topical corticosteroids and tacrolimus, may have influenced these results. Further studies are required to confirm the emollient therapy for the pruritus. We expect that the results of our questionnaire analysis will be useful to some extent for improving the ability of dermatologists to determine the appropriate role of emollients in the treatment regimen for AD.

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REFERENCES

1 Berth-Jones J, Damstra RJ, Golsch S *et al.* Twice weekly fluticasone propionate added to emollient maintenance treatment to reduce risk of relapse in atopic dermatitis: randomised, double blind, parallel group study. *BMJ* 2003; **326**: 1367.
2 Hanifin J, Gupta AK, Rajagopalan R. Intermittent dosing of fluticasone propionate cream for reducing the risk of relapse in atopic dermatitis patients. *Br J Dermatol* 2002; **147**: 528–537.
3 Pecegueiro M, Brandão M, Pinto J, Conçalo S. Contact dermatitis to Hirudoid cream. *Contact Dermatitis* 1987; **17**: 290–293.
4 Bergqvist D, Brunkwall J, Jensen N, Persson NH. Treatment of superficial thrombophlebitis. A comparative trial between placebo, Hirudoid cream and piroxicam gel. *Ann Chir Gynaecol* 1990; **79**: 92–96.
5 Smeenk G, Kerckhoffs HP, Schreurs PH. Contact allergy to a reaction product in Hirudoid cream: an example of compound allergy. *Br J Dermatol* 1987; **116**: 223–231.
6 Saeki H, Furue M, Furukawa F *et al.* Guidelines for management of atopic dermatitis. *J Dermatol* 2009; **36**: 563–577.
7 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980; **92**: 44–47.
8 Wirén K, Nohlgård C, Nyberg F *et al.* Treatment with a barrier-strengthening moisturizing cream delays relapse of atopic dermatitis: a prospective and randomized controlled clinical trial. *J Eur Acad Dermatol Venereol* 2009; **23**: 1267–1272.
9 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**: 441–446.
10 Weidinger S, Illig T, Baurecht H *et al.* Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis within allergic sensitizations. *J Allergy Clin Immunol* 2006; **118**: 214–219.
11 Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009; **129**: 543–552.

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ORIGINAL ARTICLE

Health-related quality of life assessed by the effect of bepotastine besilate in patients with pruritus: Importance of emotions score in atopic dermatitis

Tamihiro KAWAKAMI, Satoko KIMURA, Tsuneo HAGA, Risako DOI, Mikiko KYOYA, Keiko NAKAGAWA, Yoshinao SOMA

Department of Dermatology, St Marianna University School of Medicine, Kawasaki, Japan

ABSTRACT

The Skindex-16 questionnaire was recently developed as a measure of dermatological health-related quality of life (HRQoL), including symptoms, emotions and functional aspects. Bepotastine besilate is a selective histamine H_1-receptor antagonist and a second-generation non-sedating antihistamine to treat various dermatological disorders. We assessed changes of the HRQoL instrument (Skindex-16) on patients with pruritus, including those with atopic dermatitis (AD) over bepotastine treatment period. The patients' personal assessment of the intensity of pruritus was determined using the Visual Analog Scale (VAS) for pruritus. Patients answered the Skindex-16 at baseline and at week 4. Forty-eight of 51 enrolled dermatological patients completed the Skindex-16. Of the 48 patients, 11 had AD and 37 had other conditions. Improvement in the clinical evaluation and VAS score was significant in all patients, the AD group and the other disorders group between baseline and week 4. Skindex-16 showed significantly lower scores for each of the three scales (symptoms, emotions and functioning) and the global score at baseline compared to that at week 4 in all patients and the other disorders. In contrast, there was a significant reduction in the emotions and global score among the AD patients. We found a significant correlation between falls in emotions score of Skindex-16 and falls in VAS scores for pruritus in the AD group. Bepotastine could be effective in the management of patients' HRQoL and useful in patients suffering with pruritus. We suggested that pruritus of AD patients could exert a stronger emotional effect due to the skin condition compared to the symptomatic or functional effects.

Key words: atopic dermatitis, bepotastine besilate, emotions, health-related quality of life, pruritus, Skindex-16.

INTRODUCTION

Itching arises from a variety of skin conditions.[1,2] Particularly, itching is the predominant feature of atopic dermatitis (AD) and it is an essential diagnostic feature of the disease.[3,4] Learning more about how itching affects people may help us develop better treatment strategies for dermatological patients with pruritus. Histamine H_1-receptor antagonists (antihistamines) are used to treat various dermatological disorders including urticaria, eczema and AD.[5] Bepotastine besilate is a selective histamine H_1-receptor antagonist and a second-generation, non-sedating antihistamine that has recently been used for the treatment of allergic disorders in Japan.[6]

Health-related quality of life (HRQoL) has emerged as an important outcome of clinical investigation and patient care in dermatology.[7] The Skindex has been recently developed as a specific measure of HRQoL in dermatology patients, quantifying the effects of skin disease on patients' HRQoL, which has been extensively studied and refined. Initially, the Skindex was comprised of 61 questions but was then modified to 29 questions. Later, a briefer version consisting of 16 questions was introduced, which is known as Skindex-16.[8–10] The Skindex-16 is a self-administered questionnaire covering symptoms, emotions and functional aspects of dermatological conditions. Four questions (nos. 1–4) are related to symptoms, seven questions (nos. 5–11) are related to emotions, five questions (nos. 12–16) are related to functioning, and all 16 questions are related to global score. We assessed changes of the HRQoL instrument (Skindex-16) on patients with pruritus, including those with AD, over a bepotastine treatment period.

METHODS

Patients

Fifty-one Japanese patients suffering from pruritus and above the age of 20 years seen at the Department of Dermatology,

Correspondence: Tamihiro Kawakami, M.D., Ph.D., Department of Dermatology, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan. Email: tami@marianna-u.ac.jp

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St Marianna University School of Medicine between 2008 and 2010 were eligible for the study. Exclusion criteria were specific pruritus treatments that patients were currently taking or had received in the last 3 months. These treatments included phototherapy, antihistamines, corticosteroids, immunosuppressive drugs, or any other experimental or investigational drug. Patients gave written informed consent prior to the start of the study. Dermatological disorders were diagnosed by experienced dermatologists based on the Japanese Dermatological Association criteria. The diagnosis of AD was defined by the Hanifin and Rajka[11] diagnostic criteria.

Forty-eight of the 51 enrolled patients with pruritus completed the Skindex-16 questionnaire and the results of bepotastine treatment were followed over the course of 4 weeks. Of the 48 patients (26 men, 22 women; aged 55.6 ± 21.9 years), 11 (23%) had AD (six men, five women; aged 27.1 ± 10.8 years) and 37 (77%) had other conditions (20 men, 17 women; age 64.1 ± 16.4 years). In the other disorder group, 10 patients were diagnosed with cutaneous pruritus of unknown origin, eight with urticaria, seven with prurigo chronica and seven with chronic eczema. The mean duration of their disorder was 46.9 ± 78.3 months for all 48 patients, 135.6 ± 118.9 months for the AD group and 19.2 ± 25.8 months for the other disorder group. The ages and duration of disorder differed significantly between the AD group and the other disorders group.

Methods

All 51 patients with associated pruritus who enrolled in the study were treated with an oral non-sedating H_1 antihistamine, bepotastine besilate 10 mg, twice daily (morning, evening) for 4 weeks. The patients continued using any topical corticosteroid and/or emollient they were currently using during the 4 weeks. Clinical evaluation for pruritus was graded as "none", "mild", "moderate", "severe" or "very severe" according to same dermatologists who examined the patients with pruritus at baseline and at week 4. All adverse events were recorded throughout the study from the time of patient consent to the final visit. The patients similarly reported on the intensity of pruritus at baseline and week 4 using the Visual Analog Scale (VAS) for pruritus.[12,13] Patients graded their pruritus using a 10-cm VAS, with "none" on the left side of the scale and "severe" on the right. All patients answered the Skindex-16 questionnaire at baseline and at week 4. HRQoL was assessed using the Skindex-16 questionnaire, which consists of 16 items within three scales: symptoms, emotions and functioning. Patients answered each question using a 7-point scale from 0–6 (0 = "never bothered" and 6 = "constantly bothered"). The mean score was determined for each scale. The global score was calculated by averaging the mean scores for each item. Further details of scoring are provided in previous publications.[10] The questionnaire was distributed to each patient, and they submitted them to the clerks in our clinic on the same day.

Statistical analysis

The level of significance was set at $P < 0.05$ in all cases. Mann–Whitney *U*-test was performed to define the patient's background between two patient groups, the AD group and the group with other disorders. Clinical evaluations for pruritus assessed by dermatologists at baseline and week 4 were analyzed by Wilcoxon rank sum tests. The statistics were analyzed by paired Student's *t*-test to compare VAS scores for pruritus and Skindex-16 scores among all patients, the AD group and the other disease group. Spearman's rank correlation coefficients were used to compare the difference between the Skindex-16 scores and VAS scores for pruritus. All data are expressed as the mean ± standard deviation.

This study was based on the ethical principles of Good Clinical Practice and was approved by the St Marianna University School of Medicine Institutional Review Board for Human Subjects Research (no. 1427).

RESULTS

Clinical evaluation and VAS scores for pruritus

Clinical evaluation for pruritus assessed by dermatologists over bepotastine treatment period improved significantly in all 48 patients, the AD group and the other disease group (Fig. 1). There were no reported adverse affects during bepotastine treatment. VAS scores (27.3 ± 26.0) for pruritus assessed by each patient at week 4 were significant lower than the baseline score (60.7 ± 20.1) in all 48 patients with pruritus. VAS scores (34.5 ± 25.5) for pruritus at week 4 were significantly lower than the baseline score (60.5 ± 17.1) in the AD group. VAS scores (25.1 ± 26.1) for pruritus at week 4 were significantly lower than the baseline score (60.7 ± 21.1) in the other disease group. There was no significant difference in VAS scores for pruritus between the AD group and the other disorder group.

Changes in Skindex-16 scores

All 48 patients who completed the Skindex-16 questionnaire showed significantly lower scores for each of the three scales (symptoms, emotions and functioning) and the global score at baseline compared to that at week 4 (Fig. 2). This indicates an improvement in HRQoL among the pruritus patients who underwent

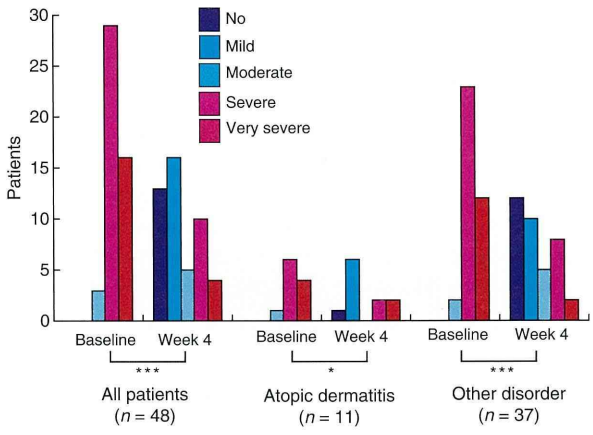

Figure 1. Clinical evaluation of pruritus assessed by dermatologists over bepotastine treatment period was significant in all patients, the atopic dermatitis group and the other disease group using Wilcoxon rank sum tests. ***$P < 0.001$, *$P < 0.05$.

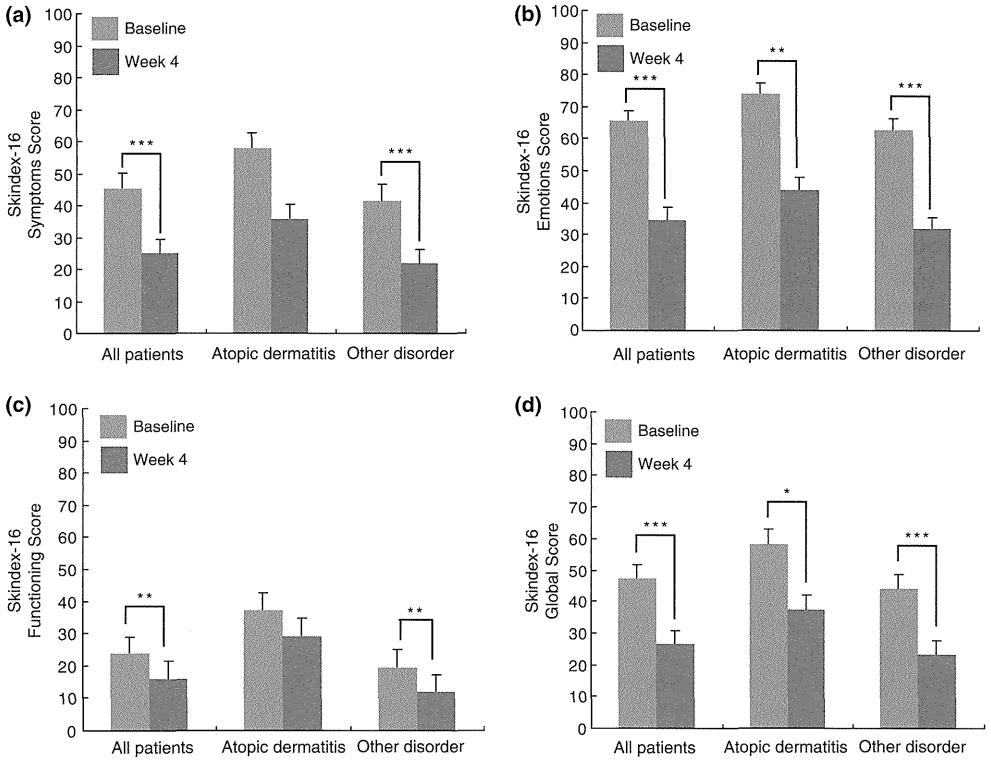

Figure 2. Skindex-16 questionnaire in symptoms score (a), emotions score (b), functioning score (c) and global score (d) at baseline and week 4 among all patients, the atopic dermatitis (AD) group and the other disorder group. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

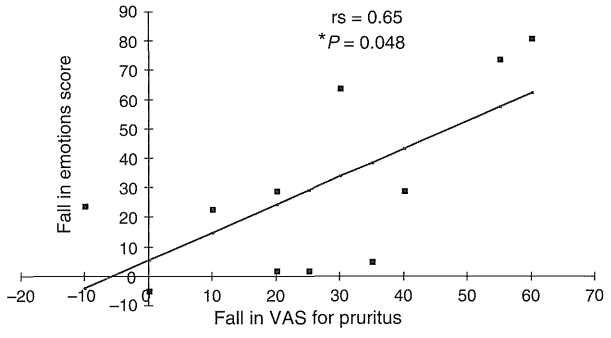

Figure 3. We found significant correlation between falls in emotions score of Skindex-16 and falls in Visual Analog Scale (VAS) score for pruritus among the atopic dermatitis group who underwent bepotastine treatment using Spearman's rank correlation coefficients.

bepotastine treatment. This same trend was seen among the other disorder group, where there was a significant reduction in four Skindex-16 scores between baseline and week 4 (Fig. 2). In contrast, the symptoms and functioning scores in the AD group did not decrease significantly between baseline and week 4 (Fig. 2a,c). However, there was a significant reduction in the emotions and global score among the AD patients (Fig. 2b,d). Furthermore, we found a significant correlation between falls in emotions score of Skindex-16 and falls in VAS scores for pruritus in the AD group (Fig. 3).

DISCUSSION

In the present study, the pruritus of our patients who received bepotastine to treat their itching improved from both the dermatologists' and patients' point of view. Skin disease-specific, the Skindex-16 HRQoL instrument is valuable for assessing patients' outcomes, especially their response to therapy. These improvements were noted against the three scales (symptoms, emotions and functioning) of the Skindex-16 questionnaire, in addition to global scores of the Skindex-16 questionnaire over the treatment period. We suggest that bepotastine could be effective in the management of patients' HRQoL and useful in patients suffering with pruritus.

Both clinical evaluation for pruritus by dermatologists and VAS scores for pruritus by patients significantly improved in the AD group after 4-week administration of bepotastine. Some investigated whether the Skindex-16 could measure disease severity and clinical change in the estimation of the effects of AD on patients' HRQoL.[14,15] The Skindex-16 questionnaire showed significantly lower scores for both emotions score and global score at baseline compared to that at week 4 in the AD group. We found that falls in the emotions score significantly correlated with falls in VAS scores for pruritus. Based on these data, we suggest that pruritus of AD

patients could exert a stronger emotional effect due to the skin condition compared to the symptomatic or functional effects, as indicated by the high scores on the emotional component of the Skindex-16 in pruritus patients with AD. Therefore, emotional impact could be an important measure of HRQoL in AD patients. Due to scratching, the primary skin disease may be confounded by secondary scratch lesions. This may occur in excoriated forms of AD. Acute phases in AD are primarily characterized by extreme pruritus, which in turn leads to excoriation. Excoriation further exacerbates the underlying inflammation, setting up an itch–scratch cycle, resulting in chronic lesions.[16] Bepotastine has favorable antihistamine properties leading to allergic inflammation beyond those directly involving the histamine H_1-receptor.[17] It is important for dermatologists to effectively treat the underlying causes of emotion associated with pruritus in AD patients. We expect that the results of our questionnaire analysis may provide some basis for dermatologists to effectively assess HRQoL in the treatment regimen for AD.

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REFERENCES

1 Ständer S, Weisshaar E, Mettang T *et al.* Clinical classification of itch: a position paper of the International Forum for the Study of Itch. *Acta Derm Venereol* 2007; **87**: 291–294.
2 Lovell P, Vender RB. Management and treatment of pruritus. *Skin Therapy Lett* 2007; **12**: 1–6.
3 Stander S, Steinhoff M. Pathophysiology of pruritus in atopic dermatitis: an overview. *Exp Dermatol* 2002; **11**: 12–24.
4 Dawn A, Papoiu AD, Chan YH, Rapp SR, Rassette N, Yosipovitch G. Itch characteristics in atopic dermatitis: results of a web-based questionnaire. *Br J Dermatol* 2009; **160**: 642–644.
5 Sidbury R. Systemic therapy of atopic dermatitis. *Clin Exp Dermatol* 2000; **25**: 559–566.
6 Nakahara T, Urabe K, Moroi Y, Morita K, Furue M. Bepotastine besilate rapidly inhibits mite-antigen induced immediate reactions in atopic dermatitis. *J Dermatol Sci* 2003; **32**: 237–238.
7 Abeni D, Picardi A, Pasquini P, Melchi CF, Chren MM. Further evidence of the validity and reliability of Skindex-29: an Italian study on 2242 dermatological outpatients. *Dermatology* 2002; **204**: 43–49.
8 Chren MM, Lasek RJ, Sahay AP, Sands LP. Measurement properties of Skindex-16: a brief quality of life measure for patients with skin diseases. *J Cutan Med Surg* 2001; **5**: 105–110.
9 AlGhamdi KM, AlShammari SA. Arabic version of Skindex-16: translation and cultural adaptation, with assessment of reliability and validity. *Int J Dermatol* 2007; **46**: 247–253.
10 Higaki Y, Kawamoto K, Kamo T, Horikawa N, Kawashima M, Chren MM. The Japanese version of Skindex-16: a brief quality-of-life measure for patients with skin diseases. *J Dermatol* 2002; **29**: 693–698.
11 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980; **92**: 44–47.
12 Kawakami T, Kaminishi K, Soma Y, Kushimoto T, Mizoguchi M. Oral antihistamine therapy influences plasma tryptase levels in adult atopic dermatitis. *J Dermatol Sci* 2006; **43**: 127–134.
13 Imaizumi A, Kawakami T, Murakami F, Soma Y, Mizoguchi M. Effective treatment of pruritus in atopic dermatitis using H1 antihistamines (second-generation antihistamines): changes in blood histamine and tryptase levels. *J Dermatol Sci* 2003; **33**: 23–29.
14 Higaki Y, Kawamoto K, Kamo T, Ueda S, Arikawa J, Kawashima M. Measurement of the impact of atopic dermatitis on patients' quality of life: a cross-sectional and longitudinal questionnaire study using the Japanese version of Skindex-16. *J Dermatol* 2004; **31**: 977–982.
15 Kawashima M, Harada S. Effect of standard medication on quality of life of patients with atopic dermatitis. *J Dermatol* 2007; **34**: 9–16.
16 Wahlgren CF. Itch and atopic dermatitis: an overview. *J Dermatol* 1999; **26**: 770–799.
17 Williams JI, Gow JA, Klier SM, McCue SL, Salapatek AM, McNamara TR. Non-clinical pharmacology, pharmacokinetics, and safety findings for the antihistamine bepotastine besilate. *Curr Med Res Opin* 2010; **26**: 2329–2338.

ORIGINAL ARTICLE

Interleukin 33 Is Induced by Tumor Necrosis Factor α and Interferon γ in Keratinocytes and Contributes to Allergic Contact Dermatitis

K Taniguchi,[1,2] S Yamamoto,[1] E Hitomi,[1] Y Inada,[1] Y Suyama,[1] T Sugioka,[2] Y Hamasaki[1]

[1]Department of Pediatrics, Faculty of Medicine, Saga University, Saga, Japan
[2]Community Medical Support Institute, Faculty of Medicine, Saga University, Saga, Japan

Abstract

Background: Interleukin (IL) 33, a novel member of the IL-1 family, is produced mainly by epithelial cells and endothelial cells in response to various types of stress, including necrosis. The effects of IL-33 on the immune cells involved in allergic contact dermatitis have recently been revealed in vitro. However, in vivo, the induction mechanism and function of IL-33 are not fully understood.
Objectives: Our objectives were to investigate induction of IL-33 in keratinocytes and to evaluate the functions of IL-33 and its inducers in a murine model of allergic contact dermatitis.
Material and Methods: KERTr cells, a human keratinocyte cell line, were cultured with various cytokines, including tumor necrosis factor (TNF) α and interferon (IFN) γ. IL-33 expression was detected using quantitative reverse transcriptase polymerase chain reaction, immunocytochemistry, and Western blotting. The functions of IL-33, TNF-α, and IFN-γ in allergic contact dermatitis were evaluated using a murine model.
Results: TNF-α and IFN-γ induced expression of IL-33 mRNA and protein in KERTr cells. Blockade of IL-33 attenuated swelling in the ears of the experimental mice. Similar effects were noted for blockade of TNF-α and IFN-γ in these mice.
Conclusions: TNF-α and IFN-γ induce expression of IL-33, and IL-33 produced by keratinocytes contributes to allergic contact dermatitis. Blockade of IL-33, TNF-α, and IFN-γ could represent novel and potent strategies to treat allergic contact dermatitis.

Key words: Interleukin 33. Keratinocyte. Tumor necrosis factor α. Interferon γ. Allergic contact dermatitis.

Resumen

Antecedentes: La Interleucina 33 (IL-33), un nuevo miembro de la familia de la IL-1, es producida fundamentalmente por las células epiteliales y endoteliales en respuesta a diferentes estímulos, incluyendo la necrosis.
Recientemente se han confirmado los efectos de esta IL sobre las células del sistema inmunológico in vitro en pacientes con dermatitis de contacto, aunque los mecanismo y función in vivo de la IL-33 no son bien conocidos.
Objetivos: El objetivo de este estudio fue analizar los factores que podrían inducir IL-33 en queratinocitos y evaluar las funciones de esta citocina y de sus inductores en un modelo murino de dermatitis alérgica de contacto.
Métodos: Para ello se cultivaron células KERTr, una línea celular de queratinocitos humanos, en presencia de varias citocinas, incluyendo TNF-α e IFN –γ. La expresión de IL-13 se detectó mediante PCR cuantitava a tiempo real, inmunocitoquímica e inmunobloting. Así mismo se evaluó la función de IL-33, TNF-α, e IFN-γ en el modelo murino.
Resultados: En cuanto a los resultados obtenidos TNF-α y IFN-γ indujeron la expresión de mRNA y expresión de proteína en las células KERTr. El bloqueo de IL-33 atenúa la inflación en la dermatitis de contacto murina.
Efectos similares se obtienen mediante el bloqueo de TNF-α y IFN-γ.
Conclusiones: En conclusión, TNF-α and IFN-γ son inductores de la producción de IL-33, y además esta citocina producida por los queratinocitos contribuye a la expresión de dermatitis alérgica de contacto. El bloqueo de no solo IL-33, sino también de TNF-α y IFN-γ podría representar una modalidad terapéutica nueva y potente en la dermatitis alérgica de contacto.

Palabras clave: Interleucina-33. Queratinocito. Factor de necrosis tumoral α. Interferón γ.

Introduction

Interleukin (IL) 33 is a cytokine of the IL-1 family IL-1F11. It acts as a specific extracellular ligand for the orphan IL-1 receptor–related protein ST2 [1]. Epithelial cells and endothelial cells were reported to be the main cellular sources of IL-33 [1-3]. IL-33 is released by cells undergoing necrosis and, in this respect, is thought to function as a damage-associated molecular pattern and alarmin [3,4]. ST2, the receptor for IL-33, is expressed on various immune cells, including type 2 helper T cells (T_H2) [5], $CD8^+$ T cells [6], mast cells [7], eosinophils [8], basophils [9], natural killer (NK) cells, NKT cells [10], and dendritic cells [11]. IL-33 induces production of T_H2 cytokines (including IL-5 and IL-13) from T_H2 cells, eosinophils, basophils, NK cells, and NKT cells [12]. Dendritic cells stimulated by IL-33 promote differentiation of T_H2 cells [13]. Natural helper cells [14] and nuocytes [15] have recently attracted attention because they produce abundant T_H2 cytokines when stimulated by IL-33. Therefore, IL-33 is closely involved in T_H2 immune responses. In fact, IL-33 is associated with a range of allergic diseases, including bronchial asthma [16], allergic rhinitis [17], allergic conjunctivitis [18], anaphylaxis [19], atopic dermatitis [20], and allergic contact dermatitis [21].

Allergic contact dermatitis is a clinical form of contact hypersensitivity. It involves a delayed-type hypersensitivity reaction and is the manifestation of an allergic response caused by contact with allergens [22]. Contact allergens are essentially soluble haptens with physical and chemical properties that allow them to cross the stratum corneum of the skin [22]. The mechanisms by which these reactions occur are complex. Allergic contact dermatitis involves a cellular immune response mediated by IL-4– and IL-13–producing T_H2 cells and by interferon (IFN)-γ–producing T_H1 cells [23]. Tumor necrosis factor (TNF) α, which is produced mainly by monocytes and macrophages, is also involved in the pathogenesis of allergic contact dermatitis [22]. Thus, T_H1 and T_H2 immune responses coexist in allergic contact dermatitis [22,23].

IL-33 plays a key role in the pathogenesis of allergic contact dermatitis by promoting T_H2 immune responses [21]. However, the induction mechanism and function of IL-33 in allergic contact dermatitis are not fully understood. In the present study, we investigated induction of IL-33 in keratinocytes and evaluated the functions of IL-33 and its inducers in a murine model of allergic contact dermatitis.

Material and Methods

Cell Culture

KERTr cells, which are a human keratinocyte cell line, were purchased from the American Type Culture Collection (ATCC). The cells were cultured in keratinocyte serum-free medium supplemented with 0.05 mg/mL of bovine pituitary extract and 35 ng/mL of human recombinant epidermal growth factor (all from Invitrogen).

Quantitative Reverse Transcriptase Polymerase Chain Reaction

KERTr cells were seeded at 3×10^5 cells/well on 6-well plates (Thermo Fisher Scientific) and incubated overnight. The cells were then incubated with recombinant TNF-α, IFN-γ, IL-6, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-1α, IL-1β, IL-4, IL-13, IL-17A (PeproTech), and polyinosinic-polycytidylic acid potassium salt (poly I:C) (Sigma-Aldrich). RNA was isolated from the KERTr cells using TRIzol (Invitrogen) and reverse-transcribed with ReverTra Ace (Toyobo) and deoxynucleotide triphosphate (Promega) according to the manufacturer's protocol. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using THUNDERBIRD SYBR qPCR Mix (Toyobo) and an Applied Biosystems StepOnePlus Real-Time PCR System (Life Technologies Japan). Oligonucleotide primers were designed using Primer BLAST software (National Center for Bioinformatics).

Western Blotting

KERTr cells were incubated with 200 ng/mL recombinant TNF-α or 20 ng/mL recombinant IFN-γ for various periods. The cells were then lysed using radioimmunoprecipitation assay buffer. Western blotting was carried out with a goat polyclonal anti–IL-33 primary antibody (R&D Systems), followed by a horseradish peroxidase (HRP)–conjugated rabbit antigoat IgG secondary antibody (Santa Cruz Biotechnology). As an internal control, Western blotting was carried out with a mouse monoclonal antiglyceraldehyde 3-phosphate dehydrogenase (GAPDH) primary antibody (Santa Cruz Biotechnology), followed by an HRP-conjugated goat antimouse IgG secondary antibody (Santa Cruz Biotechnology). The membranes were developed with an ECL Plus Western Blotting Detection System (GE Healthcare).

Immunocytochemistry

KERTr cells were seeded at 1×10^4 cells/well on 8-well Lab-Tek Chamber Slides (Thermo Fisher Scientific) and incubated overnight. One set of cells was then incubated with and without 200 ng/mL recombinant TNF-α for 6 hours, while another set of cells was incubated with and without 20 ng/mL recombinant IFN-γ for 12 hours. All the cultured cells were fixed and stained with a mouse monoclonal anti–IL-33 primary antibody (Enzo Life Sciences), followed by an Alexa Fluor 488-conjugated goat antimouse IgG secondary antibody (Invitrogen). Nuclei were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (Dojindo). An IgG1 isotype control antibody (Enzo Life Sciences) was also evaluated.

Sensitization and Induction of Allergic Contact Dermatitis

Animal experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animal Science (1987). Six-week-old female C57BL/6 mice were purchased from Kyudo. The protocol for preparing the murine model of allergic contact dermatitis was reported previously [24]. Briefly, mice

were painted with 100 μL of 5% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxa) in ethanol on their shaved abdomen on day 0. oxa challenges were performed on days 5, 7, 9, 11, and 13 after sensitization. The right ears of the mice were painted with 0.1% oxa in ethanol, while the left ears were painted with ethanol alone. An antimouse IL-33 antibody (functional grade purified; Medical & Biological Laboratory), antimouse TNF-α antibody (functional grade purified; eBiosciences), or antimouse IFN-γ antibody (functional grade purified; eBiosciences) was subcutaneously injected into the ears on day 9. An IgG1 isotype control antibody (Abcam) was also evaluated. The mice were categorized as follows: group 1, not sensitized, oxa-challenged, no antibody injected; group 2, oxa-sensitized, oxa-challenged, no antibody injected; group 3, oxa-sensitized, oxa-challenged, isotype control IgG1 injected; group 4, oxa-sensitized, oxa-challenged, anti–IL-33 antibody injected; group 5, oxa-sensitized, oxa-challenged, anti–TNF-α antibody injected; group 6, oxa-sensitized, oxa-challenged, anti–IFN-γ antibody injected.

Measurement of Serum IgE

Mice were euthanized by intraperitoneal injection of Isozol (Nichiiko) on day 15 after sensitization. Blood samples were drawn by cardiocentesis and centrifuged, and the serum samples were collected. The serum IgE concentrations were measured with a Mouse IgE ELISA Kit (Shibayagi) according to the manufacturer's protocol.

Measurement of Ear Thickness

Ear thickness was measured using a Peacock dial thickness gauge G-1A (Ozaki). Ear swelling was calculated as follows: [thickness of ears after oxa applications – thickness of ears before oxa applications] – [thickness of ears after vehicle applications – thickness of ears before vehicle applications].

Histology and Immunohistochemistry

Ear tissue was fixed and immunostained. Expression of IL-33 protein was investigated by means of cross-sections stained with a mouse monoclonal anti–IL-33 antibody [Nessy-1] (Biotin) (Abcam) and evaluated using a Histofine SAB-PO (M) Kit and a Histofine DAB Substrate Kit (Nichirei, Tokyo, Japan) according to the manufacturer's protocols. Nuclei were stained with hematoxylin.

Statistical Analysis

Statistical analyses were performed using Prism 5.0 (GraphPad Software). Data are presented as mean (SD). The significance of differences between values was assessed using an unpaired *t* test. *P* values of <.05 were considered statistically significant.

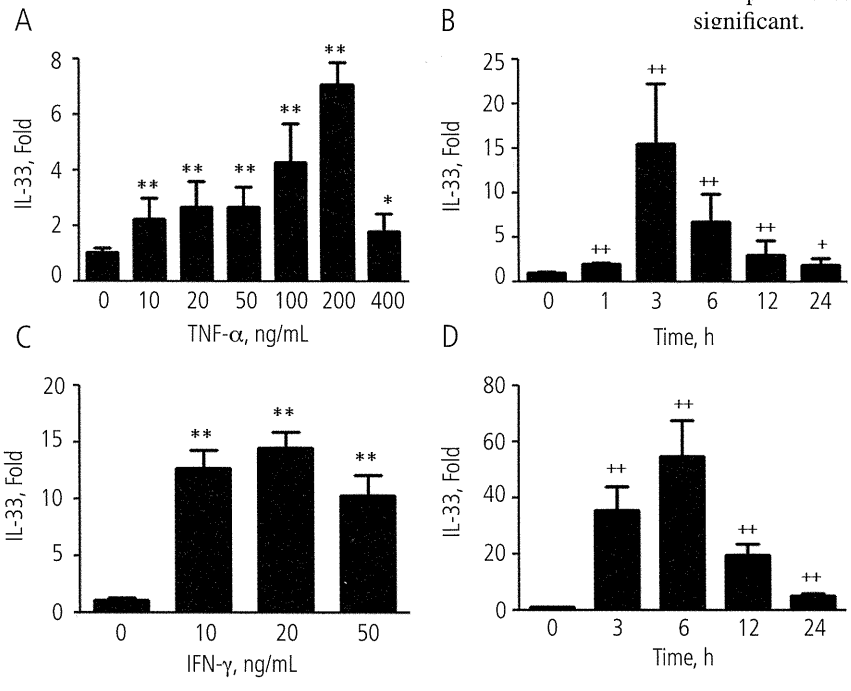

Figure 1. TNF-α and IFN–γ induce expression of IL-33 mRNA. A, KERTr cells were incubated with TNF-α at the indicated concentrations for 3 hours and then evaluated by qRT-PCR (n=4-5 wells). B, KERTr cells were incubated with 200 ng/mL TNF-α for the indicated times and then evaluated by qRT-PCR (n=3-8 wells). C, KERTr cells were incubated with IFN-γ at the indicated concentrations for 6 hours and then evaluated by qRT-PCR (n=4-5 wells). D, KERTr cells were incubated with 20 ng/mL IFN-γ for the indicated times and then evaluated by qRT-PCR (n=4-5 wells). Data are shown as mean (SD). **P*<.05 and ***P*<.01 vs 0 ng/mL; +*P*<.05 and ++*P*<.01 vs 0 hours. TNF indicates tumor necrosis factor; IFN, interferon; IL, interleukin; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction.

Results

TNF-α and IFN-γ Induce Expression of IL-33 mRNA in KERTr Cells

In order to investigate which cytokines could induce expression of IL-33 mRNA, we stimulated KERTr cells using various cytokines associated with allergic contact dermatitis. qRT-PCR revealed that TNF-α and IFN-γ were able to induce expression of IL-33 mRNA (Figure 1). The expression of IL-33 mRNA induced by TNF-α peaked at 200 ng/mL (Figure 1A). In the kinetics analysis, expression of IL-33 mRNA induced by TNF-α peaked at 3 hours after stimulation (Figure 1B); expression of IL-33 mRNA induced by IFN-γ peaked at 20 ng/mL (Figure 1C). In the kinetics analysis, expression of IL-33 mRNA induced by IFN-γ peaked at 6 hours after stimulation (Figure 1D). These findings showed that TNF-α and IFN-γ induced IL-33 mRNA expression in KERTr cells. Other cytokines, including IL-6, GM-CSF, IL-1α, IL-1β, IL-4, IL-13, and IL-17, and poly (I:C), a mimetic of double-stranded RNA, did not induce IL-33 mRNA expression (data not shown).

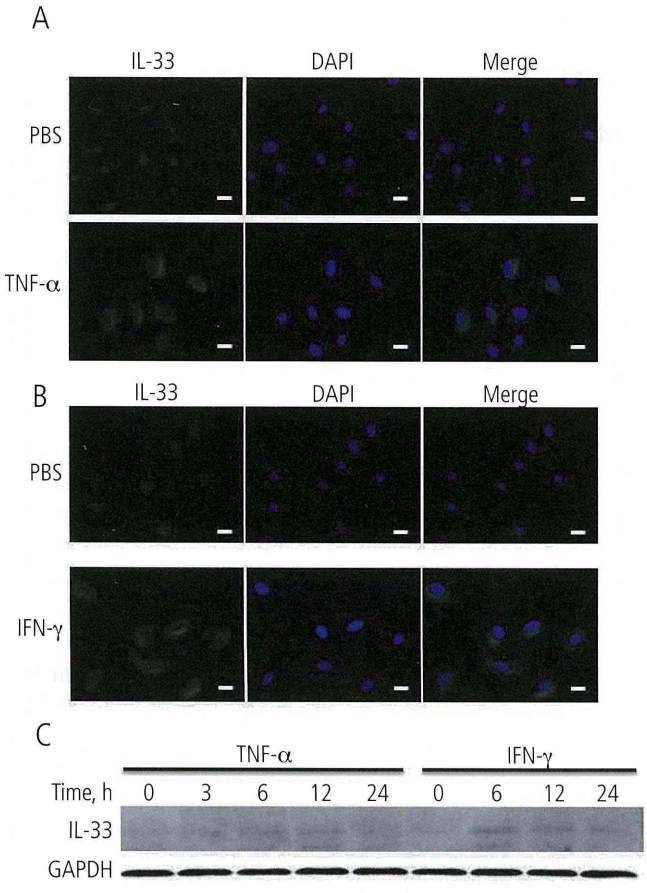

Figure 2. TNF-α and IFN-γ induce expression of IL-33 protein. A, KERTr cells were incubated with 200 ng/mL TNF-α or phosphate-buffered saline (PBS) for 6 hours. B, KERTr cells were incubated with 20 ng/mL IFN-γ or PBS for 12 hours. Scale bars, 10 μm. C, KERTr cells were incubated with 200 ng/mL TNF-α or 20 ng/mL IFN-γ for the indicated times. TNF indicates tumor necrosis factor; IFN, interferon; IL, interleukin; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

TNF-α and IFN-γ Induce Expression of IL-33 Protein in KERTr Cells

We performed immunocytochemistry and Western blotting analysis to investigate whether TNF-α and IFN-γ could induce IL-33 protein expression. In KERTr cells incubated without these cytokines, IL-33 protein was located in the nucleus (Figure 2A and B). In KERTr cells incubated with TNF-α, expression of IL-33 protein was increased, and its location shifted from the nucleus to the cytoplasm (Figure 2A). Similarly, in KERTr cells incubated with IFN-γ, expression of IL-33 protein was increased, and the protein showed the same translocation pattern as that of stimulation with TNF-α (Figure 2B). These findings suggested that IL-33 was constitutively expressed in the nucleus and shifted to the cytoplasm when its expression was upregulated.

We further examined expression of IL-33 protein using Western blotting. In KERTr cells incubated with TNF-α, upregulation of IL-33 protein began 3 hours after stimulation, peaked at 6 hours, and then gradually disappeared (Figure 2C). In KERTr cells incubated with IFN-γ, upregulation of the IL-33 protein began at 6 hours after stimulation and then gradually disappeared (Figure 2C). We investigated whether IL-33 was secreted into the culture supernatants. However, we did not detect any IL-33 in the supernatants by enzyme-linked immunosorbent assay (ELISA) (data not shown).

Preparation of the Murine Model of Allergic Contact Dermatitis

We used a murine model to evaluate the functions of IL-33, TNF-α, and IFN-γ in allergic contact dermatitis. We confirmed that sensitization was successful by measuring serum IgE concentrations using ELISA. The serum IgE levels obtained from oxa-sensitized mice (groups 2 to 6) were significantly higher than those obtained from nonsensitized mice (group 1) (Figure 3), suggesting that the model was successful. Ear swelling in group 2 mice was significantly greater than in group 1 mice (Figure 4). Immunohistochemistry revealed that expression of IL-33 protein was upregulated in group 2 mice compared with group 1 mice (Figure 5).

Blockade of IL-33 Attenuates Ear Swelling

In order to evaluate the function of IL-33, we administered anti–IL-33 antibody to the mice in our model. Ear swelling was increased in group 3 mice (isotype control IgG1–injected), as well as in group 2 mice. However, in the anti–IL-33 antibody–injected mice (group 4), ear swelling was not increased and was significantly milder than in group 3 mice (Figure 4). Immunohistochemistry showed that expression of IL-33 protein in keratinocytes was upregulated

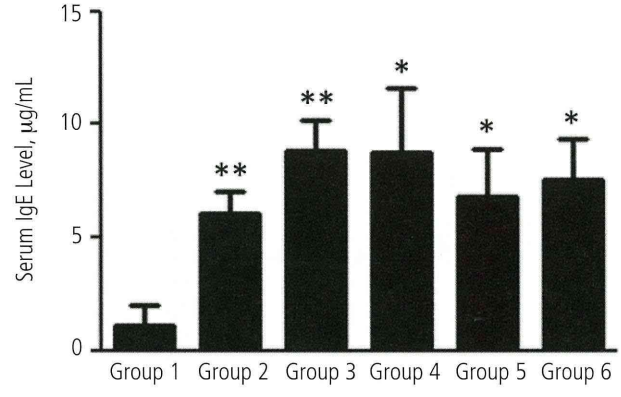

Figure 3. Serum IgE levels are increased. Serum samples were obtained from sensitized mice or nonsensitized mice as a negative control on day 15 after sensitization. The serum IgE levels were measured by enzyme-linked immunosorbent assay (n=4). The categorization of groups 1 to 6 is described in Material and Methods. Data are expressed as mean (SD). *$P<.05$ and **$P<.01$ versus group 1 (nonsensitized mice). Ig indicates immunoglobulin.

in group 3 mice compared with mice from group 1, as well as group 2 (Figure 5). Furthermore, expression of IL-33 protein in keratinocytes was not upregulated in group 4 mice (Figure 5). These findings demonstrated that blockade of IL-33 attenuated ear swelling. Incidentally, the serum IgE level in group 4 mice showed no significant differences compared with group 3 mice (Figure 3), suggesting that blockade of IL-33 could not attenuate oxa sensitization.

Blockade of TNF-α and IFN-γ Attenuates Ear Swelling

We administered an anti–TNF-α antibody or anti–IFN-γ antibody to the mice in our model in order to evaluate the functions of TNF-α and IFN-γ. In the anti–TNF-α antibody–injected mice (group 5), ear swelling was not increased and was significantly milder than in group 3 mice (Figure 4). In the anti–IFN-γ antibody–injected mice (group 6), ear swelling was not increased and was significantly milder than in group 3 mice (Figure 4). Immunohistochemistry showed that expression of IL-33 protein in keratinocytes in group 5 and group 6 mice was downregulated to the level of group 4 mice (Figure 5). These findings demonstrated that blockade of TNF-α and IFN-γ attenuated ear swelling in our model. This attenuation could be mediated by downregulation of IL-33 expression. Incidentally, the serum IgE levels obtained from group 5 and group 6 mice showed no significant differences compared with group 3 mice (Figure 3), suggesting that blockade of TNF-α and IFN-γ was unable to attenuate oxa sensitization.

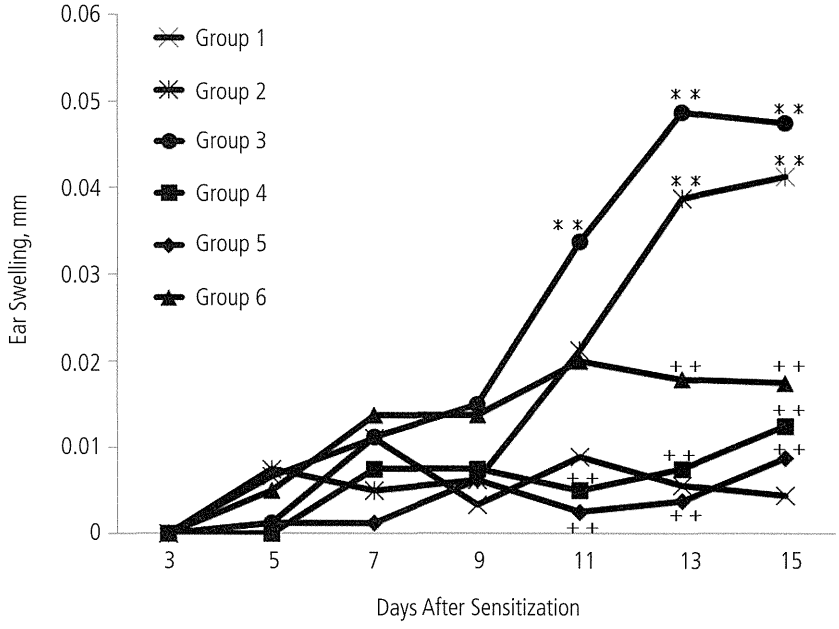

Figure 4. Ear swelling is increased and suppressed by blockade of IL-33, TNF-α, and IFN-γ. The categorization of groups 1-6 is described in Material and Methods. Ear thickness was measured on days 5, 7, 9, 11, 13, and 15 after sensitization (n=8-9). Data are expressed as mean (SD). $^{*}P<.05$ and $^{**}P<.01$ vs group 1 (nonsensitized mice); $^{+}P<.05$ and $^{++}P<.01$ vs group 3 (oxa-sensitized, isotype control IgG1–injected mice). TNF indicates tumor necrosis factor; IFN, interferon; IL, interleukin; oxa, 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one.

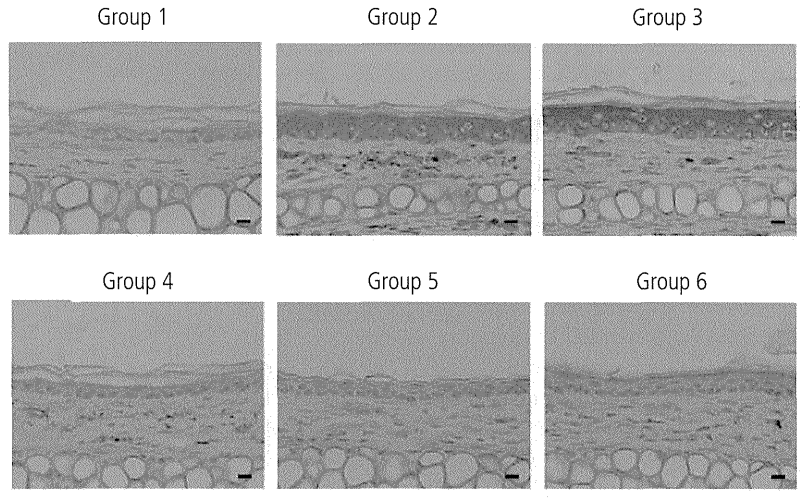

Figure 5. IL-33 expression is increased and downregulated by blockade of IL-33, TNF-α, and IFN-γ. The categorization of groups 1-6 is described in Material and Methods. The expression levels of IL-33 protein in ear tissue on day 15 after sensitization were evaluated by immunohistochemistry with an anti-IL-33 antibody and DAB, followed by nuclear staining with hematoxylin (n=8-9). Scale bars, 10 μm. IL indicates interleukin; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; oxa, 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one.

Discussion

The contribution of IL-33 to allergic diseases has been widely reported [16-21]. Although the effects of IL-33 on the immune cells that appear in allergic diseases are well known [5-11], the induction mechanism of IL-33 is not fully understood. Very recently, TNF-α and IFN-γ were shown to induce IL-33 expression in normal human epidermal keratinocytes in vitro [25]. Our data support this finding. Consequently, IL-33 plays a role in inflammatory skin diseases involving high levels of TNF-α and IFN-γ. In the present study, IL-33 was constitutively expressed in the nucleus of KERTr cells, where it was upregulated by TNF-α and IFN-γ before entering the cytoplasm. IL-33 is also a secreted protein, and this shift may be part of the process of secretion. However, IL-33 protein was not detected in the cell culture supernatants. Failure to detect secreted endogenous

IL-33 in different types of healthy cells has been reported in various studies [25-27]; secreted IL-33 was only detected once the cells had begun to undergo necrosis.

Our findings provide a mechanism that could explain upregulation of IL-33 in keratinocytes in allergic contact dermatitis. Immune responses can be roughly classified as type 1 and type 2. IFN-γ, which is mainly produced by T_H1 cells, and TNF-α, which is mainly produced by monocytes and macrophages, play an active part in type 1 immune responses. On the other hand, IL-4, IL-5, and IL-13, which are produced by T_H2 cells, play an active role in type 2 immune responses. IFN-γ has been thought to suppress type 2 immune responses through inhibition of T_H2 cell proliferation [28]. However, in chronic inflammation, expression of T_H2 cytokines occurs even in type 1 immune responses [29]. We previously reported that IFN-γ had opposite effects on IL-4–induced production of CCL26, a potent chemoattractant for eosinophils, which are effector cells in type 2 immune responses in normal human epidermal keratinocytes [30]. Our data support the coexistence of the properties of type 1 and type 2 immune responses. In the present study, we found that IFN-γ and TNF-α, which play a key role in type 1 immune responses, induced expression of IL-33, which could promote type 2 immune responses in keratinocytes. Our findings provide evidence for both upregulation of IL-33 in keratinocytes and coexistence of type 1 and type 2 immune responses in allergic contact dermatitis.

IL-33 and its receptor ST2 appear to contribute to allergic contact dermatitis. For example, ST2 was upregulated in skin lesions in a contact dermatitis rat model using a representative hapten, 2,4-dinitrofluorobenzene [31]. In an oxazolone-induced murine model of allergic contact dermatitis, inflammation was attenuated in ST2-deficient mice compared with wild-type mice, because activation of B-1 cells via IL-33 and ST2 interactions could not occur [21]. We generated our model under conditions by which a difference in epidermal thickness was observed between the control and experimental groups and edema and hypertrophy of the dermis were not observed. We found that blockade of IL-33 attenuated ear swelling in a murine model of allergic contact dermatitis. Thus, IL-33 is functionally involved in the pathogenesis of allergic contact dermatitis, especially in keratinocytes, the main structural cells in the epidermis. However, blockade of IL-33 did not attenuate sensitization with oxa. Therefore, we suggest that IL-33 is not involved in the sensitization phase, at least in our experiments. IL-33 is expressed in the nucleus of keratinocytes at low levels in the resting phase. Once inflammation occurs, IL-33 can be initially upregulated in keratinocytes and secreted as alarmin, before exerting its effects on immune cells that mediate allergic responses. Thus, inflammation may be facilitated and prolonged in allergic contact dermatitis. Expression levels of TNF-α and IFN-γ are upregulated in allergic contact dermatitis [32]. TNF-α and IFN-γ can promote expression of IL-33 in keratinocytes, thus sustaining chronic inflammation. We further showed that blockade of TNF-α and IFN-γ attenuated allergic contact dermatitis. In addition, expression of IL-33 in keratinocytes was decreased by blockade of TNF-α and IFN-γ. We suggest that blockade of TNF-α and IFN-γ suppresses upregulation of IL-33 expression in keratinocytes, resulting in the attenuation of allergic contact dermatitis. Therefore, both blockade of IL-33 and blockade of TNF-α and IFN-γ could be effective treatments for allergic contact dermatitis.

The main treatments for allergic contact dermatitis are topical and systemic (oral and injected) corticosteroids and antihistamines. These treatments successfully inhibit allergic inflammation, including the infiltration of inflammatory cells into the skin and secretion of various inflammatory cytokines [33]. However, corticosteroids and antihistamines are used to treat symptoms, rather than provide a cure, and are thus unable to completely prevent the inflammation from becoming chronic. The results of the present study suggest that blockade of IL-33, TNF-α, and IFN-γ could become a potent strategy for allergic contact dermatitis even in the chronic phase, in which type 1 and type 2 immune responses coexist.

In conclusion, TNF-α and IFN-γ were able to induce expression of IL-33 in KERTr cells. Blockade of IL-33 and blockade of TNF-α and IFN-γ attenuated allergic contact dermatitis in a murine model. These findings could pave the way for successful treatment of allergic contact dermatitis.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005;23:479-90.
2. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, Bouche G, Girard JP. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. Proc Natl Acad Sci USA. 2007;104:282-7.
3. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. J Immunol. 2011;186:4375-87.
4. Lüthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, Brumatti G, Taylor RC, Kersse K, Vandenabeele P, Lavelle EC. Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. Immunity. 2009;31:84-98.

5. Löhning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC, Levinson D, Radbruch A, Kamradt T. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci USA. 1998;95:6930-5.
6. Yang Q, Li G, Zhu Y, Liu L, Chen E, Turnquist H, Zhang X, Finn OJ, Chen X, Lu B. IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. Eur J Immunol. 2011;41:3351-60.
7. Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. J Immunol. 1998;161:4866-74.
8. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY. IL-33 exacerbates eosinophil-mediated airway inflammation. J Immunol. 2010;185:3472-80.
9. Schneider E, Petit-Bertron AF, Bricard R, Levasseur M, Ramadan A, Girard JP, Herbelin A, Dy M. IL-33 activates unprimed murine basophils directly in vitro and induces their in vivo expansion indirectly by promoting hematopoietic growth factor production. J Immunol. 2009;183:3591-7.
10. Bourgeois E, Van LP, Samson M, Diem S, Barra A, Roga S, Gombert JM, Schneider E, Dy M, Gourdy P, Girard JP, Herbelin A. The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-γ production. Eur J Immunol. 2009;39:1046-55.
11. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, Kita H. IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol. 2009;123:1047-54.
12. Oboki K, Ohno T, Kajiwara N, Saito H, Nakae S. IL-33 and IL-33 receptors in host defense and diseases. Allergol Int. 2010;59:143-60.
13. Besnard AG, Togbe D, Guillou N, Erard F, Quesniaux V, Ryffel B. IL-33-activated dendritic cells are critical for allergic airway inflammation. Eur J Immunol. 2011;41:1675-86.
14. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. Innate production of TH2 cytokines by adipose tissue-associated c-Kit+Sca-1+ lymphoid cells. Nature. 2010;463:540-4.
15. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TKA, Bucks C, Kane CM, Fallon PG, Pannell R, Jolin HE, McKenzie ANJ. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature. 2010;464:1367-70.
16. Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, Martin JG, Hamid Q. Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol. 2010;125:752-4.
17. Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, Imoto Y, Fujieda S, Yasuda M, Hisa Y, Akira S, Nakanishi K, Yoshimoto T. A critical role of IL-33 in experimental allergic rhinitis. J Allergy Clin Immunol. 2012;42:218-28.
18. Matsuba-Kitamura S, Yoshimoto T, Yasuda K, Futatsugi-Umikura S, Taki Y, Muto T, Ikeda T, Mimura O, Nakanishi K. Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. Int Immunol. 2010;22:479-89.
19. Hsu C-L, Neilsen CV, Bryce PJ. IL-33 is produced by mast cells and regulates IgE-dependent inflammation. PLoS One. 2010;5:e11944.
20. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, Karisola P, Reunala T, Wolff H, Lauerma A, Alenius H. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. J Invest Dermatol. 2012;132:1392-400.
21. Komai-Koma M, Gilchrist DS, McKenzie ANJ, Goodyear CS, Xu D, Liew FY. IL-33 activates B1 cells and exacerbates contact sensitivity. J Immunol. 2011;186:2584-91.
22. Wagner AH, Wittjen I, Stojanovic T, Middel P, Meingassner JG, Hecker M. Signal transducer and activator of transcription 1 decoy oligodeoxynucleotide suppression of contact hypersensitivity. J Allergy Clin Immunol. 2008;121:158-65.
23. Saint-Mezard P, Berard F, Dubois B, Kaiserlian D, Nicolas JF. The role of CD4+ and CD8+ T cells in contact hypersensitivity and allergic contact dermatitis. Eur J Dermatol. 2004;14:131-8.
24. Kobayashi M, Nunomura S, Gon Y, Endo D, Kishiro S, Fukunaga M, Kitahara Y, Terui T, Ra C. Abrogation of high-affinity IgE receptor-mediated mast cell activation at the effector phase prevents contact hypersensitivity to oxazolone. J Invest Dermatol. 2010;130:725-31.
25. Meephansan J, Tsuda H, Komine M, Tominaga S, Ohtsuki M. Regulation of IL-33 expression by IFN-γ and tumor necrosis factor-α in normal human epidermal keratinocytes. J Invest Dermatol. 2012;132:2593-600.
26. Nile CJ, Barksby E, Jitprasertwong P, Preshaw PM, Taylor JJ. Expression and regulation of interleukin-33 in human monocytes. Immunology. 2010;130:172-80.
27. Cayrol C, Girard JP. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. Proc Natl Acad Sci USA. 2009;106:9021-6.
28. Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-γ in immune regulation. I. IFN-γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocytes. J Immunol. 1988;12:4245-52.
29. Hayashi N, Yoshimoto T, Izuhara K, Matsui K, Tanaka T, Nakanishi K. T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN-γ and IL-13 production. Proc Natl Acad Sci USA. 2007;104:14765-70.
30. Nishi N, Yamamoto S, Ou W, Muro E, Inada Y, Hamasaki Y. Enhanced CCL26 production by IL-4 through IFN-gamma-induced upregulation of type 1 IL-4 receptor in keratinocytes. Biochem Biophys Res Commun. 2008;376:234-40.
31. Hartmann B, Staedtler F, Hartmann N, Meingassner J, Firat H. Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. Inflamm Res. 2006;55:322-34.
32. Wakabayashi T, Hu DL, Tagawa Y, Sekikawa K, Iwakura Y, Hanada K, Nakane A. IFN-γ and TNF-α are involved in urushiol-induced contact hypersensitivity in mice. Immunol Cell Biol. 2005;83:18-24.
33. Lee PW, Elsaie ML, Jacob SE. Allergic contact dermatitis in children: common allergens and treatment: a review. Curr Opin Pediatr. 2009;21:491-8.

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Kazuto Taniguchi

Department of Pediatrics
Faculty of Medicine
Saga University
5-1-1 Nabeshima
Saga 849-8501, Japan

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Research Article Open Access

Blockade of Interleukin-33 Attenuates Allergic Contact Dermatitis in Model Mice: Possible Mechanism via Eosinophil Infiltration

Kazuto Taniguchi[1,2*], Shuichi Yamamoto[1], Emiko Hitomi[1], Yukiko Inada[1], Takashi Sugioka[2], and Yuhei Hamasaki[1]

[1]*Department of Pediatrics, Faculty of Medicine, Saga University, Japan*
[2]*Community Medical Support Institute, Faculty of Medicine, Saga University, Japan*

Abstract

Background: Interleukin (IL)-33, a novel member of the IL-1 family, is mainly produced by epithelial cells and endothelial cells. Effects of IL-33 on allergic diseases have been reported. Allergic contact dermatitis is a clinical form of contact hypersensitivity that involves a delayed-type hypersensitivity reaction. We previously reported that IL-33 is induced by tumor necrosis factor-alpha and interferon-gamma in keratinocytes and plays a critical role in allergic contact dermatitis. However, the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis is not fully understood. We investigated the role of IL-33 in allergic contact dermatitis using model mice.

Methods: Allergic contact dermatitis model mice were generated. Epidermal thickness and eosinophil infiltration in the dermis were evaluated by histology. The function of IL-33 was investigated by *in vivo* administration of an anti-IL-33 antibody.

Results: Epidermal thickness was increased in the ear lesions of allergic contact dermatitis model mice. We showed that eosinophil infiltration in the dermis was increased in the ear lesions. We further found that blockade of IL-33 attenuated not only the epidermal thickness but also the eosinophil infiltration in the dermis in the ear lesions.

Conclusions: IL-33 may promote inflammation via eosinophil infiltration in allergic contact dermatitis. Blockade of IL-33 may represent a novel and potent therapeutic strategy for allergic contact dermatitis.

Keywords: Interleukin-33; Allergic contact dermatitis; Epidermal thickness; Eosinophil

Abbreviations: Oxa: 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one; IL: Interleukin; TNF-α: Tumor Necrosis Factor-alpha; IFN-γ: Interferon-gamma

Introduction

Interleukin (IL)-33 was identified as a novel member of the IL-1 family, IL-1F11 [1]. IL-33 was reported as DVS 27 in 1999 and also reported as nuclear factor from high endothelial venules (NF-HEV) in 2003 [2,3]. Epithelial cells and endothelial cells are the main cellular sources of IL-33 [1,4,5]. IL-33 is released by cells undergoing necrotic cell death and functions as a Damage-Associated Molecular Pattern (DAMP) and alarming [5]. The receptor for IL-33, ST2, is expressed on various immune cells including T-helper type 2 (Th2) cells $CD8^+$ T cells mast cells eosinophils basophils NK cells, NKT cells and dendritic cells [6-12]. Natural helper cells and nuocytes produce abundant Th2 cytokines when stimulated by IL-33 [13,14].

IL-33 is closely related to type 2 immune responses. It has been reported that IL-33 is involved in various allergic diseases, including bronchial asthma allergic rhinitis allergic conjunctivitis atopic dermatitis and allergic contact dermatitis [15-19]. Allergic contact dermatitis is a clinical form of contact hypersensitivity that involves a delayed-type hypersensitivity reaction, and its mechanism is complex.

In the previous study, we found that IL-33 plays a critical role in allergic contact dermatitis model mice [20]. However, we could not fully reveal the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis. In the present study, we further investigated the role of IL-33 in allergic contact dermatitis using model mice. Here, we show that not only epidermal thickness but also eosinophil infiltration in the dermis are increased in the ear lesions of allergic contact dermatitis model mice. We further demonstrate that blockade of IL-33 attenuates the epidermal thickness and eosinophil infiltration in the dermis in the ear lesions. These findings suggest that IL-33 functions like a chemoattractant for eosinophils in allergic contact dermatitis model mice, and that IL-33 is a novel therapeutic target for allergic contact dermatitis.

Materials and Methods

Generation of allergic contact dermatitis model mice

All animal experiments were undertaken in accordance with the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animal Science (1987). Six-week-old female C57BL/6 mice were purchased from Kyudo (Saga, Japan). The protocol for preparing allergic contact dermatitis model mice was previously reported [20,21]. Briefly, mice were sensitized with 100 μl of 5% 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxa) in ethanol on their shaved abdomen on day 0. Oxa challenges were performed on days 5, 7, 9, 11, and 13 after sensitization. The right ears of the mice were applied with 0.1% oxa, while the left ears were applied with ethanol alone. An anti-mouse IL-33 antibody (Functional Grade Purified; Medical & Biological Laboratory, Research Triangle Park, NC) was

***Corresponding author:** Kazuto Taniguchi, Department of Pediatrics, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan, Tel: +81-952-34-2314; Fax: +81-952-34-2091; Email: taniguck@cc.saga-u.ac.jp

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subcutaneously injected into the ears on day 9. An IgG1 isotype control antibody (Abcam, Cambridge, MA) was also evaluated.

Histological analysis

For histological examination, specimens were obtained from the ears at 48 hours after the last oxa challenge. The specimens were fixed in formalin and embedded in paraffin. Cross-sections were prepared and subjected to H&E staining. To evaluate eosinophil infiltration, the total numbers of eosinophils in the dermis in five high power fields (magnification, ×400) were counted.

Statistical analysis

Statistical analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, CA). Data are presented as means ± SD. The significance of differences between values was assessed using an unpaired Student's *t*-test. Values of $P<0.05$ were considered statistically significant.

Results

Generation of allergic contact dermatitis model mice

To investigate the function of IL-33 in allergic contact dermatitis, we initially generated allergic contact dermatitis model mice using oxa (Figure 1a). The epidermal thickness of the ear tissue was increased in allergic contact dermatitis model mice compared with control mice that were not sensitized with oxa (Figure 1b). This finding indicated that the preparation of allergic contact dermatitis model mice was successful. There was no difference of the ears between allergic contact dermatitis model mice and control mice in appearance (data not shown).

Blockade of IL-33 attenuates the epidermal thickness in allergic contact dermatitis model mice

To evaluate the function of IL-33, we administered the anti-IL-33 antibody into the lesions of allergic contact dermatitis model mice (Figure 2a). Administration of the anti-IL-33 antibody suppressed the epidermal thickness in allergic contact dermatitis model mice compared with administration of the IgG1 isotype control antibody (Figure 2b). This finding suggested that blockade of IL-33 attenuated allergic contact dermatitis.

Eosinophil infiltration in the dermis is suppressed by blockade of IL-33 in allergic contact dermatitis model mice

We confirmed that IL-33 was functionally involved in the pathogenesis of allergic contact dermatitis. IL-33 activates the eosinophil, a potent mediator of allergic inflammation, and also increases eosinophil survival in humans [22]. We investigated the relationship between IL-33 and eosinophils in our allergic contact dermatitis model mice. We found that the eosinophil infiltration in the dermis was significantly increased in allergic contact dermatitis model mice compared with control mice that were not sensitized with oxa (Figure 3). On the other hand, administration of the anti-IL-33 antibody significantly suppressed the eosinophil infiltration in the dermis compared with administration of the IgG1 isotype control antibody (Figure 3). Very few lymphocytes, neutrophils, basophils, and macrophages appeared in the skin lesions in our allergic contact dermatitis model mice. These data suggest that IL-33 functions like a chemoattractant for eosinophils, which facilitate allergic inflammation.

Discussion

IL-33 is involved in various allergic diseases [15-19]. We previously

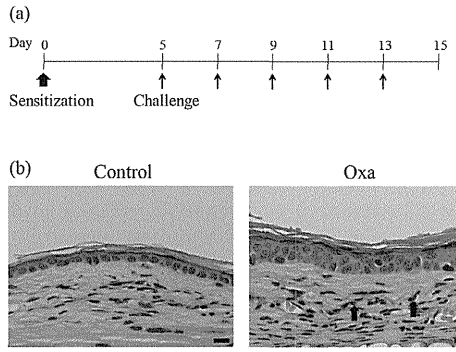

Figure 1: Epidermal thickness is increased in allergic contact dermatitis model mice. (a) Mice were sensitized with 100 µl of 5% oxa in ethanol on their shaved abdomen on day 0. Oxa challenges were performed on days 5, 7, 9, 11, and 13 after sensitization. (b) Ear tissues obtained from the mice on day 15 were subjected to H&E staining. (n=8–9/group). Left, control mouse; right, oxa-sensitized mouse; arrows, eosinophils. Scale bars, 10 µm.

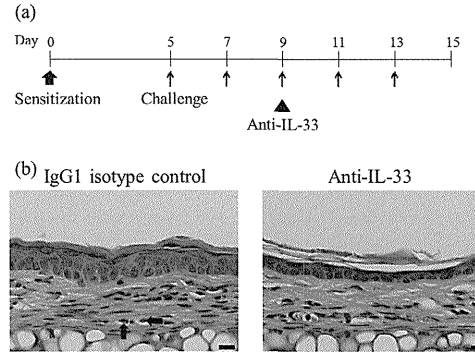

Figure 2: Blockade of IL-33 attenuates the epidermal thickness in allergic contact dermatitis model mice. (a) An anti-mouse IL-33 antibody or IgG1 isotype control antibody, 10 µg each, was subcutaneously injected into the ears of allergic contact dermatitis model mice on day 9 after sensitization. (b) Ear tissues obtained from the mice on day 15 were subjected to H&E staining (n=8–9/group). Left, IgG1 isotype control antibody-injected mouse; right, anti-IL-33 antibody-injected mouse; arrows, eosinophils. Scale bars, 10 µm.

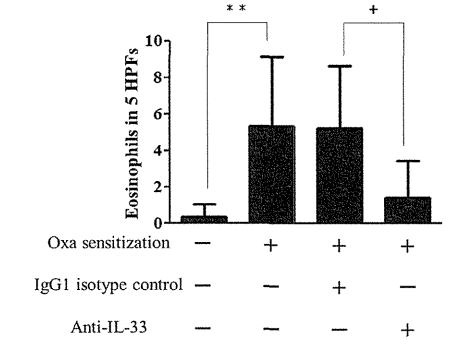

Figure 3: Eosinophil infiltration is promoted in allergic contact dermatitis model mice and suppressed by blockade of IL-33. Ear tissues obtained from the mice on day 15 after sensitization were subjected to H&E staining. The total numbers of eosinophils in the dermis in five high power fields (HPFs; magnification, ×400) were counted (n=8–9/group). The data are means ± SD. $^{**}P<0.01$, versus control mice. $^{*}P<0.05$, versus IgG1 isotype control antibody-injected mice.

reported that IL-33 contributes to allergic contact dermatitis, as do tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [20]. However, the mechanism underlying how IL-33 is involved in the pathogenesis of allergic contact dermatitis is not fully understood. To investigate the role of IL-33, we generated allergic contact dermatitis model mice under the condition that relatively mild inflammation was sustained to omit the involvement of excessive immune cells. Although a difference of epidermal thickness between the control and experimental mice appeared, edema and hypertrophy of the dermis, the characteristics of severe dermatitis, did not appear in our model mice. In addition, the serum IgE concentrations were increased in our model mice. This mouse model is suitable for analyses of the early stage of skin allergic inflammation. We previously reported that IL-33 protein was upregulated in keratinocytes in the lesions of allergic contact dermatitis model mice [20].

IL-33 and its receptor ST2 appear to contribute to allergic contact dermatitis. For example, ST2 was upregulated in the lesions of contact dermatitis model rats by a representative hapten, 2,4-dinitrofluorobenzene [23]. In oxazolone-induced allergic contact dermatitis model mice, inflammation was attenuated in ST2-deficient mice compared with wild-type mice, because activation of B-1 cells via IL-33 and ST2 interactions could not occur [19]. Allergic contact dermatitis, which is a clinical form of contact hypersensitivity, has properties both of type 1 and type 2 immune responses, especially in chronic inflammation. IL-33 closely relates to type 2 immune responses. On the other hand, TNF-α and IFN-γ closely relate to type 1 immune responses. We have reported that TNF- α and IFN-γ induce IL-33 production in KERTr cells, normal human keratinocyte cell line [20]. These are supporting data for the coexistence of the properties of type 1 and type 2 immune responses.

Effects of IL-33 on various immune cells have been reported [6-14]. We paid the attention to eosinophils, which express ST2 and are involved in the pathogenesis of various allergic diseases, including contact dermatitis. IL-33 induces eosinophil superoxide anion production and degranulation, and also increases eosinophil survival in humans [22]. In the present study, we showed a relationship between IL-33 and eosinophils in allergic contact dermatitis model mice. We further showed that blockade of IL-33 attenuated epidermal thickness in allergic contact dermatitis model mice, together with decreased eosinophil infiltration in the dermis. These data suggest that IL-33 is functionally involved in the pathogenesis of allergic contact dermatitis model mice as if it were a chemoattractant for eosinophils. IL-33 does not influence the eosinophil migration directly. It has reported that IL-33 induced the production of eotaxin, a potent chemoattractant for eosinophils, in fibroblasts in allergic bronchial asthma model mice [24]. Accordingly, IL-33 influences the eosinophil migration indirectly. Lymphocytes, neutrophils, basophils, and macrophages seldom appeared in the skin lesions at least in our allergic contact dermatitis model mice. IL-33 may be initially upregulated in keratinocytes, epithelial cells in the epidermis, and secreted as an alarmin when keratinocytes are damaged and exposed to TNF- α and IFN-γ [5,20]. We suggest that IL-33 subsequently attracts eosinophils that mediate allergic responses in the skin lesions (Figure 4). Thus, inflammation may be facilitated and sustained in allergic contact dermatitis. Blockade of IL-33 may attenuate allergic contact dermatitis by suppressing eosinophil infiltration into the skin lesions.

A corticosteroid and antihistamine drugs with external application and systemic administration are currently the main treatments for contact dermatitis. These treatments successfully inhibit the allergic inflammation [25]. However, the corticosteroid therapy and antihistamine therapy are symptomatic therapies, rather than curative therapies [25]. The data obtained in the present study suggest that blockade of IL-33 could become a novel strategy for allergic contact dermatitis as a molecularly targeted therapy.

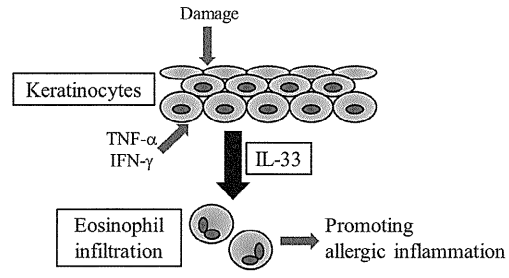

Figure 4: The possible function of IL-33 in allergic contact dermatitis model mice. Keratinocytes produce IL-33 when damaged and exposed to TNF-α and IFN-γ. IL-33 functions like a chemoattractant for eosinophils, which facilitate allergic inflammation, in allergic contact dermatitis model mice.

In Conclusion, blockade of IL-33 attenuated allergic contact dermatitis in our model mice. This process may be mediated by suppression of eosinophil infiltration in the dermis. These findings could pave the way for successful treatment of allergic contact dermatitis.

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References

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, et al. (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 23: 479-490.
2. Onda H, Kasuya H, Takakura K, Hori T, Imaizumi T, et al. (1999) Identification of genes differentially expressed in canine vasospastic cerebral arteries after subarachnoid hemorrhage. J Cereb Blood Flow Metab 19: 1279-1288.
3. Baekkevold ES, Roussigné M, Yamanaka T, Johansen FE, Jahnsen FL, et al. (2003) Molecular characterization of NF-HEV, a nuclear factor preferentially expressed in human high endothelial venules. Am J Pathol 163: 69-79.
4. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, et al. (2007) IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor *in vivo*. Proc Natl Acad Sci U S A 104: 282-287.
5. Lüthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, et al. (2009) Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. Immunity 31: 84-98.
6. Löhning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, et al. (1998) T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. Proc Natl Acad Sci U S A 95: 6930-6935.
7. Yang Q, Li G, Zhu Y, Liu L, Chen E, et al. (2011) IL-33 synergizes with TCR and IL-12 signaling to promote the effector function of CD8+ T cells. Eur J Immunol 41: 3351-3360.
8. Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R (1998) The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. J Immunol 161: 4866-4874.
9. Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY (2010) IL-33 exacerbates eosinophil-mediated airway inflammation. J Immunol 185: 3472-3480.

10. Schneider E, Petit-Bertron AF, Bricard R, Levasseur M, Ramadan A, et al. (2009) IL-33 activates unprimed murine basophils directly *in vitro* and induces their *in vivo* expansion indirectly by promoting hematopoietic growth factor production. J Immunol 183: 3591-3597.

11. Bourgeois E, Van LP, Samson M, Diem S, Barra A, et al. (2009) The pro-Th2 cytokine IL-33 directly interacts with invariant NKT and NK cells to induce IFN-gamma production. Eur J Immunol 39: 1046-1055.

12. Rank MA, Kobayashi T, Kozaki H, Bartemes KR, Squillace DL, et al. (2009) IL-33-activated dendritic cells induce an atypical TH2-type response. J Allergy Clin Immunol 123: 1047-1054.

13. Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, et al. (2010) Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature 463: 540-544.

14. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, et al. (2010) Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 464: 1367-1370.

15. Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, et al. (2010) Increased IL-33 expression by epithelial cells in bronchial asthma. J Allergy Clin Immunol 125: 752-754.

16. Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, et al. (2012) A critical role of IL-33 in experimental allergic rhinitis. J Allergy Clin Immunol 130: 184-194.

17. Matsuba-Kitamura S, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Taki Y, et al. (2010) Contribution of IL-33 to induction and augmentation of experimental allergic conjunctivitis. Int Immunol 22: 479-489.

18. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, et al. (2012) IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. J Invest Dermatol 132: 1392-1400.

19. Komai-Koma M, Gilchrist DS, McKenzie AN, Goodyear CS, Xu D, et al. (2011) IL-33 activates B1 cells and exacerbates contact sensitivity. J Immunol 186: 2584-2591.

20. Taniguchi K, Yamamoto S, Hitomi E, Inada Y, Suyama Y, et al. (2013) Interleukin-33 is induced by tumor necrosis factor-alpha and interferon-gamma in keratinocytes, and contributes to allergic contact dermatitis. J Investig Allergol Clin Immunol.

21. Kobayashi M, Nunomura S, Gon Y, Endo D, Kishiro S, et al. (2010) Abrogation of high-affinity IgE receptor-mediated mast cell activation at the effector phase prevents contact hypersensitivity to oxazolone. J Invest Dermatol 130: 725-731.

22. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H (2008) A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. J Allergy Clin Immunol 121: 1484-1490.

23. Hartmann B, Staedtler F, Hartmann N, Meingassner J, Firat H (2006) Gene expression profiling of skin and draining lymph nodes of rats affected with cutaneous contact hypersensitivity. Inflamm Res 55: 322-334.

24. Kurokawa M, Matsukura S, Kawaguchi M, Ieki K, Suzuki S, et al. (2011) Expression and effects of IL-33 and ST2 in allergic bronchial asthma: IL-33 induces eotaxin production in lung fibroblasts. Int Arch Allergy Immunol 155 Suppl 1: 12-20.

25. Lee PW, Elsaie ML, Jacob SE (2009) Allergic contact dermatitis in children: common allergens and treatment: a review. Curr Opin Pediatr 21: 491-498.

Citation: Taniguchi K, Yamamoto S, Hitomi E, Inada Y, Sugioka T, et al. (2013) Blockade of Interleukin-33 Attenuates Allergic Contact Dermatitis in Model Mice: Possible Mechanism via Eosinophil Infiltration. J Clin Exp Dermatol Res 4: 183. doi:10.4172/2155-9554.1000183

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