

## ORIGINAL RESEARCH ARTICLE

# Activation of proteinase-activated receptor-2 (PAR-2) simultaneously induces interleukin 8 (IL-8) and suppresses monocyte chemoattractant protein-1 (MCP-1) in HaCaT cells

Yoshinobu Murakami<sup>1,2\*</sup>, Seisho Azuma<sup>2,3</sup>, Kimiye Baba<sup>2</sup>, Masahiko Taniguchi<sup>2</sup>, Fukumi Furukawa<sup>4,5</sup>

<sup>1</sup> Department of Aesthetics and Health Sciences, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8509, Japan. E-mail: ymurakam@wakayama-med.ac.jp

<sup>2</sup> Department of Natural Products Research, Faculty of Pharmacy, Osaka Medical and Pharmaceutical University, Takatsuki, Osaka 569-1094, Japan.

<sup>3</sup> Jabara Laboratory Co., Ltd., Wakayama 640-8425, Japan.

<sup>4</sup> Takatsuki Red Cross Hospital, Takatsuki, Osaka 569-1096, Japan.

<sup>5</sup> Department of Forensic Medicine, Wakayama Medical University, Wakayama 641-8509, Japan.

## ABSTRACT

Allergic diseases are currently considered diseases of excessive type 2 inflammation created by orchestration between the innate and acquired immune systems. Since pattern recognition receptors (PRRs) are present in epidermal keratinocytes, it is noteworthy that aggravating factors of allergic diseases act directly on keratinocytes via PRRs. To investigate the relationship between the activation of PRRs and inflammation, we stimulated a keratinocyte cell line (HaCaT cells) with agonists against proteinase-activated receptor-2 (PAR-2), Toll-like receptor (TLR)2, and TLR4, alone or in combination, and we evaluated the changes in inflammatory cytokines and chemokines. Activation of TLR2 or TLR4 alone induced interleukin 6 (IL-6), IL-8, and monocyte chemoattractant protein-1 (MCP-1) in an agonist concentration-dependent manner. Simultaneous activation of TLR2 and TLR4 induced IL-8 synergistically, MCP-1 in an additive trend, and IL-6 weakly but synergistically. PAR-2 activation of HaCaT cells induced IL-6 and IL-8 but suppressed MCP-1 in an agonist concentration-dependent manner. The enhancement of IL-8 and the suppression of MCP-1 by PAR-2 activation were both neutralized by the PAR-2 antagonist AZ3451, supporting the possibility that PAR-2 activation simultaneously induces the following opposing effects in inflammation: enhancement of IL-8 and suppression of MCP-1. The nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway inhibitor BAY 11-7082 neutralized the induction of IL-8 but not the suppression of MCP-1 by PAR-2 activation, indicating that PAR-2 activation induces activation of the NF- $\kappa$ B pathway, and that the suppression of MCP-1 by PAR-2 activation is not related to the NF- $\kappa$ B pathway.

**Keywords:** Pattern Recognition Receptor (PRR); Proteinase-activated Receptor-2 (PAR-2); Inflammation; Allergic Diseases; Induced Interleukin 8 (IL-8), Monocyte Chemoattractant Protein-1 (MCP-1)

## ARTICLE INFO

Received 19 May 2022  
Accepted 27 May 2022  
Available online 9 June 2022

## COPYRIGHT

Copyright © 2022 Yoshinobu Murakami, *et al.*  
EnPress Publisher LLC. This work is licensed  
under the Creative Commons Attribution-NonCommercial 4.0 International License  
(CC BY-NC 4.0).  
<https://creativecommons.org/licenses/by-nc/4.0/>

## 1. Introduction

Allergic diseases have traditionally been considered diseases created by activation of the acquired immune system, but it was recently proposed to be diseases of excessive type 2 inflammation created by orchestration between the innate and acquired immune systems<sup>[1,2]</sup>. Aggravating factors for allergic diseases include house dust mites (HDMs)<sup>[3,4]</sup>, fungus<sup>[5,6]</sup>, and bacteria<sup>[7]</sup>, which activate the acquired immune system but also the innate immune system. Immune cells involved in innate immunity recognize foreign substances and pathogens through pattern recognition receptors (PRRs). PRRs are

also present in epithelial cells located at the interface of the body with the outside world<sup>[8]</sup>. Thus it should be aware that aggravating factors in the environment can act directly on epidermal cells<sup>[9,10]</sup>. Although we have studied the component analysis of natural products<sup>[11]</sup> and the effects of them on allergic diseases in clinical trials<sup>[12,13]</sup>, their mechanisms of action also need to be further investigated from the perspective of innate immunity. As in the case of HDMs, when considered at the molecular level even a single aggravating factor is composed of various types of molecules, and a single factor is thus thought to stimulate multiple PRRs<sup>[14]</sup>. Since the above aggravating factors<sup>[4-7]</sup> activate proteinase-activated receptors-2 (PAR-2), Toll-like receptor (TLR)2, and TLR4, we focused on these PRRs in this study.

In this study, we used agonists of these PRRs to investigate the direct effects of environmental aggravators of allergic diseases on epidermal keratinocytes, and we report interesting results on the activation of PRRs and inflammatory responses.

## 2. Materials and methods

### 2.1 Materials

The human epidermal keratinocyte line HaCaT cells were obtained from Cosmo Bio (Tokyo). Dulbecco's modified Eagle's medium (DMEM) (high glucose) and Penicillin-Streptomycin solution were obtained from Wako Pure Chemical Industries (Osaka, Japan). Fetal bovine serum (FBS) was obtained from Corning (Corning, NY, USA). The NF- $\kappa$ B pathway inhibitor BAY 11-7082, Zymosan A, and lipopolysaccharide (LPS) from *Escherichia coli* O55 were purchased from Wako Pure Chemical Industries. The PAR-2 agonist SLIGKV-NH<sub>2</sub> was purchased from Abcam (Cambridge, UK). The PAR-2 antagonist AZ3451 was purchased from MedChemExpress (Monmouth Junction, NJ). The Cell Counting Kit-8 was from Dojindo Laboratories (Kumamoto, Japan). Enzyme-linked immunosorbent assay (ELISA) kits for human interleukin 6 (IL-6), IL-8, monocyte chemoattractant protein-1 (MCP-1), thymic stromal lymphopoietin (TSLP), and tumor necrosis factor-alpha (TNF- $\alpha$ ) were ob-

tained from BioLegend (San Diego, CA).

### 2.2 Cell culture

HaCaT cells were maintained in DMEM containing high glucose (4,500 mg/L), sodium pyruvate (110 mg/L), and *L*-glutamine (584 mg/L), supplemented with 10% FBS, 100 units/mL of penicillin, and 100  $\mu$ g/mL of streptomycin. Cells were incubated at 37 °C with 5% CO<sub>2</sub> in a humidified atmosphere.

### 2.3 Cell stimulation experiments

HaCaT cells were seeded in 24-well plates at a density of  $3.0 \times 10^5$  cells/well in DMEM containing high glucose, sodium pyruvate, and *L*-glutamine, supplemented with 1% FBS and antibiotics at 37 °C for 24 h. The cells were then stimulated with the agonists Zymosan<sup>[15,16]</sup>, LPS<sup>[17]</sup>, and SLIGKV-NH<sub>2</sub><sup>[18]</sup>, respectively for an additional 24 h. After treatment, the culture supernatants were collected and stored at -20 °C until assayed. For the evaluation of the effect of an antagonist or an inhibitor, i.e., AZ3451<sup>[19]</sup> or BAY 11-7082<sup>[20]</sup>, were pre-treated for 2 h prior to agonist stimulation for 24 h.

### 2.4 Cell viability assay

A Cell Counting Kit-8 was used to measure cell viability according to the manufacturer's instructions. The absorbance was read at the wavelength of 450 nm using a microplate reader (model 680XR, Bio-Rad, Hercules, CA).

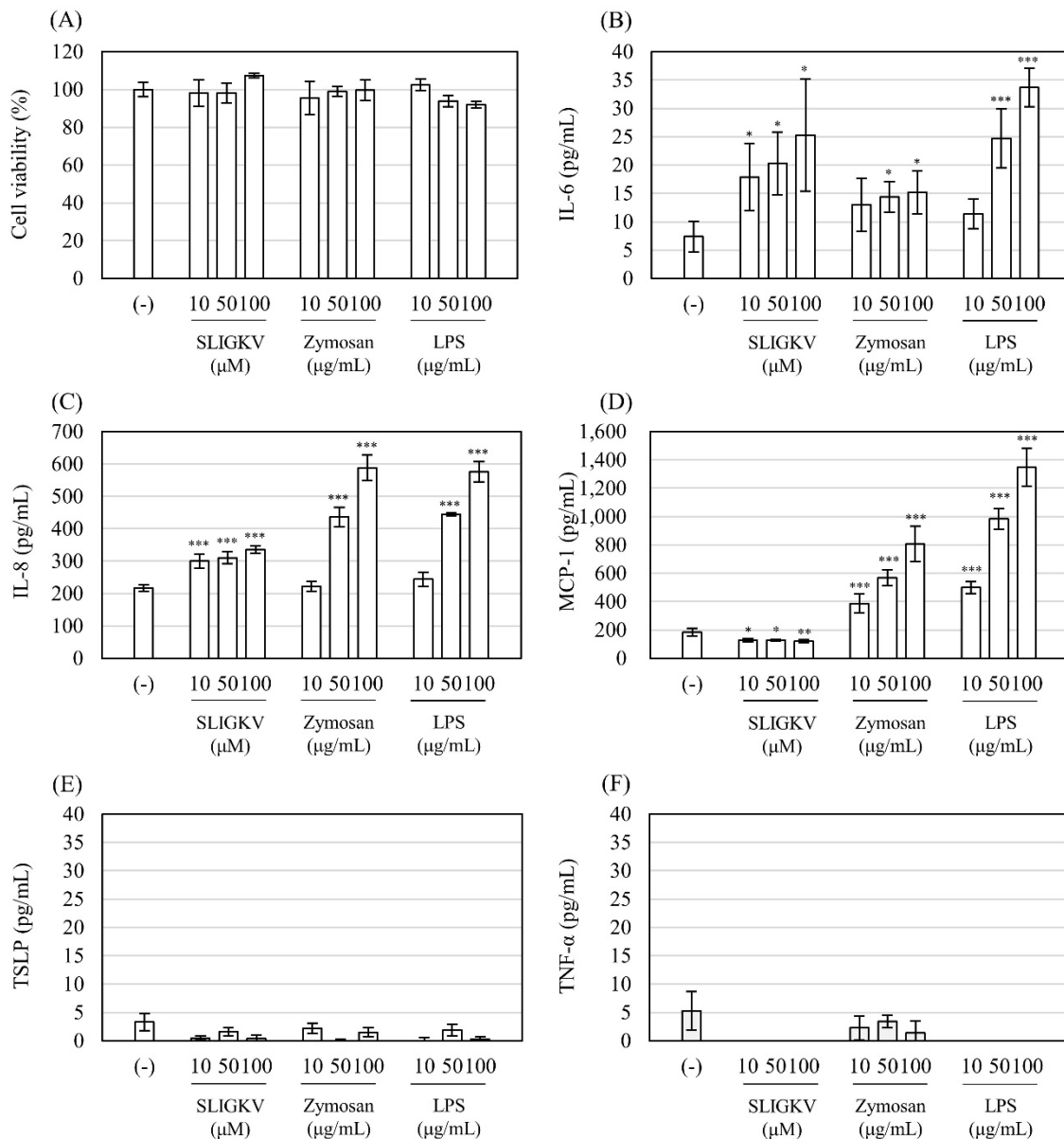
### 2.5 Measurement of protein levels of cytokines/chemokines in the cell culture supernatants

ELISA kits were used to measure the protein levels of IL-6, IL-8, MCP-1, TSLP, and TNF- $\alpha$  in the cell culture supernatants. Assays were performed according to the manufacturers' instructions. The absorbance was read at the wavelength of 450 nm using the 680XR microplate reader.

### 2.6 Statistical analysis

The results were examined using Student's *t*-test and are presented as the mean  $\pm$  standard deviation (SD) (*n* = 4). Statistical significance is de-

noted as \* $p < 0.05$ , \*\* $p < 0.01$ , or \*\*\* $p < 0.001$ .



**Figure 1.** Stimulation of PRRs by their respective agonists in HaCaT cells. SLIGKV-NH<sub>2</sub>, Zymosan, or LPS, which are agonists of PAR-2, TLR2, or TLR4, respectively, was added to HaCaT cells alone, and cell viability (A) and the levels of inflammatory cytokines and chemokines in supernatants, such as IL-6 (B), IL-8 (C), MCP-1 (D), TSLP (E), and TNF-α, were examined after 24-h culture. To assess cell viability (A), the optical density of cells incubated in medium alone without agonists was set at 100%. Results are presented as mean ± standard deviation (SD) (n = 4). Statistical analyses were calculated using the Student's *t*-test. Statistical significance was marked as \* $p < 0.05$ , \*\* $p < 0.01$ , or \*\*\* $p < 0.001$  compared to no agonists.

### 3. Results

#### 3.1 Stimulation of PRRs by their respective agonists in HaCaT cells

SLIGKV-NH<sub>2</sub> (a PAR-2 agonist), Zymosan (a TLR2 agonist), or LPS (TLR4 agonist) was added to HaCaT cells alone, and the cells' viability and production of inflammatory cytokines and chemo-

kines were examined after 24-h culture. No major changes in cell viability were observed with the addition of these agonists (Figure 1A). The addition of any of the three agonists resulted in large changes in the productions of IL-8 and MCP-1 and small but significant changes in that of IL-6, but no regular or significant changes in TSLP or TNF-α (Figure 1B-F). IL-6 and IL-8 were increased in a dose-dependent manner by each of the agonists

(Figure 1B,C). For MCP-1, although a concentration-dependent production was observed when Zymosan or LPS was added to the cells, the addition of SLIGKV-NH<sub>2</sub> resulted in a very slight but significant decrease, as in the no-addition group (Figure 1D).

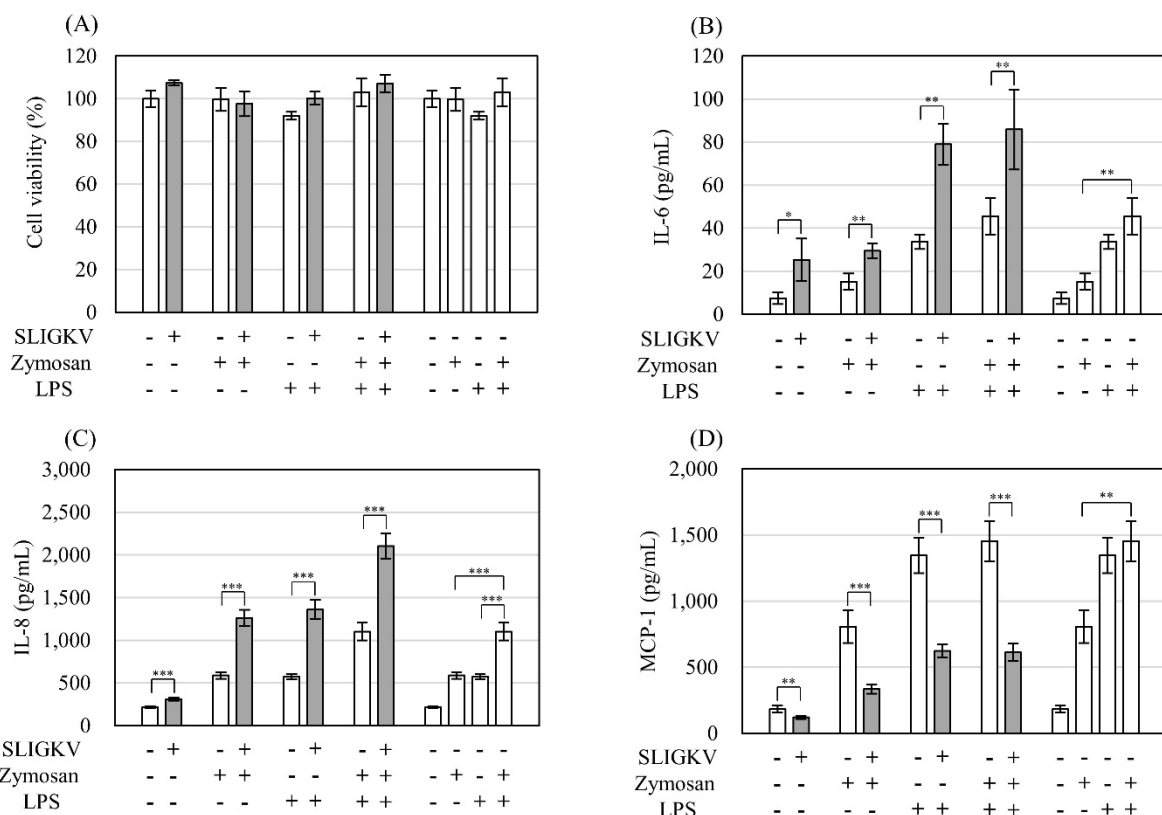
### 3.2 Stimulation of PRRs by mixed agonists in HaCaT cells

The single agonists or mixtures of SLIGKV-NH<sub>2</sub>, Zymosan, and LPS were added to HaCaT cells, and the cells' viability and production of inflammatory cytokines and chemokines were measured after 24-h culture. No major changes in cell viability were observed with the addition of these agonists (Figure 2A). Regarding the production of IL-8, a synergistic effect was observed with each combination of agonists (Figure 2C). A synergistic effect on IL-6 production was also observed with the two-agonist mixture of SLIGKV-NH<sub>2</sub> (100 μM) and LPS (100 μg/mL) and the three-agonist mixture of SLIGKV-NH<sub>2</sub> (100 μM), Zymosan (100

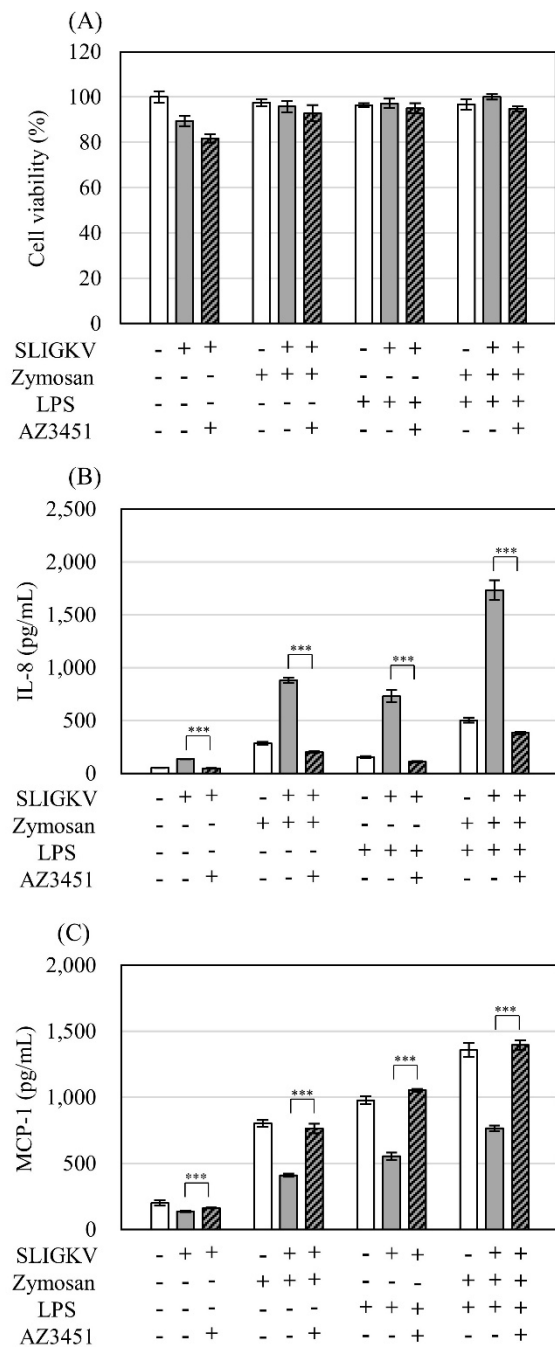
μg/mL), and LPS (100 μg/mL) (Figure 2B). In contrast, MCP-1 was suppressed by the addition of SLIGKV-NH<sub>2</sub>, and the addition of a mixture of other agonists had an additive trend effect, not a synergistic effect as in the case of IL-8 (Figure 2D).

### 3.3 Neutralization of the effect of SLIGKV-NH<sub>2</sub> by the PAR-2 antagonist AZ3451

As shown in Figure 2, the addition of SLIGKV-NH<sub>2</sub>, a PAR-2 agonist, to HaCaT cells induced IL-8 and suppressed MCP-1; we thus examined the effect of SLIGKV-NH<sub>2</sub> on IL-8 and MCP-1 in the presence of the PAR-2 antagonist AZ3451 (10 μM), which blocks PAR-2 signaling. No major changes in cell viability were observed with the addition of AZ3451 (Figure 3A). For IL-8, the increase by SLIGKV-NH<sub>2</sub> alone and the synergistic increase by mixing SLIGKV-NH<sub>2</sub> with other agonists were both neutralized by AZ3451 (Figure 3B). The suppression of MCP-1 by SLIGKV-NH<sub>2</sub> was also neutralized by AZ3451 (Figure 3C).



**Figure 2.** Stimulation of PRRs by mixed agonists in HaCaT cells. Single or mixtures of SLIGKV-NH<sub>2</sub> (100 μM), Zymosan (100 μg/mL), or LPS (100 μg/mL) were added to HaCaT cells, and cell viability (A) and the levels of IL-8 (B), MCP-1 (C) in supernatants were examined after 24-h culture. To assess cell viability (A), the optical density of cells incubated in medium alone without agonists was set at 100%. Results are presented as mean ± SD (n = 4). Statistical significance was marked as \**p* < 0.05, \*\**p* < 0.01, or \*\*\**p* < 0.001.



**Figure 3.** Neutralization of the effect of SLIGKV-NH<sub>2</sub> by the PAR-2 antagonist AZ3451. HaCaT cells were pre-treated with PAR-2 antagonist AZ3451 (10  $\mu$ M) for 2 h, and incubated with single or mixtures of SLIGKV-NH<sub>2</sub> (100  $\mu$ M), Zymosan (100  $\mu$ g/mL), and LPS (100  $\mu$ g/mL). Cell viability (A) and the levels of IL-8 (B), MCP-1 (C) in supernatants were examined after 24-h culture. To assess cell viability (A), the optical density of cells incubated in medium alone without agonists was set at 100%. Results are presented as mean  $\pm$  SD (n = 4). Statistical significance was marked as \* $p$  < 0.05, \*\* $p$  < 0.01, or \*\*\* $p$  < 0.001.

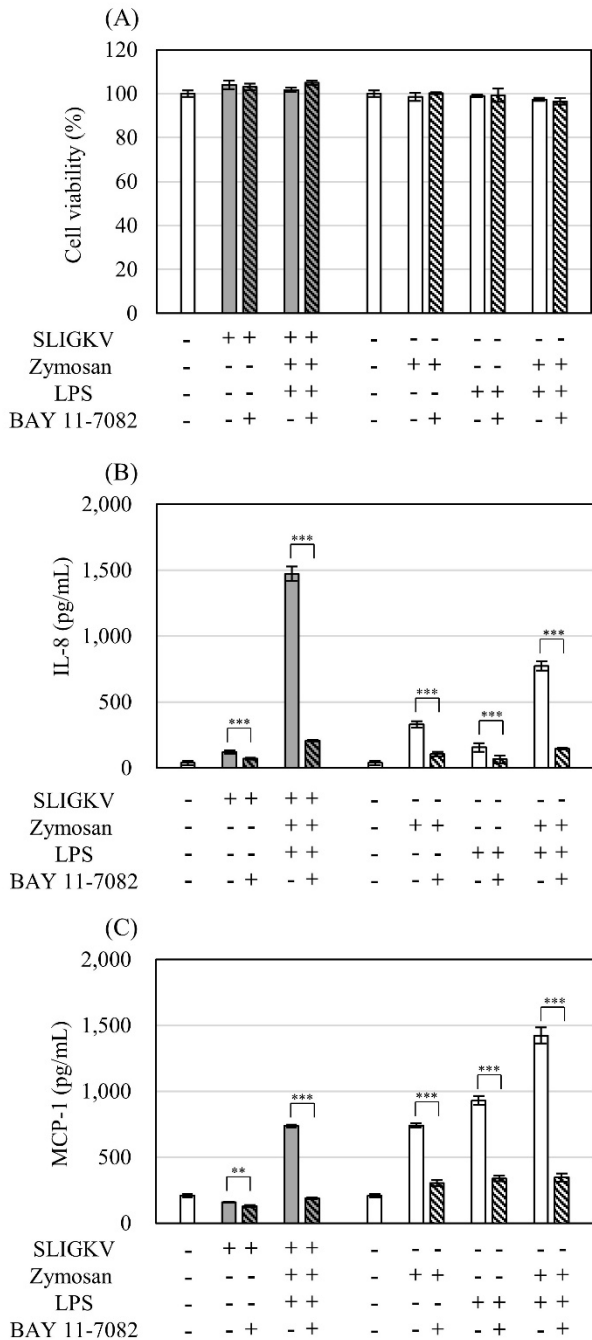
### 3.4 Effect of the NF- $\kappa$ B pathway inhibitor BAY 11-7082 on IL-8 and MCP-1

As shown in **Figure 2**, the addition of SLIGKV-NH<sub>2</sub>, a PAR-2 agonist, to HaCaT cells induced IL-8 and suppressed MCP-1; we therefore investigated the effect of SLIGKV-NH<sub>2</sub> on IL-8 and MCP-1 in the presence of the NF- $\kappa$ B pathway inhibitor BAY 11-7082 (10  $\mu$ M). No major changes in cell viability were observed with the addition of BAY 11-7082 (**Figure 4A**). The increase of IL-8 induced by SLIGKV-NH<sub>2</sub> alone and its synergistic increase by the combination of SLIGKV-NH<sub>2</sub> with other agonists were both neutralized by BAY 11-7082 (**Figure 4B**). The suppression of MCP-1 by SLIGKV-NH<sub>2</sub> was not neutralized by the addition of BAY 11-7082 (**Figure 4C**).

## 4. Discussion

Environmental aggravators of allergic diseases such as HDMs, fungus, and bacteria not only act as allergens on acquired immunity; they also activate the innate immune system by being recognized through PRRs such as TLRs and PARs. Since PRRs are also present in epidermal keratinocytes, it is noteworthy that aggravating factors of allergic diseases act directly on keratinocytes via PRRs. In this study, to investigate the relationship between the activation of PRRs and inflammation, we stimulated the keratinocyte cell line HaCaT with agonists against PAR-2, TLR2, and TLR4, alone or in combination, and we observed the changes in inflammatory cytokines and chemokines. IL-8 and MCP-1 are major mediators of acute neutrophil- and chronic macrophage-mediated inflammation, respectively<sup>[21]</sup>. Thus, these chemokines influence the pathogenesis of allergic diseases.

The activation of TLR2 or TLR4 alone induced productions of IL-6, IL-8, and MCP-1 in an agonist concentration-dependent manner, although in varying amounts (**Figure 1**). The simultaneous activation of TLR2 and TLR4 induced IL-8 synergistically, MCP-1 additively, and IL-6 weakly but synergistically (**Figure 2**). These inductions were neutralized by the addition of BAY11-7082 (10  $\mu$ M), indicating that they were due to activation of the NF- $\kappa$ B pathway (**Figure 4**).



**Figure 4.** Effect of NF- $\kappa$ B pathway inhibitor BAY 11-7082 on IL-8 and MCP-1. HaCaT cells were pre-treated with NF- $\kappa$ B pathway inhibitor BAY 11-7082 (10  $\mu$ M) for 2 h, and incubated with single or mixtures of SLIGKV-NH2 (100  $\mu$ M), Zymosan (100  $\mu$ g/mL), and LPS (100  $\mu$ g/mL). Cell viability (A) and the levels of IL-8 (B), MCP-1 (C) in supernatants were examined after 24-h culture. To assess cell viability (A), the optical density of cells incubated in medium alone without agonists was set at 100%. Results are presented as mean  $\pm$  SD (n = 4). Statistical significance was marked as \* $p$  < 0.05, \*\* $p$  < 0.01, or \*\*\* $p$  < 0.001.

In the activation of PAR-2, contrasting results were observed for IL-6 and IL-8, and for MCP-1. That is, the results obtained for IL-6 and IL-8 were similar to those observed with the activations of

TLR2 and TLR4 but not MCP-1, in which an agonist concentration-dependent suppression was observed (Figures 1, 2). The enhancement of IL-8 and the suppression of MCP-1 by PAR-2 activation was neutralized by AZ3451 (10  $\mu$ M), an inhibitor of PAR-2, supporting the possibility that PAR-2 activation induces opposing effects: an enhancement of IL-8 and a suppression of MCP-1 (Figure 3). BAY 11-7082 (10  $\mu$ M), an inhibitor of the NF- $\kappa$ B pathway, neutralized the induction of IL-8 by PAR-2 activation, but it did not neutralize the suppression of MCP-1, indicating that PAR-2 activation induces activation of the NF- $\kappa$ B pathway, and the inhibition of MCP-1 by PAR-2 activation is not related to the NF- $\kappa$ B pathway (Figure 4).

What is noteworthy about this finding is that the activation of PAR-2 had two opposing effects at the same time, i.e., both inducing and suppressing inflammation (Figures 2, 3). These results are not surprising, since there are conflicting reports of PAR-2 activation regarding the induction<sup>[22,23]</sup> and suppression<sup>[24,25]</sup> of inflammation, but it is extremely interesting that the activation of PAR-2 simultaneously exerted conflicting effects. The prior conflicting findings may be due to the different experimental conditions used, but they may also suggest that PAR-2 plays a dual role in inducing and protecting against inflammation *in vivo*.

In the HaCaT cells in this experiment, as in other reports<sup>[26]</sup>, the activation of PAR-2 is thought to activate the NF- $\kappa$ B pathway, but MCP-1, which is expected to be induced by activation of the NF- $\kappa$ B pathway, was suppressed, and we thus speculate that the results regarding MCP-1 may be due to a different mechanism of action. It has been reported that the activation of TLR2<sup>[16,17]</sup> and TLR4<sup>[17]</sup> induces inflammation in keratinocytes, and that HDMs, a causative and aggravating agent of allergic diseases, mediates innate immune responses through TLR2/4 co-activation in alveolar macrophages<sup>[27]</sup>. In the present study, inflammation was also induced by the activation of TLRs, and a synergistic effect of TLR2/4 co-activation was observed in IL-8 production (Figures 1 and 2).

However, we observed no regular or significant changes in the production of TSLP or TNF- $\alpha$

(**Figure 1E**). Earlier studies demonstrated that a production of TSLP in keratinocytes was induced by the activation of TLR3<sup>[28]</sup>, TLR5<sup>[29]</sup>, and TLR2/6<sup>[30]</sup> and by the simultaneous activation of IL-1R and PAR-2<sup>[31]</sup>, whereas it was not induced by peptidoglycan<sup>[30]</sup>, an agonist of TLR2, and was weakly induced by PAR-2 activation alone<sup>[31]</sup>. It thus seems reasonable that no induction of TSLP was observed in the present study. It was reported that TNF- $\alpha$  in keratinocytes is induced by the activations of TLR2 and TLR4<sup>[32]</sup>, as well as by the activation of PAR-2<sup>[33]</sup>. Although there are differences in the origin and primary culture cells/cell lines between the above reports and the present study, it is not clear why TNF- $\alpha$  was not induced by PRR activation in the present investigation.

In this experiment, HaCaT cells were used instead of normal human epidermal keratinocyte cells. It is true that HaCaT cells are a heterogeneous population in terms of differentiation and are affected by the FBS in the medium during subculture, and we do not know to what extent our experimental system mimics the biological response. However, the fact that PAR-2 activation simultaneously induced and inhibited inflammatory responses under the same conditions supports the existence of PAR-2-mediated inflammatory and anti-inflammatory pathways, as suggested by Cocks and Moffatt<sup>[34]</sup>. This mechanism remains to be elucidated. Since there are many molecules with protease activity in the environment (e.g., fungi and HDMs), the relationship between PAR-2 activation and inflammation will require further study.

## 5. Conclusion

PAR-2 activation of HaCaT cells induced the production of IL-6 and IL-8 but suppressed the production of MCP-1 in an agonist concentration-dependent manner. The enhancement of IL-8 and the suppression of MCP-1 by PAR-2 activation were both neutralized by its antagonist, supporting the concept that PAR-2 activation simultaneously induces opposing effects in inflammation, i.e., the enhancement of IL-8 and the suppression of MCP-1. PAR-2 activation induces activation of the NF- $\kappa$ B pathway, and the suppression of MCP-1 by PAR-2

activation is not related to the NF- $\kappa$ B pathway.

## Conflict of interest

Yoshinobu Murakami belongs to the Department of Aesthetics and Health Sciences, Wakayama Medical University, which is funded by Jabara Laboratory Co., Ltd.

## Acknowledgements

This study was partially supported by the Wakayama Prefectural Government project “Research and Development Support Project for Pioneering Industrial Technology”. We sincerely thank Professor Toshikazu Kondo of the Department of Forensic Medicine, Wakayama Medical University for his valuable guidance and advice in conducting this study.

## References

1. Honda T, Kabashima K. Reconciling innate and acquired immunity in atopic dermatitis. *Journal of Allergy and Clinical Immunology* 2020; 145(4): 1136–1137. doi: 10.1016/j.jaci.2020.02.008
2. Thiriou D, Morianos I, Xanthou G, *et al.* Innate immunity as the orchestrator of allergic airway inflammation and resolution in asthma. *International Immunopharmacology* 2017; 48: 43–54. doi: 10.1016/j.intimp.2017.04.027
3. Jacquet A. The role of the house dust mite-induced innate immunity in development of allergic response. *International Archives of Allergy and Immunology* 2011; 155(2): 95–105. doi: 10.1159/000320375
4. Wang JY. The innate immune response in house dust mite-induced allergic inflammation. *Allergy, Asthma & Immunology Research* 2013; 5(2): 68–74. doi: 10.4168/aair.2013.5.2.68
5. Matsuwaki Y, Wada K, White T, *et al.* *Alternaria* fungus induces the production of GM-CSF, interleukin-6 and interleukin-8 and calcium signaling in human airway epithelium through protease-activated receptor 2. *International Archives of Allergy and Immunology* 2012; 158(Suppl 1): 19–29. doi: 10.1159/000337756
6. Hayes T, Rumore A, Howard B, *et al.* Innate immunity induced by the major allergen Alt a 1 from the fungus *Alternaria* is dependent upon toll-like receptors 2/4 in human lung epithelial cells. *Frontiers in Immunology* 2018; 9: 1507. doi: 10.3389/fimmu.2018.01507
7. Bitschar K, Wolz C, Krismer B, *et al.* Keratinocytes as sensors and central players in the immune defense against *Staphylococcus aureus* in the skin. *Journal of Dermatological Science* 2017; 87(3): 215–220. doi: 10.1016/j.jdermsci.2017.06.003

8. Köllisch G, Kalali BN, Voelcker V, *et al.* Various members of the Toll-like receptor family contribute to the innate immune response of human epidermal keratinocytes. *Immunology* 2005; 114(4): 531–541. doi: 10.1111/j.1365-2567.2005.02122.x
9. Chieosilapatham P, Kiatsurayanon C, Umehara Y, *et al.* Keratinocytes: Innate immune cells in atopic dermatitis. *Clinical & Experimental Immunology* 2021; 204(3): 296–309. doi: 10.1111/cei.13575
10. Boonpiyathad T, Sözener ZC, Satitsuksanoa P, *et al.* Immunologic mechanisms in asthma. *Seminars in Immunology* 2019; 46: 101333. doi: 10.1016/j.smim.2019.101333
11. Azuma S, Murakami Y, Azuma E, *et al.* Anti-inflammatory effects of flavonoids in Citrus jabara fruit peels. *Trends in Immunotherapy* 2020; 4(1): 5–14. doi: 10.24294/ti.v2i2.844
12. Inaba Y, Furukawa F, Azuma S, *et al.* Evaluation of the safety and usefulness of Citrus jabara fruit peel powder cream for patients with atopic dermatitis. *Trends in Immunotherapy* 2020; 4(1): 42–46. doi: 10.24294/ti.v4.i1.1230
13. Azuma S, Murakami Y, Taniguchi M, *et al.* Daily intake of Citrus jabara fruit peel powder (Japanese Patent No. 5,323,127) improves allergy-like symptoms: A randomized double-blind parallel-group comparative study. *Trends in Immunotherapy* 2020; 5(2): 21–31. doi: 10.24294/ti.v5.i2.1390
14. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends in Immunology* 2011; 32(9): 402–411. doi: 10.1016/j.it.2011.06.006
15. Sato M, Sano H, Iwaki D, *et al.* Direct binding of Toll-like receptor 2 to zymosan, and zymosan-induced NF- $\kappa$ B activation and TNF- $\alpha$  secretion are down-regulated by lung collectin surfactant protein A. *The Journal of Immunology* 2003; 171(1): 417–425. doi: 10.4049/jimmunol.171.1.417
16. Lee HM, Yuk JM, Shin DM, *et al.* Apurinic/aprimidinic endonuclease 1 is a key modulator of keratinocyte inflammatory responses. *The Journal of Immunology* 2009; 183(10): 6839–6848. doi: 10.4049/jimmunol.0901856
17. Olaru F, Jensen LE. Chemokine expression by human keratinocyte cell lines after activation of Toll-like receptors. *Experimental Dermatology* 2010; 19(8): e314–e316. doi: 10.1111/j.1600-0625.2009.01026.x
18. Kim HY, Goo JH, Joo YA, *et al.* Impact on inflammation and recovery of skin barrier by nordihydroguaiaretic acid as a protease-activated receptor 2 antagonist. *Biomolecules & Therapeutics* 2012; 20(5): 463–469. doi: 10.4062/biomolther.2012.20.5.463
19. Huang X, Ni B, Xi Y, *et al.* Protease-activated receptor 2 (PAR-2) antagonist AZ3451 as a novel therapeutic agent for osteoarthritis. *Aging (Albany NY)* 2019; 11(24): 12532–12545. doi: 10.18632/aging.102586
20. Jia Q, Zang D, Yi J, *et al.* Cytokine expression in trichloroethylene-induced hypersensitivity dermatitis: An *in vivo* and *in vitro* study. *Toxicology Letters* 2012; 215(1): 31–39. doi: 10.1016/j.toxlet.2012.09.018
21. Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemotactic and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine & Growth Factor Reviews* 1998; 9(1): 9–23. doi: 10.1016/s1359-6101(97)00022-1
22. Hou L, Kapas S, Cruchley AT, *et al.* Immunolocalization of protease-activated receptor-2 in skin: Receptor activation stimulates interleukin-8 secretion by keratinocytes *in vitro*. *Immunology* 1998; 94(3): 356–362. doi: 10.1046/j.1365-2567.1998.00528.x
23. Asaduzzaman M, Nadeem A, Arizmendi N, *et al.* Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation. *Clinical & Experimental Allergy* 2015; 45(12): 1844–1855. doi: 10.1111/cea.12628
24. Moffatt JD, Jeffrey KL, Cocks TM. Protease-activated receptor-2 activating peptide SLIGRL inhibits bacterial lipopolysaccharide-induced recruitment of polymorphonuclear leukocytes into the airways of mice. *American Journal of Respiratory Cell and Molecular Biology* 2002; 26(6): 680–684. doi: 10.1165/ajrcmb.26.6.4693
25. Khoufache K, LeBouder F, Morello E, *et al.* Protective role for protease-activated receptor-2 against influenza virus pathogenesis via an IFN- $\gamma$ -dependent pathway. *The Journal of Immunology* 2009; 182(12): 7795–7802. doi: 10.4049/jimmunol.0803743
26. Macfarlane SR, Sloss CM, Cameron P, *et al.* The role of intracellular Ca<sup>2+</sup> in the regulation of proteinase-activated receptor-2 mediated nuclear factor  $\kappa$ B signalling in keratinocytes. *British Journal of Pharmacology* 2005; 145(4): 535–544. doi: 10.1038/sj.bjp.0706204
27. Liu CF, Drocourt D, Puzo G, *et al.* Innate immune response of alveolar macrophage to house dust mite allergen is mediated through TLR2/-4 co-activation. *PLoS ONE* 2013; 8(10): e75983. doi: 10.1371/journal.pone.0075983
28. Vu AT, Chen X, Xie Y, *et al.* Extracellular double-stranded RNA induces TSLP via an endosomal acidification- and NF- $\kappa$ B-dependent pathway in human keratinocytes. *Journal of Investigative Dermatology* 2011; 131(11): 2205–2212. doi: 10.1038/jid.2011.185
29. Le TA, Takai T, Vu AT, *et al.* Flagellin induces the expression of thymic stromal lymphopoietin in human keratinocytes via toll-like receptor 5. *International Archives of Allergy and Immunology* 2011; 155(1): 31–37. doi: 10.1159/000318679
30. Vu AT, Baba T, Chen X, *et al.* Staphylococcus aureus membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway.



- Journal of Allergy and Clinical Immunology 2010; 126(5): 985–993.e3. doi: 10.1016/j.jaci.2010.09.002
31. Redhu D, Franke K, Kumari V, *et al.* Thymic stromal lymphopoietin production induced by skin irritation results from concomitant activation of protease-activated receptor 2 and interleukin 1 pathways. *British Journal of Dermatology* 2020; 182(1): 119–129. doi: 10.1111/bjd.17940
  32. Sumikawa Y, Asada H, Hoshino K, *et al.* Induction of beta-defensin 3 in keratinocytes stimulated by bacterial lipopeptides through toll-like receptor 2. *Microbes and Infection* 2006; 8(6): 1513–1521. doi: 10.1016/j.micinf.2006.01.008
  33. Maeda S, Maeda S, Ohno K, *et al.* Protease-activated receptor-2 induces proinflammatory cytokine and chemokine gene expression in canine keratinocytes. *Veterinary Immunology and Immunopathology* 2013; 153(1–2): 17–25. doi: 10.1016/j.vetimm.2013.01.018
  34. Cocks TM, Moffatt JD. Protease-activated receptor-2 (PAR2) in the airways. *Pulmonary Pharmacology & Therapeutics* 2001; 14(3): 183–191. doi: 10.1006/pupt.2001.0285.32