



Skin scratching switches immune responses from Th2 to Th1 type in epicutaneously immunized mice

Hironori Matsushima, Satoru Hayashi, Shinji Shimada*

Department of Dermatology, Faculty of Medicine, University of Yamanashi 1110, Shimokato, Tamaho, Nakakoma, Yamanashi 409-3898, Japan

Received 12 February 2003; received in revised form 14 April 2003; accepted 17 April 2003

KEYWORDS

Epicutaneous sensitization;
T helper cells;
Atopic dermatitis;
Immunoglobulin;
Cytokines

Summary Background: The balance between Th1 and Th2 subsets is important with respects to susceptibility and resistance to particular infection or autoimmune diseases. However, the mechanism controlling Th1/Th2 balance remains unclear, although several factors have been reported to induce Th1/Th2 differentiation. Atopic Dermatitis (AD) that is a chronic skin disorder has been known as Th2 biased nature characterized by high expression of IgE in the serum. In contrast, the chronic skin lesions express IFN γ and some patients don't show IgE in the serum. Thus, the pathology is also complicated now. **Objective:** We focused on skin scratching that is common feature in the patients. In this study, we investigated in order to determine whether skin scratching regulates immune responses in murine epicutaneous sensitization model. **Methods:** The scratched mice on abdominal skin using wire brush were applied with keyhole limpet hemocyanin (KLH) on the skin using occlusive patch. We examined the immune responses including delayed type hypersensitivity (DTH) reaction, antigen-specific serum immunoglobulin formation, and cytokine expressions on the local skin in comparison with mice without scratching. **Results:** We found that the epicutaneously sensitized mice with KLH on abdominal skin showed Th2 biased immune response including expression of antigen-specific IgE in the serum and IL-13 in the local skin. Surprisingly, scratching on local abdominal skin using wire brush exchanged the immune response from Th2 dominance to Th1, because the mice displayed DTH reaction and significant level of antigen-specific IgG2a and IgG2b but not IgE in the serum. Furthermore, the abdominal skin showed significant level of IFN γ but not IL-13. **Conclusion:** These data demonstrate that skin scratching switches immune response from Th2 biased response to Th1. This suggests that skin scratching play critical roles as one of exogenous immune modulator. This murine sensitization model may help to understand natures of several allergic disorders including AD.

© 2003 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The patterns of cytokine production induced by exogenous antigens from Th cells play an important role in determining the characteristics of immune responses and developments [1–3]. Th1 cells characterized by the production of IL-2, IFN- γ ,

Abbreviations: AD, atopic dermatitis; DTH, delayed type hypersensitivity; KLH, keyhole limpet hemocyanin.

*Corresponding author. Tel.: +81-55-273-6766; fax: +81-55-273-6766.

E-mail address: sshimada@res.yamanashi-med.ac.jp (S. Shimada).

and TNF- β are critical in the cell mediated immunity, which is associated with defense against intracellular pathogens eliciting phagocyte-mediated response [2,4–6]. Th1 dominant responses illustrated by the delayed type hypersensitivity (DTH) reaction are associated with inflammation and injury [2,7]. In contrast, Th2 cells characterized by the production of IL-4, IL-5, IL-6 and IL-13 play an important role in humoral immunity including atopic and allergic disorders [2,4–7]. These two Th subsets regulate each other function through the antagonistic activity of their respective cytokines [1,2]. Increasing evidences indicate that the balance of immune responses between Th1 and Th2 determines outcomes of diseases [1–3].

Several studies have demonstrated that polarization of Th cells are determined by the dose of antigen administration and the routes, the kinds of antigen, the strength of T cell receptor (TCR)–ligand interactions, the genetic background, and the hormonal status of host, etc [8–14]. Epicutaneous sensitization by haptens, such as trinitrophenyl (TNP), fluoresceine, or metals, induces contact hypersensitivity reaction characterized by Th1 dominant response [15–20]. Haptens are capable of binding directly to soluble or cell-associated proteins and are caught by epidermal Langerhans cells (LC). LC induces differentiation of hapten specific Th1 cells after migration from skin to draining lymph nodes. In contrast, sensitization with protein antigens induces Th2 dominant response via oral or airway routes [21,22]. Furthermore, several studies have been reported that epicutaneous sensitization with soluble protein antigens induces antigen specific Th2 dominant responses in mice [21,23–27]. Thus, it is not clear how the balance between Th1 biased immune response and Th2 is regulated, although several factors have been reported to induce Th1/Th2 differentiation.

Atopic dermatitis (AD) is a chronic skin disease characterized by production of Th2 cytokines in the local skin lesions and IgE in the peripheral blood [28–30]. Although contact dermatitis is induced by hapten shows representative Th1 biased response, it is speculated that hypersensitivity to environmental macromolecular protein antigens such as house dust mite, rather than to small molecular haptens, plays an important role in AD etiology [31]. However, several studies have indicated that both Th1 and Th2 derived cytokines and transcripts exist in the lesional skin of AD patients and murine model [25,28,32]. In this way, the mechanisms of sensitization by soluble protein antigens are complicated now in AD pathology. Skin scratching is a

common feature and one of aggravate factor in AD patients. Therefore, we sought to determine whether skin scratching modulates the immune response induced by epicutaneous sensitization with soluble protein antigen in mice.

2. Material and methods

2.1. Sensitization with protein antigen

Age matched (4–6 weeks) female BALB/c mice were purchased from SLC Japan (Hamamatsu, Japan). For epicutaneous sensitization, the mice anesthetized with ether were shaved, an occlusive patch (Torii Pharmaceutical Co., Ltd, Tokyo, Japan) which was applied by 100 μ l of either keyhole limpet hemocyanin (KLH) (Sigma Chemical Co., St. Louis, MO, USA) (2 mg/ml) in phosphate buffered saline (PBS) or PBS alone into a gauze in the center was then affixed on their abdominal skin. Patches were left intact for 4 days, followed by application of a second patch on day 7. The application was repeated one time per week for 5 weeks.

2.2. Treatment of skin by brusher scratching

The mice anesthetized with ether were shaved and scratched on abdomen by ten times with wire brush, followed by application with patches. The area scratched by brushing was almost 1 cm in diameter.

2.3. Measurement of footpad swelling reaction

In order to determine KLH specific Th1 biased response, KLH specific DTH reaction was analyzed by measurement of food pad swelling. The mice epicutaneously sensitized with KLH were challenged with KLH (2 mg/ml) in PBS or PBS alone at both sides of footpad. KLH specific footpad swelling was determined by measuring footpad thickness with a micrometer.

2.4. Antibody measurement

Serum was collected on week 1, 3, 5 after sensitization with KLH. In order to determine KLH specific antibody in the serum, 96 well microtiter plates were coated with KLH (2 mg/ml) in 0.1 M NaHCO₃ at 4 °C overnight. The plates were washed three times by PBS/0.05% Tween 20 between each

step. Blocking was performed with 1% BSA in borate saline (BSA-BS) for 1 h at 37 °C. The serum was diluted serially in 1% BSA/0.05% Tween 20 was incubated for 1 h at 37 °C, followed by incubation with biotin labeled secondary rat anti-mouse antibodies including anti-IgG1, anti-IgG2a, anti-IgG2b, anti-IgE. Streptavidin-conjugated horseradish peroxidase was incubated for 30 min at 37 °C, followed by addition and incubation with 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution for 30 min. Addition of 6 N HCl terminated the reaction. Color change was read at 405 nm on a microplate ELISA reader.

2.5. Detection of cytokine in local skin

The abdominal skin of mice sensitized for 5 weeks was prepared to obtain protein for cytokines detection. Cytokines were detected using commercially available ELISA kits (IFN- γ , IL-4, IL-13) purchased from R&D Systems (Minneapolis, MN, USA).

2.6. Statistical analysis

Statistical comparisons between different groups of mice were performed with ANOVA. The data were expressed in mean $\pm$ S.E.M. unless otherwise specified. Unpaired pairwise contrasts between group means were done by using the Student Newman-Keuls test multiple comparison procedure. A value of *, $P < 0.01$ was taken as significant.

3. Results

3.1. The mice epicutaneously sensitized with soluble protein antigen show DTH reaction by scratching on abdominal skin

In this study, we focused on skin scratching which is a common feature in AD patients and studied the association with immune responses. First, we examined DTH reaction in the mice epicutaneously sensitized with protein antigen on abdominal skin. BALB/c mice were epicutaneously sensitized by KLH with or without brusher scratching on abdomen one time every week for 5 weeks (Fig. 1). The thickness was measured using micrometer after 24 and 48 h of injection with KLH into footpad. Surprisingly, skin scratching significantly induced DTH reaction in the mice sensitized by KLH. The reaction was strongest after 24 h of KLH injection. On the other hand, footpad swelling was not shown

in the mice sensitized by KLH without skin scratching (Fig. 2).

3.2. Abdominal skin scratching switches Th2 biased immune response toward Th1 in subclasses of immunoglobulin production

Subclasses of immunoglobulin produced by activated B cells are different between Th1 and Th2 biased response. IgG1 and IgE subtypes are predominant in Th2 biased response and IgG2a and IgG2b subtypes in Th1. To examine the effect of antigen specific immunoglobulin formation by skin scratching, BALB/c mice were epicutaneously sensitized by KLH with or without brusher scratching on abdomen one time every week. After 5 weeks of sensitization, the sera were collected and measured KLH specific immunoglobulin including IgG1, IgG2a, IgG2b and IgE by ELISA. Only epicutaneous sensitization significantly induced high amount of KLH specific IgG1 and IgE but not IgG2a and IgG2b. In contrast, skin scratching induced the significant level of KLH specific IgG2a and IgG2b but not IgE in the epicutaneously sensitized mice (Fig. 3). Unexpectedly, serum IgG1 on scratched mice was greater level to that on unscratched mice. To analyze the development of immunoglobulin subclasses, the sera were collected after 1, 3, 5 weeks of the sensitization (Fig. 1). The production of immunoglobulin subtypes increased in the time dependent manner from 1 to 5 weeks. Interestingly, significant IgE production was delayed in the mice without scratching (Fig. 4). When the sensitization was stopped at the point of 5 weeks, the expression of immunoglobulin had been kept for 1 month at least (data not shown).

3.3. Abdominal skin scratching shows different cytokine profile in the local skin

Local cytokine profile reflects substantial immune response in several diseases. In order to determine cytokine expression in local skin lesions, biopsy of abdomen was prepared from the epicutaneously sensitized mice with or without scratching after 1 week of the sensitization. IFN- γ , IL-4 and IL-13 by ELISA was measured in the homogenized skin after adjustment of protein concentration (Fig. 1). Interestingly, only epicutaneous sensitization induced significant level of IL-13 but not IFN- γ and IL-4. In contrast, skin scratching induced expression of IFN- γ , IL-4 and IL-13. (Fig. 5).

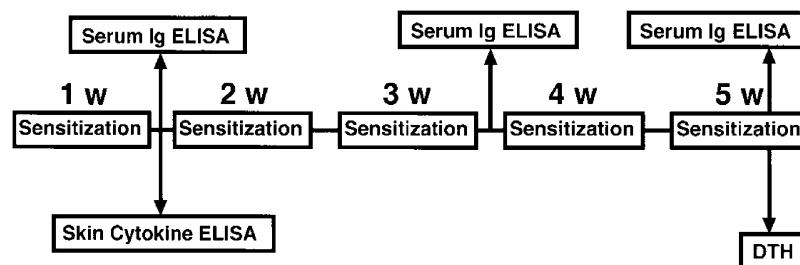


Fig. 1 Sensitization protocol. Mice were sensitized with KLH (2 mg/ml) applied in 100 μ l to a sterile patch by placement on abdominal skin with (patch + scratching) or without scratching (patch) for a week then removed. This application was continued one time every week for 5 weeks. Control mice were treated by saline instead of KLH without scratching in same management.

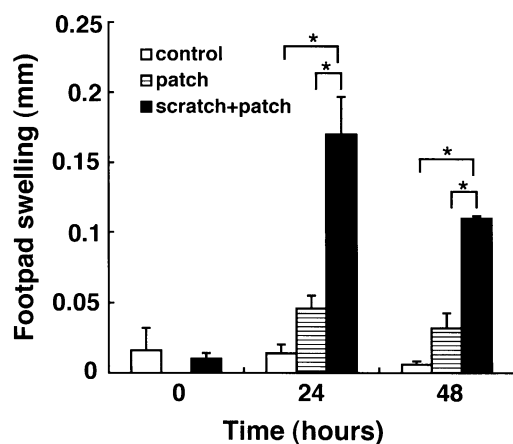


Fig. 2 KLH-specific DTH response induced by epicutaneous sensitization. The mice epicutaneously sensitized with KLH for 5 weeks were injected with KLH into the right footpad, and with saline into the left footpad. After 24 or 48 h, the footpad swelling was estimated as the distinction between the footpad thickness right and left. The data points represent the mean $\pm$ S.E.M. values ($n = 5$).

4. Discussion

The present results suggest that skin scratching modulates immune response, which was epicutaneously induced by soluble protein antigen, from Th2 biased response to Th1. Protein antigens such as ovalbumin (OVA) were reported to induce Th2 biased response by epicutaneous sensitization without adjuvant in mice [21,24–26]. In this study, BALB/c mice, which were epicutaneously sensitized by KLH, also showed significant expression of KLH specific IgG1 and IgE in the sera, and expressed IL-13 but not IFN- γ in the abdominal skin lesions. Contrary, brusher scratching treated on the abdomen before application with KLH induced significant expression of KLH specific IgG2a and IgG2b but not IgE in the sera, and expressed IFN- γ in the abdominal skin lesions. These results indicate that soluble protein antigen induces Th2 biased re-

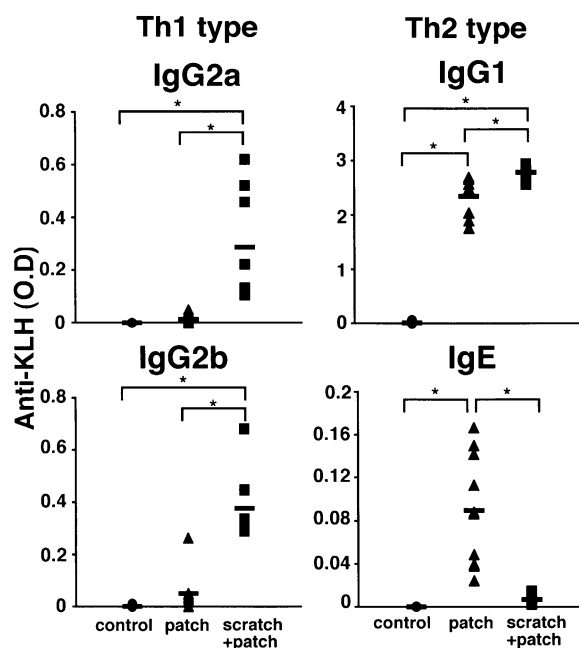


Fig. 3 KLH-specific antibody formations induced by epicutaneous sensitization. Sera were collected from the mice epicutaneously sensitized with KLH for 5 weeks, and were measured for KLH-specific antibodies detection by ELISA. Results from each individuals and mean values are shown ($n = 10$).

sponse by epicutaneous sensitization, then, skin scratching exactly modulates immune response from Th2 biased response to Th1 in this sensitization system. Most important of all, the present study is first to show that skin scratching works as a natural adjuvant in the epicutaneous sensitization with soluble protein antigens. Although many studies have reported several vaccine therapies for cancer and allergic disorders, this system, the epicutaneous immunization with skin scratching, may be an easy and safe tool in vaccination therapy.

Several reports suggested that the chronicity of AD is related to expression of IFN- γ in the local skin

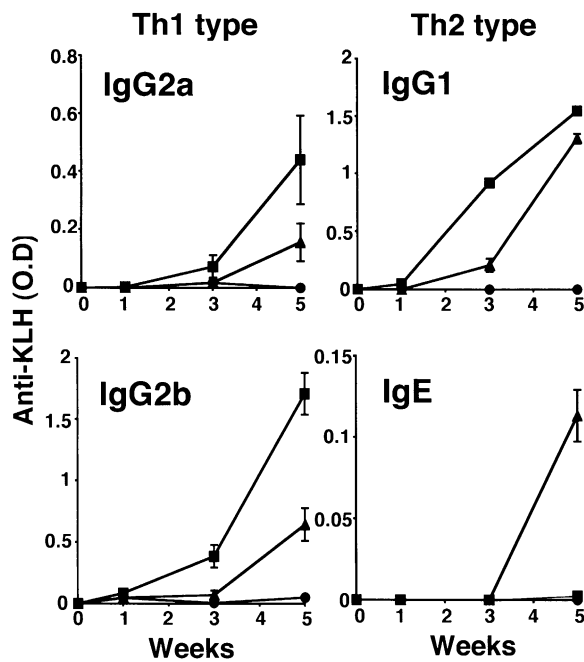


Fig. 4 Time dependent production of KLH-specific antibody in epicutaneously sensitized mice. Sera were collected from the mice epicutaneously sensitized with KLH for 1, 3 or 5 weeks, and were measured for KLH-specific antibodies detection by ELISA. Closed circles show results of control mice, closed triangles show that of sensitized mice without scratching and closed squares show that of sensitized mice with scratching. The data points represent the mean $\pm$ S.E.M. values ($n = 10$).

lesions [25,28,33,34], although AD is a chronic skin disease characterized by production of Th2 cytokines in the local skin lesions and IgE in the peripheral blood [28–30]. Furthermore, it was suggested that the expression of IFN- γ is correlated with low amount of IgE derived from peripheral blood mononuclear cells (PBMC) in IgE negative population with AD [35]. Our results are consistent with these reports, because we were able to detect IFN- γ in the abdominal skin derived from scratched mice but not antigen specific IgE in the sera. Atopic eczema is thought to be caused by skin-infiltrating CD4 $^{+}$ T cells of the Th1-like and/or Th2-like subtype [33]. The KLH-sensitized mice with skin scratching but not without scratching also showed DTH reaction mediated by antigen-specific CD4 $^{+}$ Th1 cells. Taken together, sensitization with protein antigen probably induces antigen specific CD4 $^{+}$ Th1 cells on the scratched mice. It is possible that CD4 $^{+}$ Th1 cells, which produce IFN γ , infiltrate into the local skin lesions of the scratched mice. Wang et al. reported that local but not systemic administration with IFN γ promotes IgG2a production, and suppresses IgE production in

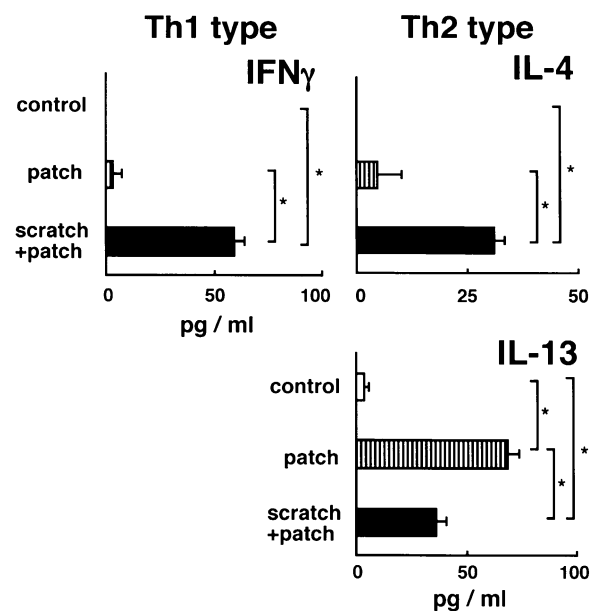


Fig. 5 Cytokine expressions in the abdominal skin lesions of epicutaneously sensitized mice. Abdominal skin lesions were obtained after treatment with KLH for 1 week. Protein was obtained from abdominal skin by lysis buffer and measured for cytokine detection by ELISA. The data points represent the mean $\pm$ S.E.M. values ($n = 5$).

the mice epicutaneously sensitized with OVA [36]. Their and our finding suggest the significance of local IFN γ expression for Th1 switching in sensitization phase of epicutaneous immunization with protein antigens. Recently, STAT6 (signal transducer and activator of transcription 6)-deficient NC/Nga mice were studied whether AD like skin lesions are induced. NC/Nga mice are characterized by the expression of AD like skin lesions and high level of IgE in the serum. Surprisingly, STAT6-deficient NC/Nga mice also showed AD like skin lesions [37]. Interestingly, STAT6-deficient NC/Nga mice showed up-regulated IFN- γ production in spleen derived CD4 $^{+}$ T cells. This report indicated Th2 cell independent nature of the AD pathogenesis in NC/Nga mice. Thus, IFN- γ may be critical cytokine in the pathogenesis with AD. Scratching action is a common feature in the patients with AD. It has been suggested that scratching action is associated with severity in the pathology of patients with AD. In present study, the effect of wire brusher scratching on abdomen in mice may represent scratching action in AD patients. Thus, our study indicates the importance of scratching action in AD pathology, because skin scratching significantly induced IFN- γ expression on abdominal skin in KLH sensitized mice.

In contrast, only epicutaneous sensitization significantly induced IL-13 on the abdominal skin and KLH specific IgE in the sera, although IL-4 was not significantly detected on the skin (Fig. 5). IL-13, which is important factor for IgE and IgG4 synthesis by B cells [38], is critical in Dp specific IgE production from mite sensitive AD patients [35,39]. In our study, the high amount of IL-13 on abdominal skin and antigen specific serum IgE was induced by epicutaneous sensitization without skin scratching. In contrast, the sensitization after skin scratching induced neither IL-13 nor IgE. Thus, the level of IL-13 on local abdominal skin was correlated with the antigen specific serum IgE levels in the mice. These data indicate that IL-13 is an important cytokine for production of serum IgE in the mice sensitized with soluble protein antigen.

IL-4, a major Th2 cytokine, is related with IgE class-switch by B cells [1,2,4]. It has been suggested that IL-4 plays a primary role in the overproduction of IgE in allergic diseases including AD [35,39]. In contrary, IL-4 independent class-switch mechanism was reported, and IL-4-deficient mice suggested that IL-4 is not necessary for Th2 biased response epicutaneously induced by OVA in the mice [26,40]. In our study, we could not detect significant level of IL-4 on the abdomen in the mice sensitized without skin scratching, although the mice expressed significant level of antigen specific IgE in the sera (Fig. 5). Thus IL-13 may be important for high amount of antigen specific IgE production rather than IL-4 in the epicutaneously sensitized mice by soluble protein antigen. On the other hand, skin scratching induced expression of IL-4 on the abdomen in the epicutaneously sensitized mice (Fig. 5). Kondo et al. reported to show IL-4 mRNA expression on the skin lesions in the epicutaneously sensitized mice by PiCl or mite antigens after stripping of skin using adhesive cellophane tape [23]. Furthermore, Werfel et al. demonstrated that majority of infiltrating house dust mite specific T cells express IFN γ mRNA, and IFN γ protein in chronic skin lesions of sensitized adult patients with AD in combination with IL-4 [41]. Our result is correlated with these previous reports with regard to IL-4 expression on local skin lesions.

In similarly to finding of IL-4 expression, it is difficult to simply explain the reason why IgG1 that has been thought as Th2 associated antibody was produced both KLH-sensitized mice with or without skin scratching. Recently, Yagi et al. reported that STAT6-deficient NC/Nga mice that develop AD-like skin lesions despite IgE deficiency produce IgG1, although IL-4 production completely defected because of deficiency of STAT6 [37]. Likewise, Herrick et al. also found the phenomenon both in IL-4 and

in IL-13 deficient mice [42]. These may suggest that IgG1 expression is not critically associated with Th2 dominant responses in epicutaneous sensitization with soluble protein antigens.

In time course experiment for serum Ig production, we found that Th1 development induced by KLH-sensitization with skin scratching is faster than Th2 development by KLH-sensitization without skin scratching (Fig. 4). Wood et al. demonstrated that keratinocytes directly produce several cytokines, such as TNF α , IL-1 α and GM-CSF, in response to skin barrier disruption by mechanical stimulation [43]. Furthermore, these cytokines up-regulate expression of MHC class II and co-stimulatory molecules on epidermal LC [44]. Thus, skin barrier disruption induces not only altered permeability but also cutaneous immunoregulatory function. Skin scratching may induce sufficient activation of LC by presentation with high dose antigen and potent induction of activity for antigen presentation with regard to the difference of kinetics between scratched and unscratched mice.

Kinetics of antigen specific IgE production was slow in epicutaneous sensitization without skin scratching in comparison with production of other Ig subtypes (Fig. 4). Repeated epicutaneous application may be required for antigen specific IgE induction by soluble protein antigen, such as the patients of latex allergy, which is IgE-mediated allergy to natural rubber latex [45–48].

In this study, it remains unclear the reason why skin scratching switches immune responses. Recently, it was reported that directing Th1 and Th2 cell development is dependent on antigen dose. Distinct dendritic cell (DC) subsets, such as myeloid and plasmacytoid precursor DC cultured from bone marrow precursors, induced Th2 cell development on low dose of protein antigen. On the other hand, high dose antigen induced Th1 cell development in presence of distinct DC subsets [49]. This result may explain the mechanism of immune modulation mediated by skin scratching in our study. Because skin barrier disruption allows antigens to penetrate easily, epidermal LC and dermal DC may capable of contact to antigens in scratching skin. We can think another possibility that Th1 switching induced with skin scratching results in potent activation of DC mediated by cytokines induced with skin scratching from epidermis. Cutaneous barrier perturbation stimulates productions of cytokine that up-regulates expression of MHC class II and co-stimulatory molecules on epidermal LC [44] in the epidermis of mice. As shown in report by Wood et al., acute barrier disruption with tape stripping induced elevated mRNA levels of TNF, IL-1 α , IL-1 β , and GM-CSF after 2.5 h [43]. In our study, skin scratch-

ing mediated barrier disruption may potentially enhance LC and DC activation, and induce Th1 biased immune response. Anyway, it is our future study to determine why skin scratching switches the immune response from Th2 dominance to Th1.

In summary, we demonstrate that skin scratching modulates immune response from Th2 dominance to Th1. While questions remain outstanding regarding the regulatory mechanism underlying skin scratching and switching from Th2 dominant response to Th1, the findings presented here have several clinical implications. In addition, this murine model may help understand the mechanism of AD and provide easy and safety vaccination strategy.

Acknowledgements

This work was supported by grants from the Ministry of Education, Science and Culture of Japan (S.S.; 12470177).

References

- [1] Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th1/Th2 polarization. *Immunol Today* 2000;21:479–83.
- [2] D'Ambrosio D, Iellem A, Colantonio L, Clissi B, Pardi R, Sinigaglia F. Localization of Th-cell subsets in inflammation: differential thresholds for extravasation of Th1 and Th2 cells. *Immunol Today* 2000;21:183–6.
- [3] Kim SH, Cho D, Hwang SY, Kim TS. Efficient induction of antigen-specific, T helper type 1-mediated immune responses by intramuscular injection with ovalbumin/interleukin-18 fusion DNA. *Vaccine* 2001;19:4107–14.
- [4] Mosmann TR, Sad S. The expanding universe of T-cell subset. *Immunol Today* 1996;17:138–46.
- [5] Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. *J Immunol* 1986;136:2348–57.
- [6] Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J Exp Med* 1987;166:1229–44.
- [7] O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subset. *Immunity* 1998;8:275–83.
- [8] Renz H, Smith HR, Henson JE, Ray BS, Irvin CG, Gelfand EW. Aerosolized antigen exposure without adjuvant causes increased IgE production and increased airway responsiveness in the mouse. *J Allergy Clin Immunol* 1992;89:1127–38.
- [9] Reiner SL, Locksley RM. The regulation of immunity to *Leishmania major*. *Annu Rev Immunol* 1995;13:151–77.
- [10] Constant SL, Bottomly K. Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu Rev Immunol* 1997;15:297–322.
- [11] Guery JC, Galbiati F, Smiroldo S, Adorini L. Selective development of T helper (Th) 2 cells induced by continuous administration of low dose soluble proteins to normal and beta (2)-microglobulin-deficient BALB/c mice. *J Exp Med* 1996;183:485–97.
- [12] Guery JC, Galbiati F, Smiroldo S, Adorini L. Non-MHC-linked Th2 cell development induced by soluble protein administration predicts susceptibility to *Leishmania major* infection. *J Immunol* 1997;159:2147–53.
- [13] Malherbe L, Filippi C, Julia V, Foucras G, Moro M, Appel H, Wucherpfennig K, Guery JC, Glaichenhaus N. Selective activation and expansion of high-affinity CD4+ T cells in resistant mice upon infection with *Leishmania major*. *Immunity* 2000;13:771–82.
- [14] Blander JM, Sant'Angelo DB, Bottomly K, Janeway CA. Alteration at a single amino acid residue in the T cell receptor α chain complementarily determining region 2 changes the differentiation of naïve CD4T cells in response to antigen from T helper cell type 1 (Th1) to Th2. *J Exp Med* 2000;191:2065–74.
- [15] Kapsenberg ML, Wierenga EA, Stiekema FEM, Tiggeleman AMBC, Bos JD. Th1 lymphokine production profiles of nickel-specific CD4+ T-lymphocyte clones from nickel contact allergic and non-allergic individuals. *J Invest Dermatol* 1992;98:59–63.
- [16] Sinigaglia F, Scheidegger D, Garotta G, Scheper R, Pletscher M, Lanzavecchia A. Isolation and characterization of Ni-specific T cell clone from patients with Ni-contact dermatitis. *J Immunol* 1985;135:3929–32.
- [17] Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. *J Immunol* 1997;159:2484–91.
- [18] Sinigaglia F. The molecular basis of metal recognition by T cells. *J Invest Dermatol* 1994;102:398–401.
- [19] Enk AH, Katz SI. Contact sensitivity as a model for T-cell activation in skin. *J Invest Dermatol* 1995;105:80S–3S.
- [20] Cavani A, Hackett CJ, Wilson KJ, Rothbard JB, Katz SI. Characterization of epitopes recognized by hapten-specific CD4+ T cells. *J Immunol* 1995;154:1232–8.
- [21] Wang LF, Lin JY, Hsieh KH, Lin RH. Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. *J Immunol* 1996;156:4079–82.
- [22] Hurst SD, Seymour BW, Muchamuel T, Kurup VP, Coffman RL. Modulation of inhaled antigen-induced IgE tolerance by ongoing Th2 responses in the lung. *J Immunol* 2001;166:4922–30.
- [23] Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur J Immunol* 1998;28:769–79.
- [24] Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;101:1614–22.
- [25] Spergel JM, Mizoguchi E, Oettgen H, Bhan AK, Geha RS. Roles of Th1 and Th2 cytokines in a murine model of allergic dermatitis. *J Clin Invest* 1999;103:1103–11.
- [26] Herrick CA, MacLeod H, Glusac E, Tigelaar RE, Bottomly K. Th2 responses induced by epicutaneous or inhalational protein exposure are differentially dependent on IL-4. *J Clin Invest* 2000;105:765–75.
- [27] Seo N, Tokura Y, Nishijima T, Hashizume H, Furukawa F, Takigawa M. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Proc Natl Acad Sci* 2000;97:371–6.

- [28] Grewe M, Bruijnzeel-Koomen CAFM, Schopf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann JA. Role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998;19:359–61.
- [29] Akdis M, Simon HU, Weigl L, Kreyden O, Blaser K, Akdis CA. Skin homing (cutaneous lymphocyte-associated antigen-positive) CD8+ positive T cells respond to superantigen and contribute to eosinophilia and IgE production in atopic dermatitis. *J Immunol* 1999;163:466–75.
- [30] Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic investigation. *J Allergy Clin Immunol* 2000;105:860–76.
- [31] Terui T, Sano K, Okada M, Shirota H, Honda M, Ozawa M, Hirasawa N, Tamura G, Tagami H. Production and pharmacologic modulation of the granulocyte-associated allergic responses to ovalbumin in murine skin models induced by injecting ovalbumin-specific Th1 or Th2 cells. *J Invest Dermatol* 2001;117:236–43.
- [32] Werfel T, Morita A, Grewe M, Renz H, Wahn U, Krutmann J, Kapp A. Allergen specificity of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. *J Invest Dermatol* 1996;107:871–6.
- [33] Grewe M, Gyufko K, Schopf E, Krutmann J. Lesional expression of interferon-gamma in atopic eczema. *Lancet* 1994;343:25–6.
- [34] Grewe M, Walther S, Gyufko K, Czech W, Schopf E, Krutmann J. Analysis of the cytokine pattern expressed in situ in inhalant allergen patch test reactions of atopic dermatitis patients. *J Invest Dermatol* 1995;105:407–10.
- [35] Wakugawa M, Hayashi K, Nakamura K, Tamaki K. Evaluation of mite allergen-induced Th1 and Th2 cytokine secretion of peripheral blood mononuclear cells from atopic dermatitis patients: association between IL-13 and mite-specific IgE levels. *J Dermatol Sci* 2001;25:116–26.
- [36] Wang L-F, Wu J-T, Sun C-C. Local but not systemic administration of IFN γ during the sensitization phase of protein antigen immunization suppress Th2 development in a murine model of atopic dermatitis. *Cytokine* 2002;19:147–52.
- [37] Yagi R, Nagai H, Iigo Y, Akimoto T, Arai T, Kubo M. Development of atopic dermatitis-like skin lesions in STAT6-deficient NC/Nga mice. *J Immunol* 2002;168:2020–7.
- [38] Punnonen J, Aversa G, Cocks BG, McKenzie ANJ, Menon S, Zurawski G, Malefyt RW, Vries JE. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci* 1993;90:3730–4.
- [39] Kanda N, Watanabe S. Intracellular 3',5'-adenosine cyclic monophosphate level regulates house dust mite-induced Interleukin-13 production by T cells from mite-sensitive patients with atopic dermatitis. *J Invest Dermatol* 2001;116:3–11.
- [40] Hamid Q, Boguniewicz M, Leung DYM. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;94:870–6.
- [41] Werfel T, Morita A, Grewe M, Renz H, Wahn U, Krutmann J, Kapp A. Allergen specific of skin-infiltrating T cells is not restricted to a type-2 cytokine pattern in chronic skin lesions of atopic dermatitis. *J Invest Dermatol* 1996;107:871–6.
- [42] Herrick CA, Xu L, McKenzie ANJ, Tigelaar RE, Bottomly K. IL-13 is necessary, not simply sufficient, for epicutaneously induced Th2 responses to soluble protein antigen. *J Immunol* 2003;170:2488–95.
- [43] Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 1992;90:482–7.
- [44] Nishijima T, Tokura Y, Imokawa G, Seo N, Furukawa F, Takigawa M. Altered permeability and disordered cutaneous immunoregulatory function in mice with acute barrier disruption. *J Invest Dermatol* 1997;109:175–82.
- [45] Morawetz RA, Gabriele L, Rizzo LV, Trauth NN, Kuhn R, Rajewsky K, Muller W, Doherty TM, Finkelman F, Coffman RL, Morse III HC. Interleukin (IL)-4-independent immunoglobulin class switch to Immunoglobulin (Ig) E in the mouse. *J Exp Med* 1996;184:1651–61.
- [46] Sussman GL, Tario S, Dolovich J. The spectrum of IgE-mediated responses to latex. *J Am Med Assoc* 1991;265:2844–7.
- [47] Kurup VP, Fink JN. The spectrum of immunologic sensitization in latex allergy. *Allergy* 2001;56:2–12.
- [48] Zucker-Pinchoff B, Stadtmayer GJ. Latex allergy. *Mt Sinai J Med* 2002;69:88–95.
- [49] Boonstra A, Asselin-Paturel C, Gilliet M, Crain C, Trinchieri G, Liu YJ, O'Garra A. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential Toll-like receptor ligation. *J Exp Med* 2003;197:101–9.