined. The scTLR3 expression level was correlated with the peripheral blood eosinophil count but was not significantly correlated with serum total IgE level, serum lactate dehydrogenase (LDH) level, or serum TARC level (Supplementary Table S1).

To investigate the mechanism responsible for the elevated scTLR3 expression seen in AD lesions, we examined the effects of various cytokines and TLR3 ligand poly(I:C) on TLR3 expressions in undifferentiated keratinocytes incubated in low- Ca^{2+} or differentiated keratinocytes incubated in high-Ca^{2+} medium. We examined whether TLR3 mRNA expression could be induced in adult human epidermal keratinocytes (HEKa) by poly(I:C) or inflammatory cytokines, such as IL-4, IL-13, IL-17, TNF-α, or IL-1β, using PCR. TLR3 mRNA expression was upregulated by stimulation with poly(I:C) in both low- and high-Ca^{2+} conditions, but not by stimulation with IL-4, IL-13, IL-17, TNF-α, or IL-1β (Fig. 2a). TLR3 expression level was significantly higher in low-Ca^{2+} medium compared with high-Ca^{2+} medium. Next, we detected TLR3 expressions in HEKa via surface and intracellular staining using flow cytometry. When TLR3 was assessed via intracellular staining, the TLR3 expression level was upregulated in the cells that were stimulated with poly(I:C) in both low- and high-Ca^{2+} conditions and higher in low-Ca^{2+} medium (Fig. 2b). On the other hand, the TLR3 expression level on the surface of HEKa was not changed after stimulation with poly(I:C) (Fig. 2b). These findings indicate that TLR3 ligands but not cytokines induce the elevation of intracellular TLR3 expressions in keratinocytes. TLR3 levels might be decreased in the upper layers of the epidermis.

In this study, we demonstrated for the first time that scTLR3 expression was elevated in AD patients and was closely associated with the skin lesion severity scores and stratum corneum hydration. These findings suggest that TLR3 may participate in the pathogenesis of acute and chronic dermatitis and the skin barrier functions as seen in AD. The scTLR3

https://doi.org/10.1016/j.jdermsci.2019.05.005

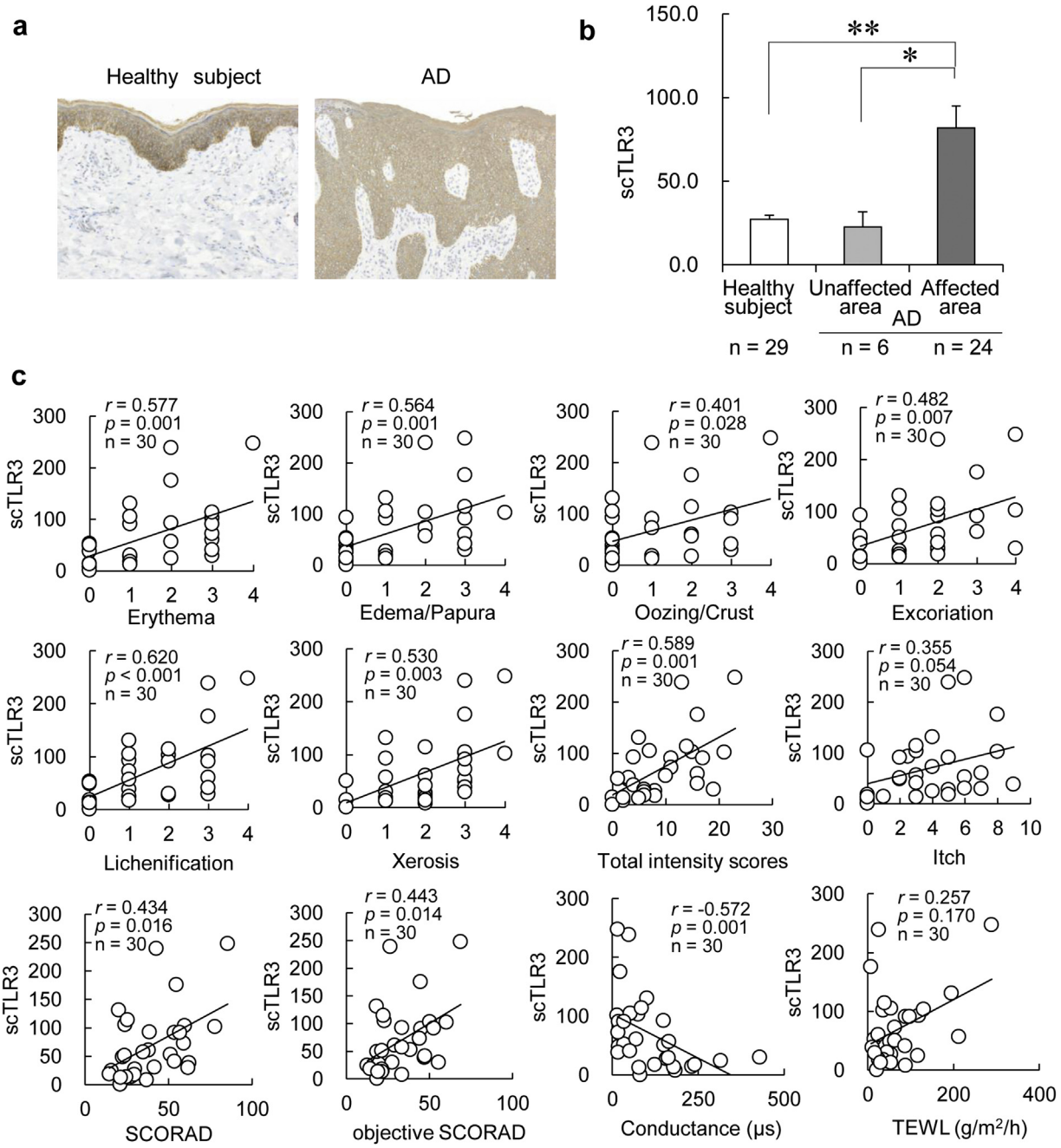

Fig. 1. (a) The TLR3 expressions in the skin of AD patients and healthy controls. Immunochemical staining was performed on formalin-fixed, paraffin-embedded sections of the trunk skin from AD patients and healthy controls. Representative results obtained from the patients with AD and healthy controls are shown. Scale bar =100 μm. (b) The scTLR3 expression levels in AD patients and healthy controls. In AD patients, samples were taken from affected and non-affected areas of the trunk. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ (c) Correlation between the scTLR3 expression and the skin symptom severity score, the SCORAD, objective SCORAD, or epidermal barrier function. Skin samples were obtained from of the affected trunk skin of AD patients via the tape-stripping method.

expression level was also correlated with the peripheral blood eosinophil count. We have previously found that TLR3 signaling enhances eosinophil infiltration in experimental allergic conjunctivitis [5]. We have also demonstrated that keratinocytes produce type 2 cytokines such as thymic stromal lymphopoietin and IL-33 via the stimulation of TLR3 with poly(I:C) [3,5]. These findings suggest that TLR3 might influence type 2 cytokine-driven inflammation including eosinophil recruitment via cytokine release from keratinocytes in AD. Serum levels of IgE, LDH, or TARC did not correlate with the scTLR3 expressions. This might be due to the fact that these markers are released from keratinocytes and/or different cell types.

TLR3 expression in keratinocytes was upregulated after stimulation with poly(I:C), but not after stimulation with Th2, Th17, or proinflammatory cytokines. It was reported that TLR3 recognizes RNA which was released from keratinocytes after UVB exposure and stimulates the production of inflammatory cytokines from keratinocytes in a TLR3-dependent manner

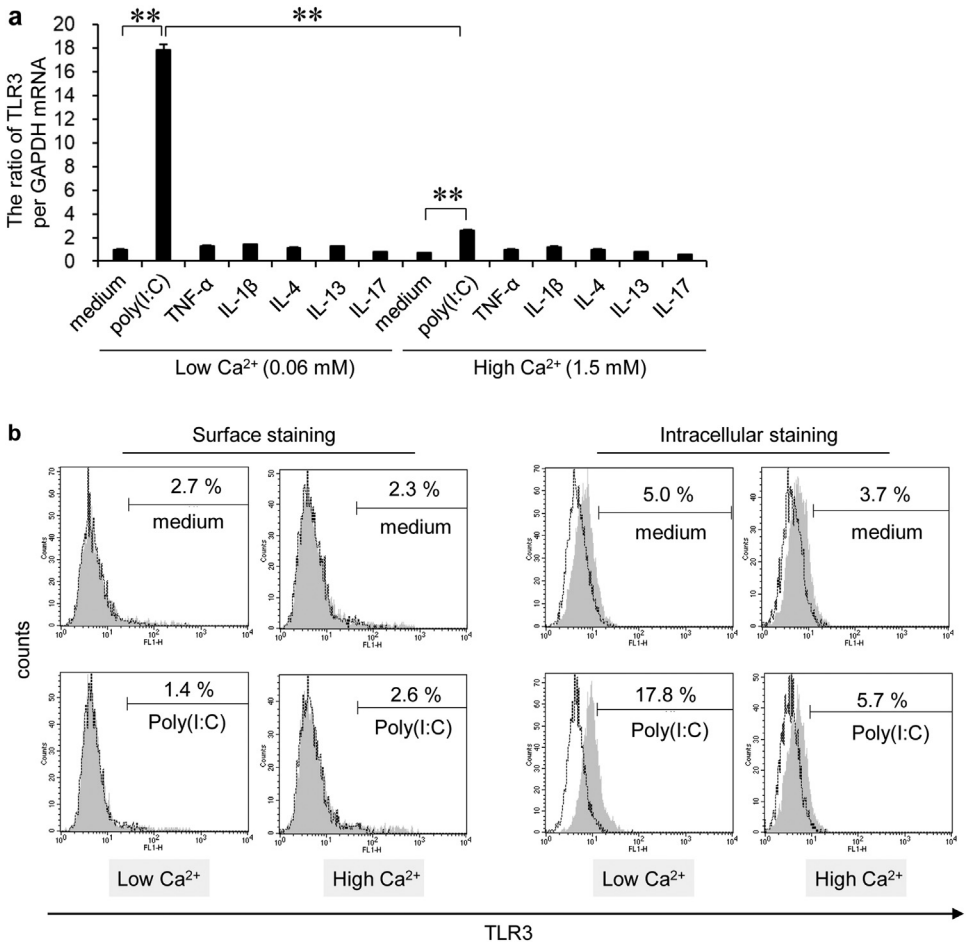

Fig. 2. Mechanisms responsible for the elevated TLR3 expression in the keratinocytes. (a) The HEKa cells cultured in low-Ca^{2+} or high-Ca^{2+} medium were incubated with 10 μg/ml poly(I:C), TNF-α, IL-1β, IL-4, IL-13, or IL-17 (all cytokine concentrations were 100 ng/ml), or medium for 6 h, and the expression of TLR3 mRNA in HEKa were assessed by Quantitative RT-PCR analysis. Data are expressed as the means ± SD of quadruplicate wells and are representative of three independent experiments. (b) The HEKa cells cultured in low-Ca^{2+} or high-Ca^{2+} medium were incubated with 20 μg/ml poly(I:C) or vehicle for 24 h, and the cell surface and intracellular expression of TLR3 in HEKa were assessed by flow cytometry. The dotted lines indicate the isotype control. The data shown are representative of two separate experiments. $^{**}P<0.01$.

[6]. Therefore, our results suggest that endogenous TLR3 ligands, such as the self-RNA released from cells that have been damaged by AD inflammation, might activate TLR3 in keratinocytes, leading to the exacerbation of inflammation in AD.

Funding sources

This study was supported in part by grants from the Japanese Ministry of Education, Science, Sports, and Culture to N.N., R.T.M., and N.K., by grants from the Maruho Takagi Dermatology Foundation to R.T.M., by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government (BioBank Japan Project) to M.U., and by the JSPS Core-to-Core Program A, Advanced Research Networks to M.U..

Conflicts of interest

H.M., Y.M., and E.Y. are employees of Tokiwa Pharmaceutical Co., Ltd., NOV Academic Research. None of the other authors have any conflicts of interest to declare.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.05.005.

References

[1] R. Tamagawa-Mineoka, M. Ueta, N. Katoh, TLR3 and inflammatory skin disease: from environmental factors to molecular opportunities, in: G.T. Wondrak (Ed.), Skin Stress Response Pathways, Springer, Switzerland, Gewerbestrasse, 2016, pp. 235–249.
[2] N. Nakamura, R. Tamagawa-Mineoka, M. Ueta, S. Kinoshita, N. Katoh, Toll-like receptor 3 increases allergic and irritant contact dermatitis, J. Invest. Dermatol. 135 (2015) 411–417.
[3] R. Yasuike, R. Tamagawa-Mineoka, M. Ueta, N. Nakamura, S. Kinoshita, N. Katoh, The role of Toll-like receptor 3 in chronic contact hypersensitivity induced by repeated elicitation, J. Dermatol. Sci. 88 (2017) 184–191.
[4] J. Wei, H. Jiang, H. Gao, G. Wang, Activation of toll like receptor-3 induces corneal epithelial barrier dysfunction, Biochem. Biophys. Res. Commun. 461 (2015) 555–559.
[5] M. Ueta, S. Uematsu, S. Akira, S. Kinoshita, Toll-like receptor 3 enhances late-phase reaction of experimental allergic conjunctivitis, J. Allergy Clin. Immunol. 123 (2009) 1187–1189.
[6] J.J. Bernard, C. Cowing-Zitron, T. Nakatsuji, B. Muehleisen, J. Muto, A.W. Borkowski, L. Martinez, E.L. Greidinger, B.D. Yu, R.L. Gallo, Ultraviolet radiation damages self noncoding RNA and is detected by TLR3, Nat. Med. 18 (2012) 1286–1290.

Naomi Nakamura[a,1], Risa Tamagawa-Mineoka[a,*,1], Mayumi Ueta[b], Eiichi Konishi[c], Risa Yasuike[a], Koji Masuda[a], Hiroshi Matsunaka[d], Yumi Murakami[d], Emiko Yokosawa[d], Norito Katoh[a]

[a]Department of Dermatology, Kyoto Prefectural University of Medicine, Kyoto, Japan

[b]Department of Frontier Medical Science and Technology for Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

[c]Department of Surgical Pathology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

[d]Research & Development, TOKIWA Pharmaceutical Co., Ltd., NOV Academic Research, Tokyo, Japan

[1]These authors contributed equally to this work.

* Corresponding author at: Department of Dermatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan.

E-mail address: risat@koto.kpu-m.ac.jp (R. Tamagawa-Mineoka).

Received 28 December 2018
Received in revised form 1 May 2019
Accepted 16 May 2019