Dermatology Clinics & Research
DCR, 1(2): 26-30
www.scitcentral.com

ISSN: 2380-5609

Original Review Article: Open Access

Regulation of IL-33 Expression in Normal Human Epidermal Keratinocytes

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Received April 26, 2015; Accepted June 10, 2015; Published Aug 30, 2015

ABSTRACT

IL-33 is a member of IL-1 cytokine family, which works as an alarmin to stimulate immune reaction at noxious stimuli. We have investigated the regulation of IL-33 expression and its mechanisms in normal human epidermal keratinocytes (NHEKs). In this report, we have reviewed our recent research on the regulation of IL-33 expression in NHEKs and its function in the proliferation of these cells. Epidermal keratinocytes express IL-33 in the nucleus in the lesional skin of psoriasis, atopic dermatitis, and lichen planus stained with anti-IL-33 antibody on formalin-fixed and paraffin-embedded sections, while IL-33 was not expressed in the epidermis of normal skin. IFNγ, IL-17A, and ultraviolet B radiation induced IL-33 expression in a monolayer culture of NHEKs. In addition, IFNγ and TNFα induced IL-33 digestion by calpain. IL-33 induction was dependent on the phosphorylation of EGF receptor, ERK, and p38 MAP kinase. IL-33 has various splice variants whose expression patterns differ among different cell types, suggesting that they may play cell type-specific roles. Many studies have shown that IL-33 induces Th2-type inflammation, and that IL-33 released from damaged keratinocytes may induce Th2 inflammation. IL-33 in the nucleus regulates cell proliferation and suppresses gene transcription by inhibiting NFκB activation. Suprabasal expression of IL-33 in the nucleus of epidermal keratinocytes in inflammatory skin diseases may suppress inflammation to maintain keratinocyte homeostasis. In addition, suprabasal expression of IL-33 may regulate keratinocyte proliferation and differentiation; however, this needs to be investigated further.

KEYWORDS: IL-33; Epidermal keratinocytes; Induction; Mechanism; EGF receptor; Atopic dermatitis; Psoriasis

ABBREVIATIONS: IL, interleukin; IFN, interferon;ST2, serum stimulation-2; ST2L, long form of ST2; EGF, epidermal growth factor; TNF, tumor necrosis factor; Th, T helper; NFκB, nuclear factor kappa B; ERK, extracellular signal-regulated kinase; STAT, signal transducers and activators of transcription; JAK, Janus kinase; MAP kinase, mitogen-activated protein kinase.

INTRODUCTION

The skin is the largest organ in the human body, which covers the entire body and protects the inner body homeostasis from outer environmental stimuli[1].The epidermis is the outermost tissue covering the skin surface and is mainly composed of keratinocytes. Epidermal keratinocytes receive and respond to outer environmental stimuli, activate immune reaction, resulting in skin inflammation. Inflammatory reaction in the skin also stimulates epidermal keratinocytes to produce cytokines and chemokines to further enhance inflammation.

IL-33, a member of IL-1 family, is expressed in epithelial and endothelial cells and is released upon cell damage.IL-33 functions as an alarmin for stimulating immune response. ST2L, a receptor of IL-33, is expressed by various immune cells such as Th2 cells, type 2 innate lymphoid cells (ILC2), mast cells, and basophils. IL-33 stimulation triggers robust expression of IL-13 and IL-5 in ILC2 and IL-5 in Th2 cells,

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Citation: Komine M, Meephansan J, Tsuda H, Oshio T, Tominaga S et al (2015) Regulation of IL-33 Expression in Normal Human Epidermal Keratinocytes. Dermatol Clin Res, 1(2): 26-30

which enhances Th2-type immune responses [2,3,4]. Transgenic mice expressing IL-33 specifically in the basal keratinocytes spontaneously develop atopic eczema-like dermatitis through IL-5 produced by ILC2 as previously reported [5].

Besides its role as a cytokine, IL-33 functions as a nuclear protein for regulating gene transcription. IL-33 suppresses the activity of NFκB and induction of inflammatory responses as reported by Ali et al. [6], on the other hand, Choi et al. showed that IL-33 binds NFκB to stimulate its function [7].

In our previous studies, we examined IL-33 expression in normal human epidermal keratinocytes (NHEKs) under inflammatory skin conditions [8,9,15]. NHEKs do not express IL-33 under normal conditions but express it upon stimulation. Suprabasal keratinocytes express IL-33 in the nucleus under various inflammatory and hyperplastic conditions.

In this article, we have reviewed our recent studies on the regulation of IL-33 expression in NHEKs.

Results of our recent study

We investigated IL-33 expression in various inflammatory skin diseases. Formalin-fixed, paraffin-embedded blocks of lesional skin from patients with psoriasis, atopic dermatitis, and lichen planus were obtained from the archives of Jichi Medical University. These blocks were sectioned, and were stained using mouse monoclonal anti-human IL-33 antibody (Nessy-1) to determine IL-33 expression. Epidermal keratinocytes in the lesional skin of patients with psoriasis, atopic dermatitis, and lichen planus were suprabasally stained with IL-33 in the nucleus[8]. Hyperplastic epidermis of the wound edge, above dermatofibroma, and that surrounding SCC and Bowen's disease were also stained suprabasally with IL-33 in the nucleus [unpublished data].

The pathogenesis of psoriasis is not fully elucidated, however, recent studies revealed the important roles of Th17 cells and iNOS and TNF-producing dendritic cells (Tip DCs) in the pathophysiology of psoriasis [10]. Atopic dermatitis frequently accompanies bronchial asthma and allergic rhinitis, and is thought to be a Th2 type inflammation, however, in its chronic stage, Th1 type inflammation has been considered to play significant role, and recently, Th22 is considered to be of importance [11]. IL-17 and IFNγ are the major cytokine produced by Th17 and Th1 lymphocytes respectively, both of which are involved in the pathophysiology of psoriasis. TNFα is produced not only by Tip DCs, but also by activated lymphocytes, macrophages and keratinocytes [12]. We were interested if IL-33 is induced by these inflammatory cytokines, which are involved in the pathogenesis of psoriasis and atopic dermatitis.

NHEKs were cultured in a keratinocyte serum-free medium and were stimulated using cytokines such as TNFα, IFNγ, and IL-17A. IFNγ[8] and IL-17A [9] but not TNFα induced the protein and mRNA expression of IL-33 in a dose-dependent manner.

Induction of IL-33 expression by IFNγ was suppressed after the addition of MAP kinase inhibitors PD98059 or SB202190, inhibiting ERK and p38, respectively, and EGF receptor kinase inhibitors PD153035 or PD168393, but not after the addition of NFκB inhibitors parthenolide or Bay11-7085. Therefore, we assumed that IFNγ induced IL-33 expression by activating EGF receptor, ERK, and p38 MAP kinase but not by activating NFκB. IFNγ+TNFα induced the production of a smaller variant of IL-33 (~20kDa) in addition to the full-length IL-33 (30kDa), which was inhibited by the addition of calpain inhibitors, suggesting that IFNγ+TNFα induced the digestion of IL-33 by calpain into a mature form [8].

IL-17A also induced the protein and mRNA expression of IL-33. Induction of IL-33 expression by IL-17A was suppressed by the addition of ERK, p38 MAP kinase, EGFR, and JAK inhibitors but not by the addition of NFκB inhibitors, implying that induction of IL-33 expression by IL-17A was dependent on ERK, p38 MAP kinase, EGFR, and JAK but not on NFκB. STAT1 dominant-negative construct abolished IL-17A-induced IL-33 expression, suggesting that this induction was dependent on STAT1 phosphorylation [9]. IL-17A+TNFα did not induce the production of mature IL-33 as that observed with IFNγ+TNFα.

Ultraviolet B (UVB) radiation is an environmental stimulus that frequently affects the epidermis. Low doses of UVB, and its specific wavelength such that in narrowband UVB, is frequently used to treat inflammatory skin diseases such as psoriasis, parapsoriasis, and atopic dermatitis. The therapeutic use of UVB radiation is believed to cause reduction of inflammatory lymphocytes in the skin and induction of systemic increase of regulatory T cells [13, 14]. However, higher doses or certain wavelength of UVB causes acute inflammation, resulting in sun burn.

Exposure of a monolayer culture of NHEKs to broadband UVB radiation (30mJ/cm2) induced the protein and mRNA expression of IL-33 in a dose-dependent manner. This expression was suppressed by the addition of ERK and p38 MAP kinase inhibitors, suggesting that induction of IL-33 expression by UVB radiation was dependent on ERK and p38 MAP kinase [15].

The function of IL-33 in NHEKs is unknown. We suppressed IL-33 expression by transfecting NHEKs with small interfering RNA and investigated IL-8 expression. IL-33 knockdown enhanced IL-8 expression by TNFα, suggesting that presence of IL-33 in NHEKs suppressed IL-8 expression by TNFα. IL-8 expression by TNFα was

dependent on NFκB. Nuclear expression of IL-33 has suppressed NFκB activation [5]. Therefore, we assumed that in the nucleus, IL-33 suppressed TNFα-induced IL-8 expression by suppressing NFκB activation[9].

Digestion of full-length IL-33 by calpain[16] or neutrophil elastase[17] converts it into its mature form. However, digestion by caspase-1 results in the loss of its activity [18]. Recent report showed that serine proteases produced by mast cells digest proform IL-33 into more potent mature form to stimulate ILC2 [19]. Western blotting of IL-33 produced several smaller bands, suggesting that IL-33 may be digested into several different sizes or may have several splice variants. IL-33 contains 8 exons, with exons2–8 being the coding exons. We designed primers to detect splice variants lacking exons3, 4, and 5 or a combination of these exons and performed PCR. We observed that NHEKs, HEK293 cells, Hela cells, human umbilical vein endothelial cells (HUVECs), HaCaT cells, DJM-1 cells, normal human dermal fibroblasts, and cells obtained from dermatofibrosarcoma protuberans expressed each splice variant of IL-33, with distinct expression patterns. Although the roles of these splice variants are unknown, their distinct expression patterns in different cell types suggested that they may play cell type-specific roles [20].

DISCUSSION

In our previous studies, we clarified IL-33 induction in NHEKs by using inflammatory cytokines and UVB radiation and determined its underlying mechanisms. The role of IL-33 in Th2-type immune response is well investigated, and many novel findings have been reported [2,3,4]. A recent study reported that release of IL-33 from damaged epithelial cells induced IL-13 and IL-5 production from ILC2 and IL-5 production from Th2 cells [3,4]. Thus, IL-33 expression in the epidermis may be of importance for inducing Th2-type inflammation after epidermal damage. However, the role of IL-33 in epidermal keratinocytes in inflammatory skin diseases such as psoriasis and atopic dermatitis has not been clearly understood. IL-33 is produced as a full-length proform and is located to the nucleus. Upon cell damage, IL-33 is believed to be released from cells and is cleaved into its mature form by neutrophil elastase [17] or serine proteases from mast cells [19] to function as a cytokine or is digested by caspase 1 to inhibit its activity [18]. IL-33 is also reported to be digested into active mature form by calpain inside cells [16]. Specific expression of IL-33 in epidermal keratinocytes in transgenic mice spontaneously developed atopic dermatitis, which was shown to be dependent on IL-5 produced by ILC2 [5]. In many skin inflammation such as erythema multiforme, toxic epidermal necrolysis (TEN), and graft-versus-host disease, damages in keratinocytes are apparent. In erythema multiforme, for example, keratinocytes damaged by infiltrating T lymphocytes may release proform IL-33 that may be digested by neutrophil elastase or serine proteases from mast cells to stimulate an immune response for further enhancing the inflammation. IL-33 may also be digested inside the cells into mature form by cytoplasmic enzymes such as calpain and may be released from the cells. Later, IL-33 may be digested by caspase 1 to inhibit its activity. Another function of IL-33 may reside in its nuclear form. Because hyperplastic epidermal keratinocytes express IL-33 in the nucleus, we assumed that nuclear expression of IL-33 is associated with the proliferation and differentiation of epidermal keratinocytes. Inflammatory skin disease with prominent epidermal hyperplasia such as psoriasis and chronic stage atopic dermatitis, keratinocytes damage is not so apparent as those diseases mentioned above, however, the nuclear expression of IL-33 in suprabasal epidermal keratinocytes is more prominent. We believe that nuclear expression of IL-33 is important for regulating keratinocyte homeostasis during inflammatory skin disorders. A recent report showed that forced expression of IL-33 in the cytoplasm caused prolonged inflammation resulting in robust eosinophilic infiltration to multiple organs leading to death [21], which suggested the precise control of the location of IL-33 expression is inevitable to maintain appropriate control of inflammation.

UVB irradiation is known to inhibit the inflammatory immune response and induce the expansion of regulatory T cells in the skin [22], and is effective in treating several inflammatory skin diseases, such as psoriasis, atopic dermatitis, and prurigo. Other groups reported that IL-33 is involved in the development and expansion of regulatory T cells [23, 24]. IL-33 has also been reported to be involved in immune suppression induced by UVB irradiation [25]. Thus, IL-33 induced in NHEKs by UVB irradiation may have a role in inducing regulatory T cells to suppress inflammatory immune reaction.

A common pathway for IL-33 induction involves the activation of EGF receptor, ERK, and p38 MAP kinase. EGF receptor is activated by EGF receptor ligands such as TGFα, amphiregulin, heparin-biding EGF-like growth factor, and epiregulin and is trans-activated by cytokines such as TNFα and IFNγ. Mechanisms for the transactivation of EGF receptor by factors other than EGF receptor ligands include induction of enzymes such as a disintegrin and metalloproteinase (ADAM) 17, which release EGF receptor ligands from their transmembrane forms [25, 26]. We have previously shown that expression of cutaneous T cell attracting chemokine (CTACK)/CCL27, an early inflammatory chemokine essential to skin inflammation, was suppressed by the activation of EGF receptor by IFNγ[27]. Therefore, we speculate that EGF receptor activation occurs at a later stage of inflammation and induces epidermal hyperplasia, suppresses early inflammatory cytokines, and stimulates late inflammatory cytokines, which support and maintain disease-specific histopathological changes such as neutrophil infiltration in psoriasis and epidermal hyperplasia

in chronic atopic dermatitis. IL-33 induction by EGF receptor activation may occur in the late stage of inflammation and may serve as a suppressor of early stage of inflammation by inhibiting NFκB activation. Suprabasal expression of IL-33 in hyperplastic epidermis may be associated with their differentiation and proliferation of NHEKs, however, we do not know it's precise roles or the mechanism of suprabasal expression of IL-33.

CONCLUSION

Here, we reviewed our recent studies on the regulation of IL-33 expression in NHEKs. IL-33 is an interesting cytokine that functions not only as a soluble factor but also as a nuclear protein to exert transcriptional regulation. However, further studies should be performed to elucidate its complex function in immune response and in the proliferation and differentiation of keratinocytes.

ACKNOWLEDGEMENT

Our work was supported by a grant from the Ministry of Health, Labor, and Welfare (Research for Intractable Diseases) and a grant from the Ministry of Education Culture Sports Science and Technology.

REFERENCES

1. Yano S, Komine M, Fujimoto M, Okochi H, Tamaki K (2004)Mechanical stretching in vitro regulates signal transduction pathways and cellular proliferation in human epidermal keratinocytes. J Invest Dermatol 122(3):783-90.PMID:15086566

2. Licona-Limón P, Kim LK, Palm NW, Flavell RA (2013) TH2, allergy and group 2 innate lymphoid cells. Nat Immunol 14(6):536-42. doi: 10.1038/ni.2617.

3. Endo Y, Hirahara K, Iinuma T, Shinoda K, Tumes DJ, et al. (2015) The interleukin-33-p38 kinase axis confers memory T helper 2 cell pathogenicity in the airway. Immunity 42(2):294-308. doi: 10.1016/j.immuni.2015.01.016.

4. Liu B, Lee JB, Chen CY, Hershey GK, Wang YH (2015) Collaborative interactions between type 2 innate lymphoid cells and antigen-specific CD4+ Th2 cells exacerbate murine allergic airway diseases with prominent eosinophilia. J Immunol. [Epub ahead of print]

5. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, et al. (2013) Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. Proc Natl Acad Sci U S A110(34):13921-6. doi: 10.1073/pnas.1307321110.

6. Ali S, Mohs A, Thomas M, Klare J, Ross R, et al. (2011) The dual function cytokine IL-33 interacts with the transcription factor NF-κB to dampen NF-κB-stimulated gene transcription. J Immunol187(4):1609-16. doi: 10.4049/jimmunol.1003080. Epub 2011 Jul 6.

7. Choi YS, Park JA, Kim J, Rho SS, Park H, et al. (2012) Nuclear IL-33 is a transcriptional regulator of NF-κB p65 and induces endothelial cell activation. Biochem Biophys Res Commun421(2):305-11. PMID:22708120

8. Meephansan J, Tsuda H, Komine M, Tominaga S, Ohtsuki M (2012) Regulation of IL-33 expression by IFN-γ and tumor necrosis factor-α in normal human epidermal keratinocytes. J Invest Dermatol132(11):2593-600.doi: 10.1038/jid.2012.185.

9. Meephansan J, Komine M, Tsuda H, Karakawa M, Tominaga S, et al. (2013) Expression of IL-33 in the epidermis: The mechanism of induction by IL-17. J Dermatol Sci71(2):107-14.doi: 10.1016/j.jdermsci.2013.04.014.

10. Lowes MA, Suárez-Fariñas M, Krueger JG.(2014) Immunology of psoriasis. Annu Rev Immunol 32:227-55. doi: 10.1146/annurev-immunol-032713-120225.

11. Gittler JK, Shemer A, Suárez-Fariñas M, Fuentes-Duculan J, Gulewicz KJ, et al. (2012) Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronicatopic dermatitis. J Allergy Clin Immunol130(6):1344-54. doi: 10.1016/j.jaci.2012.07.012.

12. Pinto-Almeida T, Torres T. Biologic therapy for psoriasis - still searching for the best target.(2014) An Bras Dermatol 89(2):365-7. PMID:24770527

13. Wang X, Wang G, Gong Y, Liu Y, Gu J, et al. (2015) Disruption of circulating CD4+ T-lymphocyte subpopulations in psoriasis patients is ameliorated by narrow-band UVB therapy. Cell Biochem Biophys71(1):499-507. doi: 10.1007/s12013-014-0230-z.

14. Johnson-Huang LM, Suárez-Fariñas M, Sullivan-Whalen M, Gilleaudeau P, Krueger JG, Lowes MA. (2010) Effective narrow-band UVB radiation therapy suppresses the IL-23/IL-17 axis in normalized psoriasis plaques. J Invest Dermatol130(11):2654-63. doi: 10.1038/jid.2010.166.

15. Meephansan J, Komine M, Tsuda H, Tominaga S, Ohtsuki M (2012) Ultraviolet B irradiation induces the expression of IL-33 mRNA and protein in normal human epidermal keratinocytes. J Dermatol Sci65(1):72-4.doi: 10.1016/j.jdermsci.2011.10.004.

16. Hayakawa M, Hayakawa H, Matsuyama Y, Tamemoto H, Okazaki H, et al.(2009) Mature interleukin-33 is

produced by calpain-mediated cleavage in vivo. Biochem Biophys Res Commun 387(1):218-22.doi: 10.1016/j.bbrc.2009.07.018.

17. Lefrançais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, et al. (2012) IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. Proc Natl Acad Sci U S A 109(5):1673-8.doi: 10.1073/pnas.1115884109.

18. Cayrol C, Girard JP (2009) The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. Proc Natl Acad Sci U S A 106(22):9021-6.

19. Lefrançais E, Duval A, Mirey E, Roga S, Espinosa E, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. Proc Natl Acad Sci U S A. 2014 Oct 28;111(43):15502-7. doi: 10.1073/pnas.1410700111.

20. Tsuda H, Komine M, Karakawa M, Etoh T, Tominaga S, et al.(2012) Novel splice variants of IL-33: differential expression in normal and transformed cells. J Invest Dermatol 132(11):2661-4.

21. Bessa J, Meyer CA, de Vera Mudry MC, Schlicht S, Smith SH, et al. (2014) Altered subcellular localization of IL-33 leads to non-resolving lethal inflammation. J Autoimmun 55:33-41. doi: 10.1016/j.jaut.2014.02.012.

22. Yamazaki S, Nishioka A, Kasuya S, Ohkura N, Hemmi H, et al. (2014) Homeostasis of thymus-derived Foxp3+ regulatory T cells is controlled by ultraviolet B exposure in the skin. J Immunol193:5488-97. doi: 10.4049/jimmunol.1400985.

23. Matta BM, Lott JM, Mathews LR, Liu Q, Rosborough BR, et al. (2014) IL-33 is an unconventional Alarmin that stimulates IL-2 secretion by dendritic cells to selectively expand IL-33R/ST2+ regulatory T cells. J Immunol 193:4010-20. doi: 10.4049/jimmunol.1400481.

24. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, et al. (2014) The alarmin IL-33 promotes regulatory T-cell function in the intestine. Nature 513: 564-8. doi: 10.1038/nature13577.

25. Byrne SN, Beaugie C, O'Sullivan C, Leighton S, Halliday GM.(2011) The immune-modulating cytokine and endogenous Alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation. Am J Pathol 179(1):211-22. doi: 10.1016/j.ajpath.2011.03.010.

26. Argast GM, Campbell JS, Brooling JT, Fausto N(2004) Epidermal growth factor receptor transactivation mediates tumor necrosis factor-induced hepatocyte replication. J Biol Chem 279(33):34530-6.

27. Yahata Y, Shirakata Y, Tokumaru S, Yang L, Dai X, et al. (2006) A novel function of angiotensin II in skin wound healing. Induction of fibroblast and keratinocyte migration by angiotensin II via heparin-binding epidermalgrowthfactor (EGF)-like growthfactor-mediated EGF receptor transactivation. J Biol Chem 281(19):13209-16.

28. Karakawa M, Komine M, Hanakawa Y, Tsuda H, Sayama K, et al. (2014) CCL27 is downregulated by interferon gamma via epidermal growth factor receptor in normal human epidermal keratinocytes. J Cell Physiol 229(12):1935-45.