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Letter to the Editor

Viral infection induces Thymic stromal lymphopoietin (TSLP) in human keratinocytes

To the Editor

Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7 like cytokine, originally cloned from a murine thymic cell line, which plays a key role in allergic inflammation by activating dendritic cell to induce naïve T cells to differentiate into Th2 cells [1]. Overexpression of the TSLP gene specifically in airway epithelial cells or keratinocytes led to asthma- and atopic dermatitis (AD)-like disease, respectively [1]. Furthermore, TSLP is highly expressed by keratinocytes in skin lesions of patients with AD and by bronchi of asthmatic patients, and its levels of expression is correlated with severity of diseases [2]. However, the regulation of TSLP expression in epithelial cells and molecular pathways regulating this production has not been fully elucidated. Recently, we have shown that polyinosinic-polycytidylic acid (poly I:C), a synthetic double-stranded (ds) RNA, induces the production of TSLP from skin keratinocytes, and this production is synergized with the presence of inflammatory (TNF- α) and Th2 cytokines (IL-4, IL-13) [3]. However, it remains unclear whether real virus infection can induce TSLP production as similar manner as poly I:C. Therefore, we investigate whether real virus infection induces TSLP production from human primary keratinocytes.

Human primary keratinocytes (Kurabo, Osaka, Japan) were grown in Humedia-KG2 (Kurabo) as previously described [4], then stimulated with poly I:C (5 μ g/ml; GE Healthcare), vesicular stomatitis virus (VSV), herpes simplex virus (HSV)-1 (kindly provided by Drs. K. Honda and H. Yanai at university of Tokyo) at multiplicities of infections (MOI) as indicated. Total RNA extraction, real-time PCR, ELISA (R&D systems) and small interfering (si)RNA (QIAGEN) were carried out as described [4] or according to the manufacturer's instructions.

When we infected keratinocytes with VSV, a member of negative-sense single-stranded (ss) RNA viruses, but not HSV, a single-stranded DNA virus with dsRNA intermediates, induced TSLP in primary human keratinocytes 9 h after infection at multiplicity of infection (MOI) = 3 (Fig. 1A and B). The levels of VSV-induced TSLP expression was lower compared with those stimulated with poly I:C (5 μ g/ml) (Fig. 1C). Infection of higher MOI (up to 30) to elucidate efficient infection of these viruses did not lead to further induction of TSLP by both VSV and HSV (data not shown). Moreover, we confirmed the expressed-TSLP was secreted in culture supernatant as protein at 24 and 48 h after stimulation (Fig. 1D and E). Interestingly, VSV-induced expression of TSLP was not further upregulated by the presence of TNF- α and Th2 cytokines (IL-4 and IL-13) as reported in poly I:C-stimulated keratinocyte (data not shown) [3].

Viral dsRNA and its synthetic analogue poly I:C are recognized via multiple pathways involving the Toll-like receptor 3 (TLR3), ds

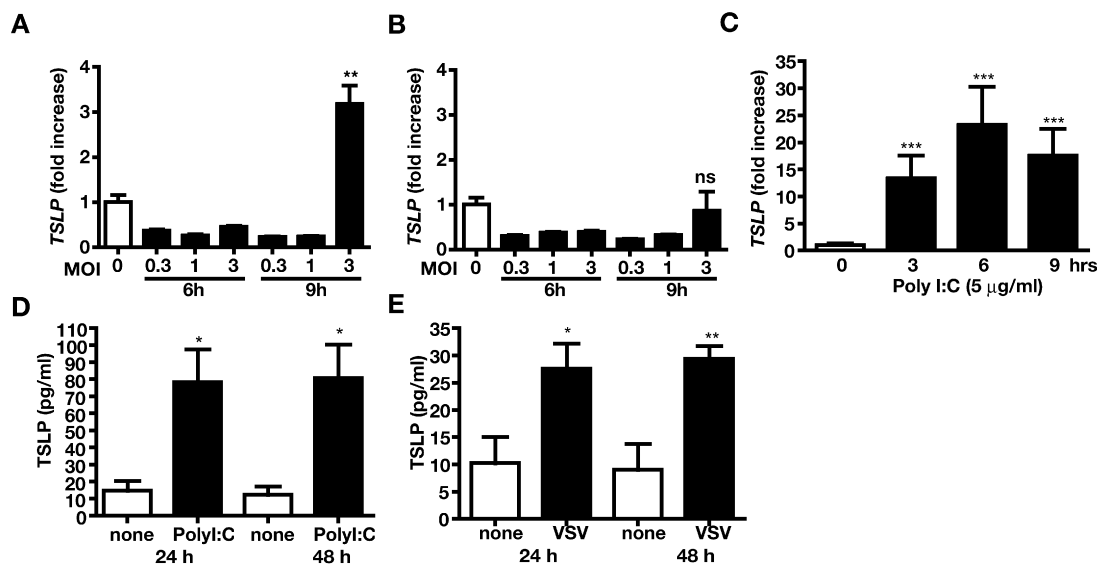


Fig. 1. VSV, but not HSV induces TSLP expression in primary human keratinocytes. Primary human keratinocytes were cultured in 6-well plates and inoculated with various concentration of VSV (A), HSV (B), and poly I:C (5 μ g/ml) (C) for the indicated time period. The expression of TSLP mRNA was measured by real-time PCR. TSLP secretion in culture supernatant stimulated with poly I:C (D) and VSV (E) at 24, 48 h was measured by ELISA. Columns show the means $\pm$ SD of three to five separate experiments. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to unstimulated cell.

RNA-activated protein kinase (PKR), and the recently described cytosolic RNA helicases, retinoic acid-inducible gene-1 (RIG-I) and melanoma differentiation-associated gene (MDA)5 [5]. To identify which pathways are involved in the induction of TSLP in keratinocytes stimulated with VSV and poly I:C, we first investigated relative expression of each receptor in keratinocytes. Unstimulated keratinocytes expressed RIG-I most abundantly, then MDA 5 and TLR3 (Fig. 2A). Their expressions were also

upregulated in keratinocytes upon stimulation with VSV and poly I:C, at variable degree, with MDA5 mRNA showing strongest (70-fold) by stimulation with poly I:C and RIG-I mRNA showing strongest (20-fold) by stimulation with VSV (Fig. 2A). Small interfering RNA analysis to identify receptors responsible for the induction of TSLP by VSV revealed that involvement of cytoplasmic dsRNA recognition receptors, both RIG-I and MDA5 (Fig. 2B). In contrast, the ability to express TSLP in response to poly I:C

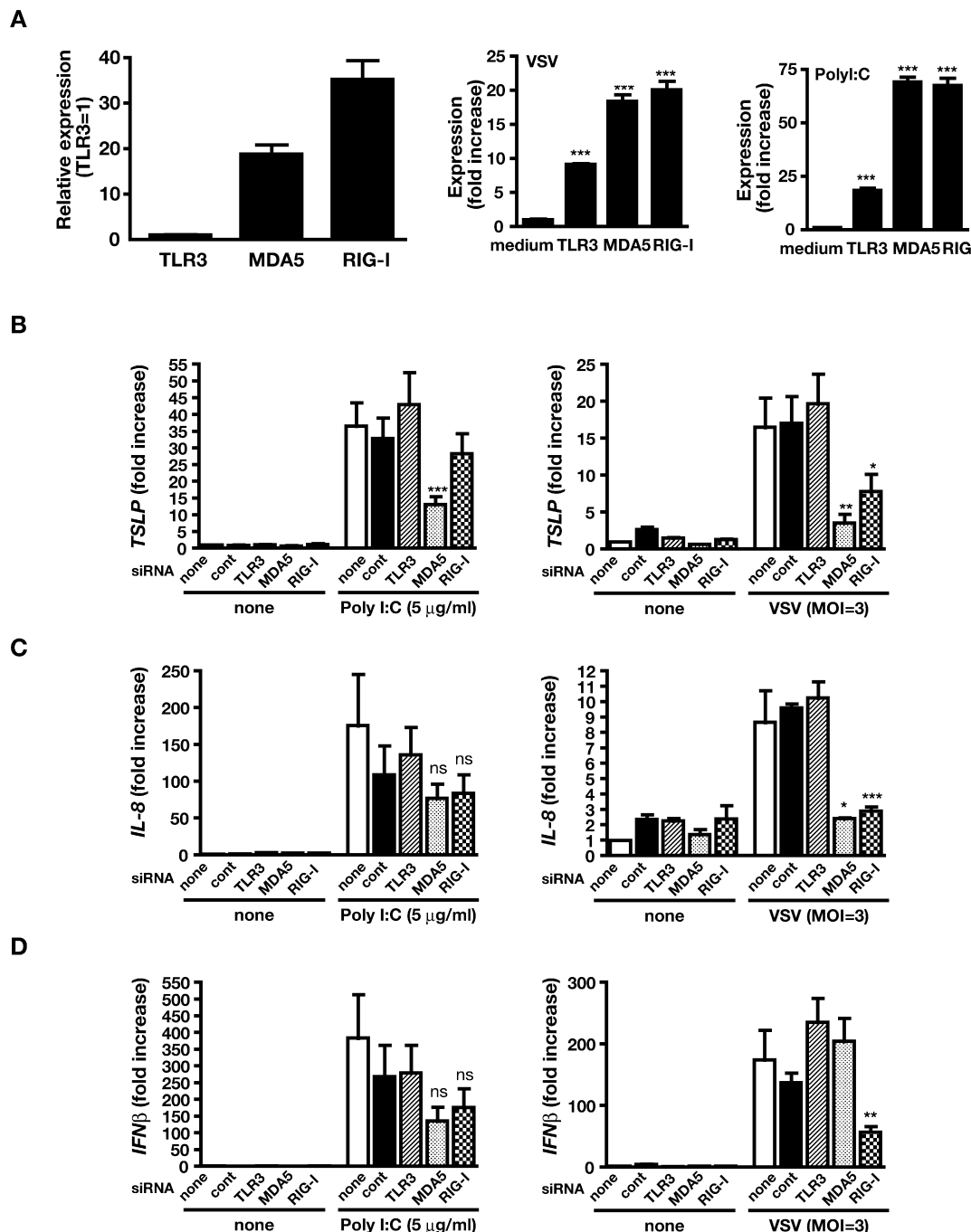


Fig. 2. Keratinocytes express dsRNA receptors, which are differently used for the recognition of VSV to induce inflammatory and anti-viral cytokine. (A) The expressions of TLR3, MDA 5 and RIG-I in unstimulated-, or in VSV (MOI = 3)- and poly I:C (5 µg/ml)- stimulated primary human keratinocytes were quantified by real-time PCR 9 h after stimulation. The values were expressed as relative expression levels of TLR3 as standard for unstimulated keratinocyte, and of unstimulated each receptor as standard for VSV, or poly I:C stimulated keratinocytes. Columns show the means $\pm$ SD of three experiments. *** p < 0.001 compared to unstimulated cell. (B) Primary human keratinocytes were stimulated with poly I:C or VSV at 24 h after corresponding siRNA transfection to inhibit specific dsRNA receptors. The expression of TSLP mRNA was measured by real-time PCR at 6 h (for poly I:C) and 9 h (for VSV) after stimulation. The expression of (C) IL-8 and (D) IFN- β was also measured by real-time PCR using same cDNA. Columns show the means $\pm$ SD of three separate experiments. ns, not significant. * p < 0.05, ** p < 0.01, *** p < 0.001 compared to control siRNA-treated cells.

appeared to be mediated by MDA5 (Fig. 2B). These results suggest that poly I:C and VSV use different recognition receptors to induce TSLP from keratinocytes. VSV-induced expression of IL-8 was similarly dependent on both MDA5 and RIG-I (Fig. 2C), however, to our surprise, IFN- β expression by VSV was totally dependent on RIG-I (Fig. 2D).

We observed clear induction of TSLP in primary human keratinocytes by VSV, but not by HSV. Although HSV is DNA virus, significant amount of dsRNA is detected during their replication [6]. Given that the length of VSV genomic RNA is 11 kb and the length of dsRNA in infected cells generated by VSV appeared to be 2.0–2.5 kbp, simple replication intermediate of VSV may not be the target of dsRNA receptors by infected cells [7]. Thus, additional virus-derived product in addition to replication intermediate might be required to stimulate TSLP induction by keratinocytes.

Intriguingly, we observed different dependency of dsRNA receptors for the production of inflammatory cytokine, TSLP, and anti-viral cytokine, IFN- β . These results are in line with a recent report showing a dominant proinflammatory response through TLR3 and a comparable proinflammatory and antiviral response through RIG-I after influenza virus infection in human bronchial epithelial cells [8]. Strong RIG-I-dependency for the induction of IFN- β but not other inflammatory cytokines might be explained by selective production of 5'-triphosphate ssRNA, another RIG-I ligand, by VSV [5]. Moreover, as the usage of RIG-I and MDA 5 is determined by length of the dsRNA, real virus infection might result in production of several dsRNA with different sizes, which stimulate several receptors at the same time and cause more complicated responses than poly I:C.

Keratinocytes play a key role not only in innate immune responses of the skin to bacterial and viral pathogens but also in initiation of acquired immunity by interacting dendritic cells and mast cells [9]. Since, TSLP protein is expressed in keratinocytes of acute and chronic skin lesions of AD but not in normal skin or nonlesional skin [2], identification of clinical relevant stimuli leading to TSLP production in keratinocytes is important strategy to prevention and treatment AD. Here, we have provided the first evidence that TSLP could be induced in keratinocytes by real virus infection, but different mechanisms from poly I:C. Moreover, our analysis indicates proinflammatory and anti-viral responses are induced in host using different receptors. As viral infections such as human herpes virus or papilloma virus infections are common in human skin, with keratinocytes as the targets for viral attack, elucidating the mechanisms to induce a sufficient anti-viral response, or inflammatory Th2 responses would be helpful to understand complex pathogenesis of AD or psoriasis, where chronic viral infections are often associated [10].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2011.02.005.

References

- [1] He R, Geha RS. Thymic stromal lymphopoietin. *Ann NY Acad Sci* 2010;1183:13–24.
- [2] Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerman A, Smith K, Gorman D, Zurawski S, Abrams J, Menon S, McClanahan T, de Waal-Malefyt Rd R, Bazan F, Kastelein RA, Liu YJ. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673–80.
- [3] Kinoshita H, Takai T, Le TA, Kamijo S, Wang XL, Ushio H, Hara M, Kawasaki J, Vu AT, Ogawa T, Gunawan H, Ikeda S, Okumura K, Ogawa H. Cytokine milieu modulates release of thymic stromal lymphopoietin from human keratinocytes stimulated with double-stranded RNA. *J Allergy Clin Immunol* 2009;123:179–86.
- [4] Niyonsaba F, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, Nagaoka I, Okumura K, Ogawa H. Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol* 2007;127:594–604.
- [5] Takeuchi O, Akira S. Innate immunity to virus infection. *Immunol Rev* 2009;227:75–86.
- [6] Weber F, Wagner V, Rasmussen SB, Hartmann R, Paludan SR. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J Virol* 2006;80:5059–64.
- [7] Kato H, Takeuchi O, Mikamo-Sato H, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med* 2008;205:1601–10.
- [8] Le Goffic R, Pothlichet J, Vitour D, Fujita T, Meurs E, Chignard M, Si-Tahar M. Cutting edge: influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. *J Immunol* 2007;178:3368–72.
- [9] Metz M, Maurer M. Innate immunity and allergy in the skin. *Curr Opin Immunol* 2009;21:687–93.
- [10] Prignano G, Ferraro C, Mussi A, Stivali F, Trento E, Bordignon V, Crescimbeni E, Salvati G, Degener AM, Ameglio F. Prevalence of human papilloma virus type 5 DNA in lesional and non-lesional skin scales of Italian plaque-type psoriatic patients: association with disease severity. *Clin Microbiol Infect* 2005;11:47–51.

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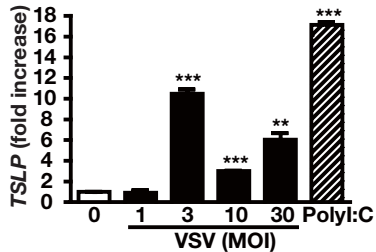
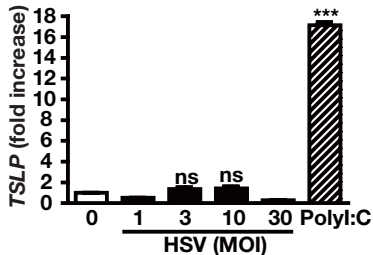
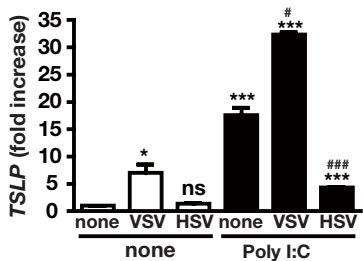
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