


RESEARCH

Open Access



Oxidative stress enhances the expression of IL-33 in human airway epithelial cells

Hiroyuki Aizawa¹, Akira Koarai^{1*} , Yutaka Shishikura¹, Satoru Yanagisawa¹, Mutsuo Yamaya², Hisatoshi Sugiura¹, Tadahisa Numakura¹, Mitsuhiro Yamada¹, Tomohiro Ichikawa¹, Naoya Fujino¹, Masafumi Noda³, Yoshinori Okada³ and Masakazu Ichinose¹

Abstract

Background: Interleukin-33 (IL-33) is a cytokine belonging to the IL-1 family, and its possible involvement in the pathophysiology of COPD and viral-induced exacerbations has been demonstrated. IL-33 has been shown to be increased in the airway epithelial cells from COPD patients, but the regulating mechanism of IL-33 expression in airway epithelial cells remains largely unknown. In the current study, we examined whether oxidative stress, which participates in the pathogenesis of COPD, affects the expression of IL-33 in airway epithelial cells and also evaluated the effect during viral infection.

Methods: The involvement of oxidative stress in the expression of IL-33, and its signal pathway was examined after stimulation with hydrogen peroxide (H₂O₂), with or without stimulation by polyinosinic-polycytidylic acid [poly (I:C)], a synthetic analogue of dsRNA that mimics viral infection, or rhinovirus infection in NCI-H292 cells and primary human bronchial epithelial cells (HBECs). In addition, the effect of antioxidant, *N*-acetylcysteine (NAC) in the expression of IL-33 was compared between HBECs from healthy subjects and those from COPD patients.

Results: Treatment with H₂O₂ significantly potentiated IL-33 expression in NCI-H292 cells, and the potentiation was reversed by NAC treatment. Mitogen-activated protein kinase (MAPK) inhibitors, but not nuclear factor-kappa B inhibitors, also significantly decreased the H₂O₂-potentiated IL-33 expression. In addition, H₂O₂ significantly potentiated the poly (I:C)- or rhinovirus-stimulated IL-33 expression. In HBECs from healthy subjects, H₂O₂-potentiated IL-33 expression and its reversal by NAC was also confirmed. Under the condition without H₂O₂-stimulation, treatment with NAC significantly decreased the expression of IL-33 in HBECs from COPD patients, but not in those from healthy subjects.

Conclusions: These results demonstrate that oxidative stress involves in the expression of IL-33 in airway epithelial cells via MAPK signal pathway and it augments IL-33 expression during viral infection. This mechanism may participate in the regulation of IL-33 expression in airway epithelial cells in COPD and the viral-induced exacerbations. Modulation of this pathway could become a therapeutic target for viral-induced exacerbations of COPD.

Keywords: COPD, Exacerbation, IL-33, Oxidative stress, Viral infection

Background

Chronic obstructive pulmonary disease (COPD) was ranked sixth as a cause of death in 1990, but it is currently the third leading cause of death in the world [1]. Exacerbations of COPD triggered by respiratory infections or environmental pollutants cause an acute deterioration in airway inflammation and lung function, and

also lead to an increase in mortality and healthcare costs [2–5]. The prevention of exacerbations remains a key therapeutic goal, but current treatments have only modest benefits and considerable adverse effects. Therefore, since new treatments are urgently needed, a better understanding of the mechanisms of COPD exacerbations is needed.

Interleukin-33 (IL-33) is a cytokine belonging to the IL-1 family, which includes IL-1 α , IL-1 β and IL-18. It is mainly a nuclear protein in several types of cells including endothelial cells, epithelial cells and fibroblasts [6, 7]. Once released

* Correspondence: koarai@rm.med.tohoku.ac.jp

¹Department of Respiratory Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryō-machi, Aoba-ku, Sendai 980-8574, Japan
Full list of author information is available at the end of the article

into the extracellular compartment in response to tissue damage or inflammation, extracellular IL-33 has been shown to act as a potent inducer of inflammation [6, 7]. Especially, IL-33 promotes T helper 2 cell (Th2) immunity, which leads to allergic pathological changes via ST2 receptor, a member of the IL-1/Toll-like receptor family [8]. IL-33 has been extensively characterized as functionally important in Th2-associated inflammatory diseases including asthma, atopic dermatitis and helminth infections [6]. Although higher expression of IL-33 has been reported in patients with asthma [9], it has also been demonstrated in bronchial epithelial cells from those with COPD, especially in a severe stage of COPD [10, 11]. In a COPD mouse model and in vitro airway epithelial cells, increases of IL-33 have been also shown, especially during viral infection or after cigarette smoke exposure [11]. Despite the increasing evidence suggesting the contribution of IL-33 to the pathophysiology of COPD and its exacerbations [12], the regulating mechanism of the expression of IL-33 in airway epithelial cells remains largely unknown.

Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) derived from cigarette smoke or released by inflammatory cells, are reported to contribute to the pathogenesis of COPD and its exacerbations [13, 14]. In fact, oxidative stress markers such as H_2O_2 are elevated in the airways of COPD patients [15–18], and higher amounts of H_2O_2 are detected in the airways during the exacerbations than in those in a stable condition [19, 20]. Pretreatment with H_2O_2 has been reported to augment the production of pro-inflammatory cytokines and chemokines in airway epithelial cells and inflammatory cells [21, 22]. Our previous report has also demonstrated that H_2O_2 augments viral-derived double-stranded RNA (dsRNA)-stimulated CXC motif chemokine ligand (CXCL) 8 release in human airway epithelial cells via the activation of Toll-like receptor 3 (TLR3) signal pathway [23]. Concerning IL-33, an antioxidant, *N*-acetylcysteine (NAC) or glutathione (GSH), has been reported to inhibit the *alternaria* extract-augmented IL-33 release in an allergic mouse model, which suggests the possible involvement of oxidative stress in the regulation of IL-33 production [24]. However, it has not been determined whether oxidative stress affects the expression of IL-33 and which signal pathways are participated in the regulating mechanisms in airway epithelial cells.

The present study, therefore, was designed to determine, using H_2O_2 and an experimental viral infection model, the following: (1) whether oxidative stress potentiates IL-33 expression in human airway epithelial cells and which signal pathways participate in the regulating mechanisms; (2) whether oxidative stress augments IL-33 expression in the dsRNA-treated or viral infected cells; and (3) whether antioxidant treatment decreases the expression of IL-33 in airway epithelial cells from COPD patients.

Methods

Materials

Poly (I:C) (polyinosinic-polycytidylic acid, sodium salt, double-stranded), SB203580, SP600125, U0126, SC-514 and BAY 11-7085 were purchased from Calbiochem (La Jolla, CA). H_2O_2 , NAC and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT) were from Sigma-Aldrich (St. Louis, MO).

Patients

Five never smokers and six former smokers with COPD took part in our study after giving written informed consent (Table 1). COPD was diagnosed according to the GOLD guidelines [25]. All subjects had undergone surgery for lung cancer after receiving pulmonary function tests. Human bronchial tissues were obtained from the 2-4 bronchi of the lobe resected at surgery, avoiding areas involved by tumors. The tissues were used for the culture of human bronchial cells. All experiments in the study were approved by the ethics committee of Tohoku University Graduate School of Medicine.

Preparation of epithelial cells

NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line (ATCC, Manassas, VA) and primary human bronchial epithelial cells (HBECs), acquired from lobes resected from patients at surgery, the details of which are shown in Table 1, were cultured as previously described [26]. Cells were grown to 80% confluence in culture plates and incubated in fetal bovine serum (FBS)- or growth factor-free medium for 24 h before treatment.

To investigate the effect of H_2O_2 on the expression of IL-33, the cells were harvested at 4 h, unless indicated otherwise, after treatment with H_2O_2 and stored at -80°C until the measurement. To examine the effect of H_2O_2 on the poly

Table 1 Characteristics of healthy subjects and patients with COPD

	Healthy subjects	COPD
Number	5	6
Male/Female	1/4	5/1
Age (years)	68.0 ± 3.4	68.0 ± 3.8
Ex-smoker/current smoker	0/0	6/0
Pack years	N/A	45.6 ± 8.5**
Lung function		
FEV ₁ /FVC (%)	86.8 ± 3.3	62.7 ± 3.0***
FEV ₁ % of predicted (%)	108 ± 6.7	75.5 ± 6.7***
DLC0/VA (%)	114 ± 4.2	85.2 ± 3.7***

Data are presented as mean ± SEM

, * represents, $p < 0.01$, $p < 0.001$ vs. healthy subjects

Abbreviations: COPD chronic obstructive pulmonary disease, FEV₁ forced expiratory volume in 1 s, FVC forced vital capacity, DLC0/VA corrected carbon monoxide diffusing capacity by alveolar volume, N/A not applicable

(I:C) or rhinovirus infection-induced IL-33 expression, H₂O₂ was added to the media 30 min before the treatment with poly (I:C) or rhinovirus [23]. To evaluate the effects of inhibitors, various concentrations of NAC, p38-MAPK inhibitor (SB203580), JNK inhibitor (SP600125), ERK inhibitor (U0126), IKK-2 inhibitor (SC-514) or IκBα inhibitor (BAY11-7085) were added to the media 1 h before H₂O₂ treatment [27, 28]. In the control group, the cells were treated with medium in the absence of H₂O₂, poly (I:C), or inhibitors.

Rhinovirus infection

A stock solution of type 14 rhinovirus (RV14) [1.0×10^7 tissue culture infectious dose (TCID₅₀)/ ml] was obtained from a patient with a common cold and the rate of RV14 release was quantified in the same manner as previously described in methods [29]. NCI-H292 cells in culture plates were infected with RV14 at a multiplicity of infection (MOI) of 1 for 90 min in RPMI-1640 medium at 33 °C before the virus was removed and replaced with RPMI-1640 medium [26, 30, 31]. NCI-H292 cells were treated with H₂O₂ or vehicle 30 min prior to RV14 infection. Cells were infected with RV14 for 90 min and the virus was removed and replaced with medium containing 200 μM H₂O₂ or vehicle. In some experiments, cells were pretreated with NAC 1 h prior to infection. After the cells were incubated for 1 to 48 h at 33 °C, the whole cell extractions were harvested and stored at -80 °C until required.

Cell viability assay

Cell viability in NCI-H292 cells were evaluated by use of the MTT assay and in HBECs were evaluated by the lactate dehydrogenase (LDH) activity using Cytotoxicity Detection KitPLUS (Sigma-Aldrich) as previously described [26]. Cell viability was calculated as the percentage of viable cells among vehicle-treated cells.

Detection of mitogen-activated protein kinase (MAPK) phosphorylation by immunoblotting

Cells were seeded in 6-well plates at a density of 1×10^5 /ml. At 80% confluence, and maintained in FBS-free medium for 24 h before stimulation. To evaluate the inhibitory effect of NAC on the expression of phospho-p38 (p-p38), c-jun N-terminal kinase (p-JNK) and Extracellular Signal-regulated Kinase 1/2 (p-ERK1/2), the cells were treated with NAC 60 min prior to H₂O₂ stimulation. After 15 min H₂O₂ stimulation, cells were washed with ice-cold HANK's balanced salt solution (HBSS) and homogenized in cell lysis buffer (0.05% TritonX, 35 mM Tris-HCl, pH 7.4, 0.4 mM EGTA, 10 mM MgCl₂, 1 μM phenylmethylsulfonyl fluoride, 100 μg/ml aprotinin and 1 μg/ml leupeptin) at 4 °C. Samples were solubilized in sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) sample buffer. Equal amounts of protein were loaded

and the separated by electrophoresis on 12.5% SDS-PAGE. After electrophoresis, the separated proteins were blotted on a PolyVinylidene DiFluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA). Rabbit monoclonal anti-phospho-p38 MAPK antibody, rabbit monoclonal anti-p38 MAPK antibody, rabbit monoclonal anti-phospho-JNK antibody, rabbit monoclonal anti-JNK antibody, rabbit polyclonal anti-phospho-ERK1/2 antibody, rabbit polyclonal anti-ERK1/2 antibody (1:1000 dilution, all from Cell Signaling Technology, Danvers, MA) and mouse monoclonal anti-β-actin antibody (1:10000 dilution; Sigma) were used to detect the target proteins. Appropriate peroxidase-conjugated secondary antibodies were used. Binding antibodies were detected using ECL-prime (Amersham Biosciences, Buckinghamshire, UK) and visualized with a chemiluminescence imaging system (LAS-4000 mini; Fujifilm, Tokyo, Japan). Each band intensity was quantified by densitometry (Quantity One software, Bio-Rad).

Detection of IL-33 by nuclear extraction and immunoblotting

Cells were treated with 200 μM H₂O₂ for 0 to 12 h. After washing with HBSS, cells were homogenized in cell lysis buffer to obtain the nuclear fraction using Nuclear Extraction Kit (Active Motif, Carlsbad, CA) according to the manufacturer's instructions. The following separation, blotting, detection and visualization procedures were performed in the same manner as for MAPK phosphorylation immunoblotting. Target proteins were detected with mouse monoclonal anti-IL-33 antibody (1:1000 dilution, Enzo Life Sciences, Exeter, UK) or mouse monoclonal anti-lamin A/C antibody (1:400 dilution; Santa Cruz Biotechnology, Santa Cruz, CA).

Real-time polymerase chain reaction (PCR)

Total RNA was isolated from the cells to prepare cDNA using High capacity of RNA to cDNA kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. cDNA was amplified with specific primers to *IL-33* and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* using the 7500 Real-Time PCR System (Applied Biosystems). Primers used to amplify cDNA were from TaqMan Gene Expression Assays (Applied Biosystems): *IL-33* (catalogue number Hs00369211_m1), *GAPDH* (Hs99999905_ml). Relative quantification of different transcripts was determined with the comparative threshold cycle (CT) method using *GAPDH* as the endogenous control. Results were calculated as fold change over control.

Statistical analysis

Data are expressed as the mean ± SEM. GraphPad Prism (GraphPad Software Inc., SanDiego, CA) was used to

perform statistical tests. Experiments with multiple comparisons were evaluated using one way analysis of variance (ANOVA) by Bonferroni's test to adjust for multiple comparisons. Wilcoxon matched-pairs signed rank test or Mann-Whitney U test was used for single comparison. Significance values were defined as $p < 0.05$.

Results

Effect of H₂O₂ on the expression of IL-33 in NCI-H292 cells

NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line, have been used for evaluation of the pathophysiology in obstructive airway disease, especially as a model for mucin production, which is one of the main features of COPD [26, 27]. NCI-H292 cells share key components of various signaling pathways including MAPK and nuclear factor-kappa B (NF- κ B) with normal HBECs and have been used to evaluate the involvement of oxidative stress in cytokine release and mucus secretion [27, 32, 33]. Here, we used NCI-H292 cells to clarify the involvement of oxidative stress in the expression of IL-33 in the airway epithelial cells and we investigated the effect of H₂O₂ on the expression of IL-33 in NCI-H292 cells. Treatment with H₂O₂ significantly increased the expression of IL-33 at concentrations of 0 to 200 μ M in a concentration-dependent manner without affecting the cell viability (Fig. 1a-b). In addition, the H₂O₂-potentiated IL-33 expression reached the peak at 4 h and the potentiated expression decreased at 24 h to the same as that at 0 h (Fig. 1c). We also confirmed that H₂O₂ significantly increased the concentration of IL-33 protein in the compartment of nuclei in the treated cells at 12 h (Fig. 1d-e). To clarify the involvement of oxidative stress in H₂O₂-potentiated IL-33 expression, the effect of NAC was evaluated. Pretreatment with 1 to 10 mM NAC significantly decreased the H₂O₂-potentiated IL-33 expression in a concentration-dependent manner (Fig. 1f).

Involvement of MAPK (p38, JNK, ERK1/2) and NF- κ B signaling pathways in H₂O₂-potentiated IL-33 expression

Previous reports indicated that oxidative stress induced the activation of MAPK [p38 MAPK, JNK, ERK1/2] and NF- κ B [23, 28, 34]. Therefore, we investigated whether the potentiated effect of H₂O₂ on IL-33 expression is mediated by MAPK and NF- κ B signaling pathways in NCI-H292 cells. Treatment with H₂O₂ significantly increased the phosphorylation of p38-MAPK, JNK and ERK1/2 (Fig. 2a). The H₂O₂-augmented phosphorylation of MAPK was inhibited by pretreatment with 10 mM NAC (Fig. 2a-d). Pretreatment with MAPK inhibitor, a p38 MAPK inhibitor, SB203580, a JNK inhibitor, SP600125, or an ERK1/2 inhibitor, U0126, inhibited the phosphorylation of p38-MAPK, JNK and ERK1/2 respectively (Additional file 1: Figure S1A-C) and dose-dependently inhibited the H₂O₂-augmented IL-33 mRNA

expression in the cells without affecting the cell viability (Fig. 2e-g). On the other hand, pretreatment with NF- κ B inhibitor (an IKK-2 inhibitor, SC-514, or an I κ B α inhibitor, BAY 11-7085) did not inhibit the H₂O₂-augmented IL-33 expression (Additional file 1: Figure S2A-B).

Effect of H₂O₂ on a synthetic viral dsRNA analogue, poly (I:C)- or viral-potentiated IL-33 expression

Rhinovirus infection is thought to be a key factor and an important modulator of COPD exacerbations [35, 36]. In our previous study, we confirmed that TLR3 was expressed in NCI-H292 cells, and poly (I:C), which is a synthetic viral dsRNA analogue and a TLR3 ligand to mimic viral infection, activated TLR3 [26, 27]. To determine whether oxidative stress augments IL-33 expression during viral infection, we investigated the effect of H₂O₂ on the expression of IL-33 in poly (I:C)-treated NCI-H292 cells. H₂O₂ slightly but significantly augmented the expression of IL-33 in the poly (I:C)-treated cells (Fig. 3a). The augmentation was significantly inhibited by 10 mM NAC treatment (Fig. 3b). To confirm that the augmented effect observed with poly (I:C) could be replicated by a virus, we performed further experiments using human rhinovirus, RV14. RV14 infection slightly but significantly increased the expression of IL-33 in NCI-H292 cells after 4 h RV14 treatment (Fig. 4a-b). In the experimental protocol for viral infection, 200 μ M H₂O₂ was added two times to medium 30 min prior to RV14 treatment and after 90 min incubation with RV14 to remove the RV14 from the supernatant and replace the medium. In this condition, H₂O₂-potentiated IL-33 mRNA expression reached a peak at 24 h, but not at 4 h, at which point H₂O₂ single treatment caused the peak in Fig. 1c (Fig. 4a). Treatment with H₂O₂ potentiated the expression of IL-33 in the RV14-infected cells and the H₂O₂-potentiated IL-33 expression reached a peak at 24 h (Fig. 4a). After 24 h RV14 infection, H₂O₂ significantly augmented IL-33 expression in RV14-infected cells (Fig. 4b). In addition, the augmentation was significantly inhibited by 10 mM NAC treatment (Fig. 4c).

Involvement of oxidative stress in the expression of IL-33 in HBECs from COPD patients

In HBECs from healthy subjects, H₂O₂ also significantly increased the expression of IL-33 and the H₂O₂-augmented IL-33 expression was inhibited by pretreatment with 10 mM NAC (Fig. 5a). To clarify the involvement of oxidative stress in the expression of IL-33 in the airway epithelial cells from COPD patients, we examined the effect of an antioxidant, NAC on the expression of IL-33 in HBECs from healthy subjects and age-matched COPD patients. Under the condition without H₂O₂-stimulation, pretreatment with 10 mM NAC significantly decreased the expression of IL-33 in HBECs from COPD

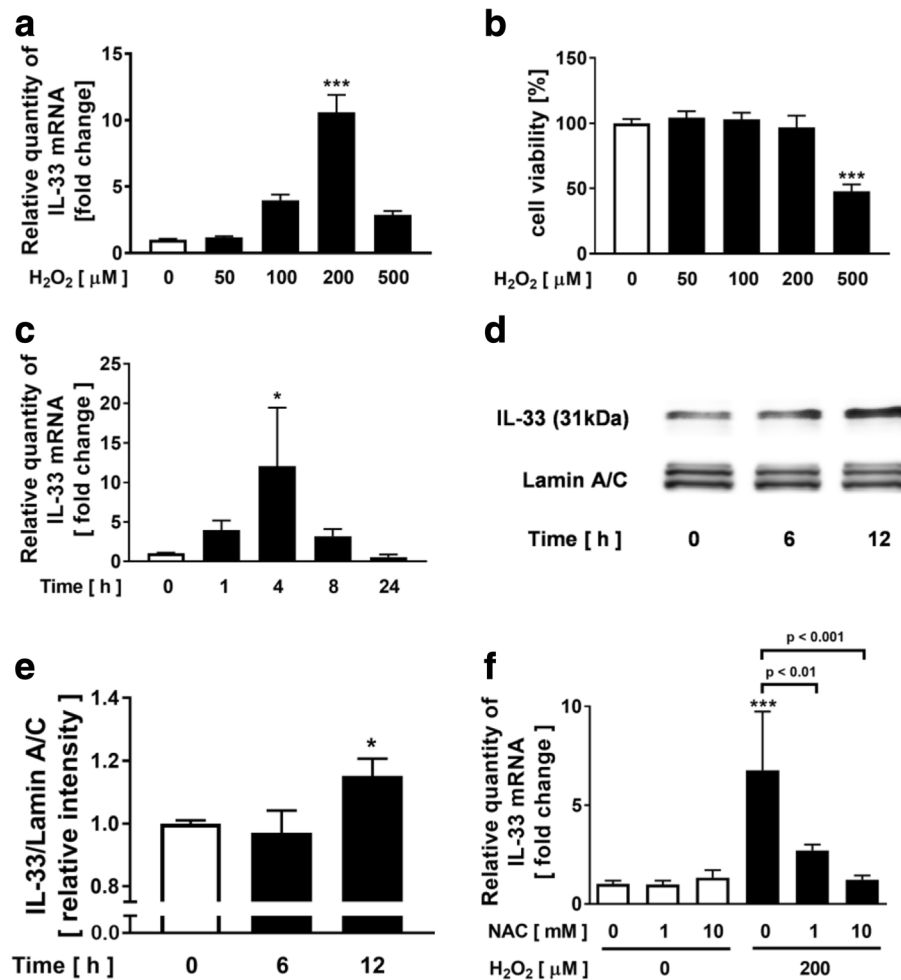


Fig. 1 Effect of H₂O₂ on the expression of Interleukin-33 (IL-33) in NCI-H292 cells. **a-c** Effect of H₂O₂ on the gene expression of IL-33 and the cell viability in NCI-H292 cells. **a, b** Cells were treated with various concentration of H₂O₂. After 4 h incubation, whole cells were harvested and assayed for the gene expression of IL-33 (**a**) and cell viability (**b**). **c** Cells were treated with 200 μM H₂O₂. At various time points after the incubation, whole cells were harvested and assayed. **d, e** Evaluation of nuclear fraction of IL-33 protein. At various time points after the treatment with 200 μM H₂O₂, the nuclear fraction of cell lysates was obtained. **d** IL-33 protein in the nuclear fraction was evaluated by immunoblotting. **e** Each band intensity was assessed by densitometry. Relative intensity was calculated by dividing the IL-33 band intensity by each appropriate lamin A/C band intensity. **f** Effect of *N*-acetylcysteine (NAC) on H₂O₂-augmented IL-33 expression. Values are the mean ± SEM (*n* = 3 - 5). **p* < 0.05, ****p* < 0.001 compared with the values of vehicle-treated cells

patients, but not in those from healthy subjects (Fig. 5b). On the other hand, concerning the basal expression of IL-33 in the airway epithelial cells, there was no increase in the expression of IL-33 in HBECs from COPD patients compared to those from healthy subjects (data not shown). In addition, to clarify the involvement of MAPK signaling pathways in the expression of IL-33 in HBECs from COPD patients, we examined the effect of MAPK inhibitor, a p38 MAPK inhibitor, SB203580, a JNK inhibitor, SP600125 and an ERK1/2 inhibitor, U0126, on the expression of IL-33 in HBECs from COPD patients. Under the condition without H₂O₂-stimulation, pretreatment with SB203580, SP600125 or U0126, significantly decreased the expression of IL-33 in HBECs from COPD patients (Fig. 5c-e).

Cell viability

The effects of the treatments on cell viability were assessed with the MTT assay in NCI-H292 cells and with the LDH assay in HBECs. Cell viability in the NCI-H292 cells was more than 77.2% after the treatment with H₂O₂, excluding the concentration at 500 μM, poly (I:C), rhinovirus infection and the inhibitors. In the HBECs, cell viability was more than 79.8% after the treatment with H₂O₂ and NAC.

Discussion

In the current study, we demonstrated that H₂O₂ potentiated the expression of IL-33 in NCI-H292 cells and primary HBECs and clarified the involvement of MAPK signal pathway by showing that inhibition of p38 MAPK,

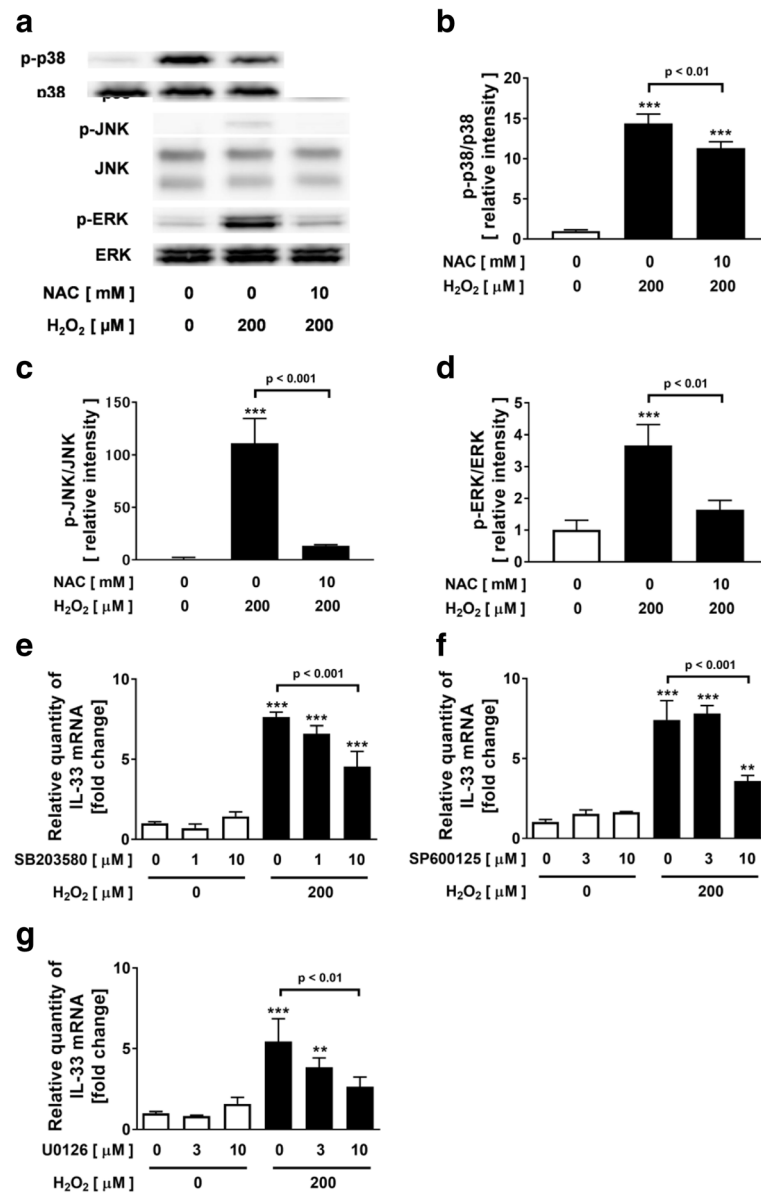


Fig. 2 Involvement of mitogen-activated protein kinase signaling pathways in H₂O₂-potentiated IL-33 expression. **a-d** Effect of H₂O₂ on the phosphorylation of MAPK (p38, JNK, ERK1/2) in NCI-H292 cells. Cells were treated with NAC or vehicle prior to treatment with H₂O₂. After 15 min, whole cell lysates were obtained. **a** Phosphorylation of p38, JNK or ERK was evaluated with immunoblotting. **b-d** Band intensities were assessed by densitometry. Relative intensity was calculated by dividing the phosphorylated p38, JNK or ERK1/2 band intensity by the p38, JNK or ERK1/2 band intensity and the results were indicated as fold change over control. **e-g** Effect of p38-MAPK inhibitor (SB203580) (**e**), JNK inhibitor (SP600125) (**f**) or ERK inhibitor (U0126) (**g**) on H₂O₂-potentiated IL-33 expression in NCI-H292 cells. Values are the mean ± SEM (n = 3). **p < 0.01, ***p < 0.001 compared with the values of vehicle-treated cells

JNK or ERK1/2 reversed the H₂O₂-augmented IL-33 expression. We also showed that pretreatment with H₂O₂ augmented the expression of IL-33 in a TLR3 ligand, poly (I:C)-treated cells and rhinovirus-infected cells. These results suggested that oxidative stress is involved in the expression of IL-33 in airway epithelial cells via MAPK signal pathway and the expression of IL-33 could be further increased in the airway epithelial cells during virus

infections, which is a main cause of COPD exacerbations (Fig. 6). In addition, we demonstrated that NAC treatment decreased the expression of IL-33 in HBECs from COPD patients, which suggested that oxidative stress was involved in the expression of IL-33 in HBECs from these patients. Although a previous study suggested the possible involvement of oxidative stress in the regulation of IL-33 production in an allergic mouse model [24], here, we

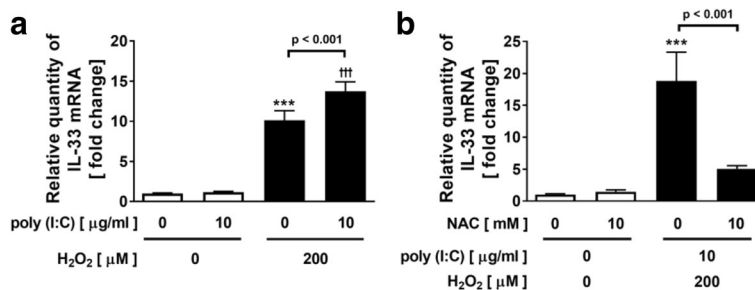


Fig. 3 Effect of H₂O₂ on a synthetic viral dsRNA analogue, poly (I:C)-potentiated IL-33 expression. **a** NCI-H292 cells were treated with H₂O₂ or vehicle 30 min prior to the treatment with poly (I:C). After 4 h, whole cells were harvested and assayed for the IL-33 gene expression. **b** Effect of NAC on H₂O₂-augmented IL-33 expression in poly (I:C)-treated cells. Cells were added with NAC before H₂O₂ treatment, and then treated with poly (I:C). Values are the mean ± SEM (n = 3 - 4). ***p < 0.001 compared with the values of vehicle-treated control. ###p < 0.001 compared with the values of poly (I:C)-treated control

demonstrated that exogenous H₂O₂ treatment potentiates the expression of IL-33 in human airway epithelial cells via MAPK signaling. In addition, we demonstrated the possibility that oxidative stress augments the expression of IL-33 during viral infection and is involved in the

expression of IL-33 in HBECs from COPD patients. Modulation of this pathway could be a therapeutic target for viral-induced exacerbations of COPD.

In the present study, we showed that exogenous H₂O₂ treatment potentiates the expression of IL-33 in NCI-

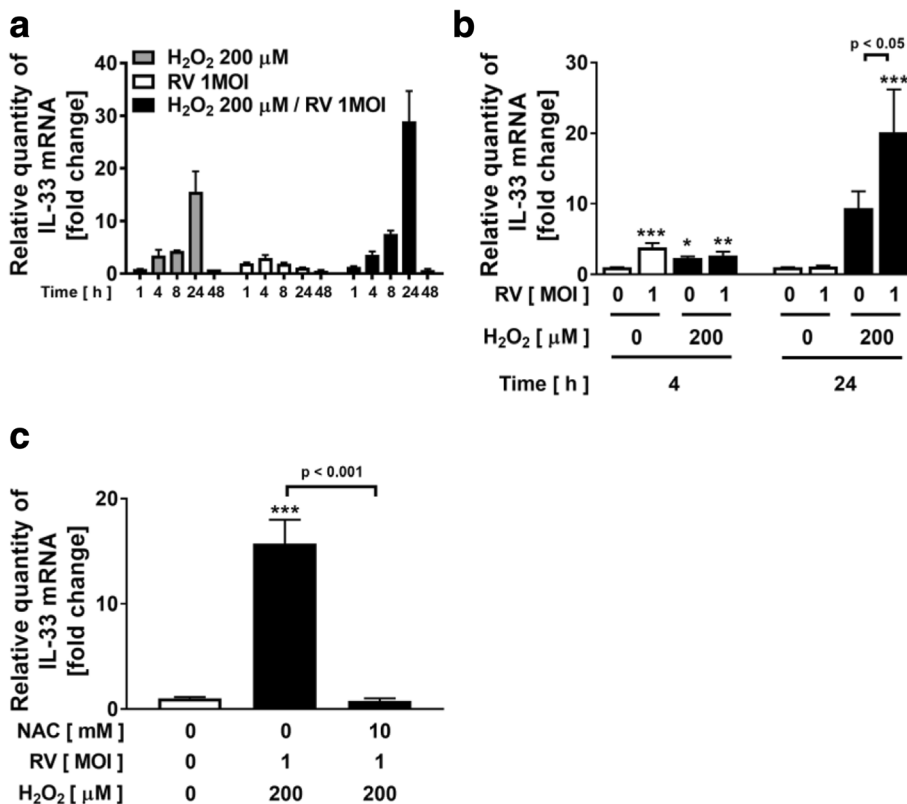


Fig. 4 Effect of H₂O₂ on viral-potentiated IL-33 expression. Time course **(a)** or comparison at 4 and 24 h **(b)** of IL-33 expression after treatment with H₂O₂ and human rhinovirus, RV14 infection. NCI-H292 cells were infected with RV14 in the presence of H₂O₂ or vehicle. At various time points after incubation **(a)** or after 4 and 24 h **(b)**, whole cells were harvested and assayed for the IL-33 gene expression. **c** Effect of NAC on H₂O₂-augmented IL-33 expression in RV14-treated cells. Cells were added with NAC before H₂O₂ treatment, and then infected with RV14. After 24 h RV14 infection, whole cells were harvested and assayed. Values are the mean ± SEM (n = 2 - 5). *p < 0.05, **p < 0.01, ***p < 0.001 compared with the values of vehicle-treated cells

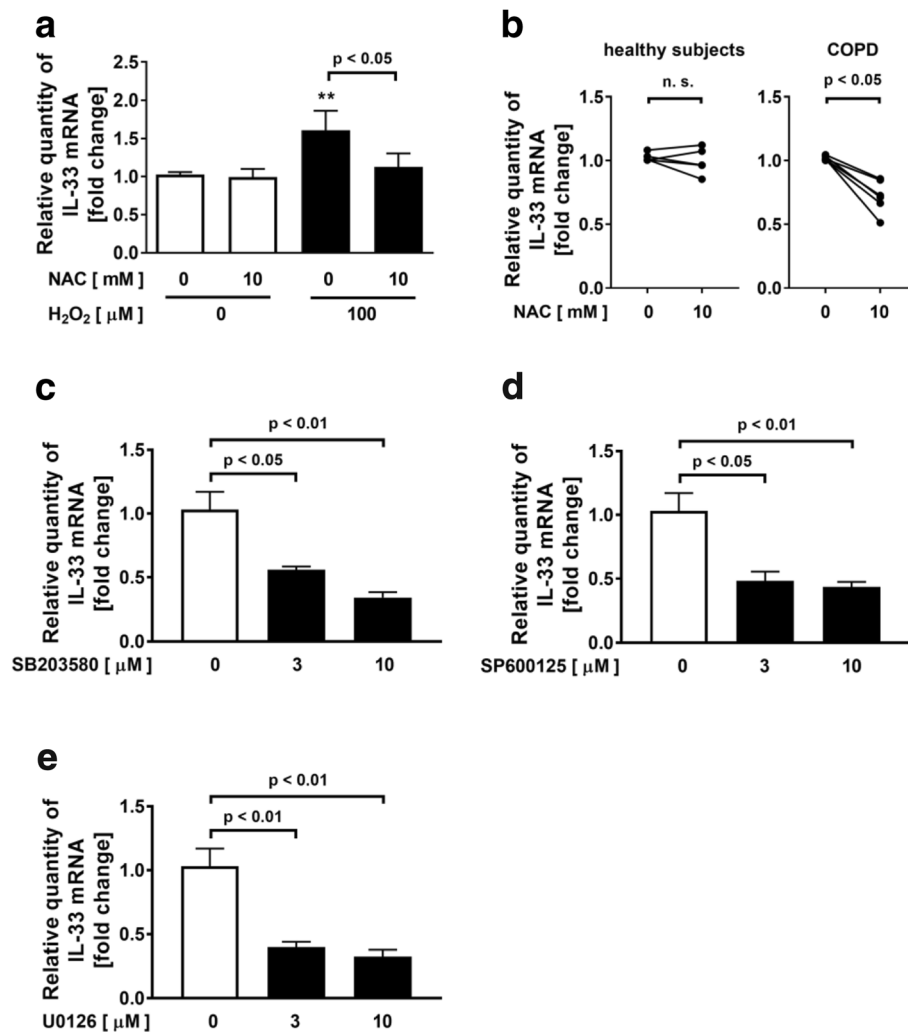
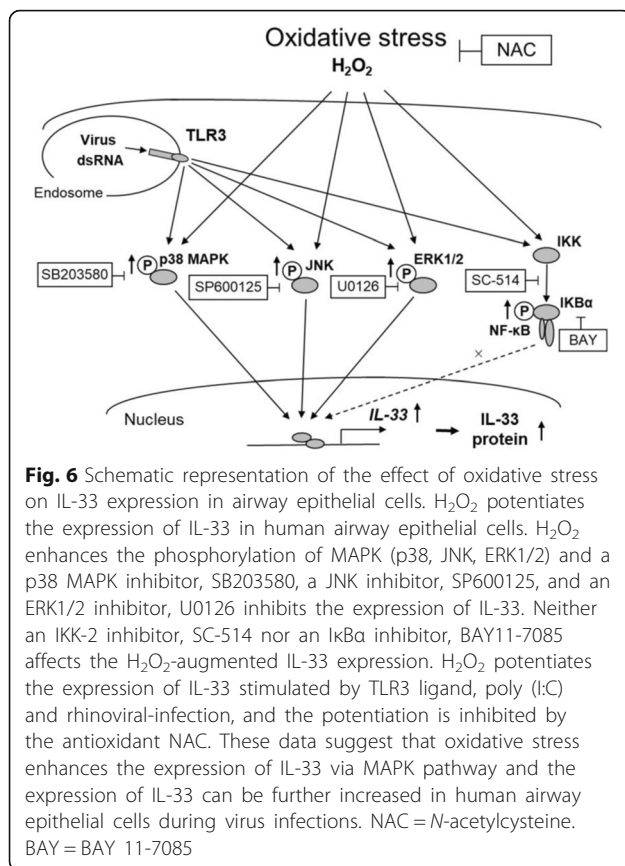


Fig. 5 Involvement of oxidative stress in the expression of IL-33 in HBECs from COPD patients. **a** Effect of H₂O₂-potentiated IL-33 expression and reversal by NAC in human bronchial epithelial cells (HBECs) from healthy subjects. Cells were treated with NAC or vehicle prior to treatment with H₂O₂. After 4 h, whole cells were harvested and assayed for the IL-33 gene expression. **b** Effect of NAC on the expression of IL-33 in HBECs from healthy subjects and COPD patients. Cells were treated with NAC or vehicle. After 4 h, whole cells were harvested and assayed. The data are expressed as the mean ± SEM of five healthy subjects or six COPD patients. **c-e** Effect of p38-MAPK inhibitor (SB203580), JNK inhibitor (SP600125) or ERK inhibitor (U0126) on the expression of IL-33 in HBECs from COPD patients. Cells were treated with the MAPK inhibitor or vehicle. After 4 h, whole cells were harvested and assayed. The data is a representative of two independent experiments with four samples performed using HBECs from two COPD patients. **p < 0.01 compared with the values of vehicle-treated cells

H292 cells and primary HBECs and the potentiation is reversed by treatment with an antioxidant, NAC. In addition, we demonstrated that NAC treatment decreased the expression of IL-33 in HBECs from COPD patients, but not in those from healthy subjects. These results suggest that oxidative stress is involved in the expression of IL-33 in airway epithelial cells. Previously, IL-33 has been reported to be upregulated in human airway epithelial cells by cigarette smoke exposure [11, 37]. As cigarette smoke contains ROS including H₂O₂ [38, 39], cigarette smoke-potentiated IL-33 expression is consistent with the current findings. In the present study, we also demonstrated that oxidative stress potentiates the amount of IL-

33 protein in the nucleus in airway epithelial cells. In a previous study, Uchida M, et al. demonstrated that an antioxidant, NAC or GSH, inhibits the *alternaria* extract-augmented IL-33 release in an allergic mice model, and they also demonstrated that NAC or GSH prevents extracellular secretion of IL-33 from IL-33-overexpressed human bronchial epithelial cells exposed to *alternaria* extract [24]. These reports support our current findings that the expression of IL-33 is regulated by oxidative stress.

H₂O₂ has been reported to activate not only the MAPK signal pathway, but also the NF-κB signal pathway in various cells including airway epithelial cells [23,



28, 34]. In the present study, we clarified the involvement of MAPK signal pathway in the mechanism of H_2O_2 -augmented IL-33 expression in airway epithelial cells by showing the inhibitory effect of p38 MAPK, JNK or ERK1/2 inhibitors. In addition, we demonstrated that these inhibitors also decreased the expression of IL-33 in HBECs from COPD patients, which suggest that MAPK signaling is involved in the regulation of IL-33 expression in airway epithelial cells in COPD. Using murine alveolar epithelial cells or macrophage cells, the involvement of the MAPK signal pathway has been demonstrated in the IL-6 group of cytokines, oncostatin M- or respiratory syncytial virus infection-augmented IL-33 expression and production [40, 41]. These reports support our current results. On the other hand, in our present study, pretreatment with NF- κ B inhibitors (an IKK-2 inhibitor, SC-514, or an I κ B α inhibitor, BAY 11-7085) did not inhibit the H_2O_2 -augmented IL-33 expression, suggesting that the NF- κ B signal pathway is not involved in the mechanism of H_2O_2 -augmented IL-33 expression in airway epithelial cells. In murine mast cells, I κ B- α inhibitor (BAY11-7085) did not also inhibit immunoglobulin E (IgE) activation-induced IL-33 expression [42], which is consistent with our results. However, in human corneal epithelial cells, cardiac cells or murine bone marrow-derived dendritic cells, I κ B- α

inhibitor (BAY11-7082) and NF- κ B inhibitor (quinazoline or dimethylfumarate) was reported to block TLR ligands-, tumor necrosis factor alpha (TNF- α) or ragweed pollen-potentiated IL-33 expression and production [43–45]. These reports are inconsistent with our present results. This discrepancy might result from differences in the stimulating substance, in the cell types used or in the experimental conditions including the inhibitor used.

In the present study, we showed that combination treatment of H_2O_2 and a synthetic viral dsRNA analogue and a TLR3 ligand, poly (I:C) or rhinovirus-infection significantly increased the expression of IL-33 compared with each treatment alone. Although the molecular mechanisms by which H_2O_2 potentiates poly (I:C)- or rhinovirus-induced IL-33 expression remain uncertain, previous reports have proposed possible mechanisms. TLR3 stimulation or rhinovirus infection has been demonstrated to increase the expression of IL-33 in human airway and corneal epithelial cells, and smooth muscle cells [43, 46, 47]. In addition, the MAPK signal pathway has been reported to be activated by TLR3 stimulation or rhinovirus infection in human airway epithelial cells [28, 48, 49]. In our previous studies, we demonstrated that oxidative stress enhances the expression of TLR3, and that cigarette smoke potentiates TLR3-ERK signal pathway in human airway epithelial cells [23, 27]. These results suggest that the MAPK signal pathway is involved in the H_2O_2 -potentiated IL-33 expression induced by poly (I:C) or rhinovirus infection. Recently, Hristova M, et al. have reported that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase homolog, dual oxidase 1 (DUOX1)-derived H_2O_2 , is involved in the *alternaria*-induced IL-33 release in airway epithelial cells. Activated DUOX1-induced H_2O_2 has been shown to stimulate A disintegrin and metalloproteinase 17 (ADAM17), which is responsible for the shedding of EGFR pro-ligand and the activation, and leads to the activation of the EGFR-ERK signal pathway [50]. These reports may suggest that oxidative stress augments the rhinovirus-stimulated IL-33 expression via TLR3-EGFR signal pathway. Further studies are necessary to prove this hypothesis.

In the comparison of the expression of IL-33 in the disease status of COPD, there was no increase in the expression of IL-33 in HBECs from COPD patients compared to HBECs from healthy subjects, which is inconsistent with a result previously reported that higher expression of IL-33 appeared in the airway epithelial cells from patients with COPD [10]. However, this higher expression of IL-33 is shown in severe COPD in GOLD stage IV. Another group has also reported that an increase of the IL-33 protein level in the whole lung appears only from severe COPD in GOLD stage III / IV [11]. In our current study, all of the patients evaluated were mild COPD in GOLD stage I-II, which might

explain the discrepancy. In the current study, we also showed that NAC treatment decreased the expression of IL-33 in HBECs from COPD patients, but not in those from healthy subjects, suggesting that oxidative stress is involved in the expression of IL-33 in airway epithelial cells in patients with COPD. We have previously reported an increased level of oxidative stress in HBECs of COPD patients compared to those in healthy subjects [51] and, therefore, it could be thought that NAC treatment decreased the increased level of oxidative stress in the HBECs of COPD patients, which affects the expression of IL-33. However, there remains the discrepancy that an increased level of oxidative stress is suggested in the HBECs from COPD patients and NAC treatment decreased the IL-33 expression from the basal levels only in COPD patients, but there is no increase in the expression of IL-33 in the HBECs from COPD patients compared to healthy subjects. Several negative regulations of IL-33 expression have been demonstrated, such as by lipopolysaccharide treatment via TLR signaling in NCI-H292 cells, or by one of short noncoding RNAs, microRNA-487b in human monocytes [52, 53]. These unknown negative regulatory mechanisms might affect the expression of IL-33 in the airway epithelial cells of COPD patients. Further studies are needed to clarify this point.

As a limitation of the present study concerning HBECs, there is a possibility that sex differences and smoking status might affect the results because HBECs were derived from 5 female healthy subjects and 1 female COPD patient. In addition, all of the healthy subjects were non-smokers, whereas all of the COPD patients were ex-smokers. Also, in the experiments in HBECs from COPD patients and healthy subjects, the evaluation of IL-33 expression was performed by measuring the levels of mRNA expression and was not confirmed by examining the protein expression.

Conclusions

We demonstrated that oxidative stress is involved in the expression of IL-33 in airway epithelial cells via the MAPK signal pathway and that it augments IL-33 expression during viral infection. This mechanism may participate in the regulation of IL-33 expression in airway epithelial cells in COPD and in viral-induced exacerbations. Modulation of this pathway could be a therapeutic target for viral-induced exacerbations of COPD.

Additional file

Additional file 1: Figure S1. Effect of mitogen-activated protein kinase (MAPK) inhibitors in H₂O₂-potentiated phosphorylation of MAPK (p38, JNK, ERK1/2). **Figure S2.** Effect of NF-κB inhibitors in H₂O₂-potentiated IL-33 expression. (PDF 449 kb)

Abbreviations

ADAM17: A disintegrin and metalloproteinase 17; ANOVA: Analysis of variance; COPD: Chronic obstructive pulmonary disease; CXCL8: CXC motif chemokine ligand 8; DLCO: Diffusing capacity of the lung for carbon monoxide; dsRNA: Double-stranded RNA; DUOX1: Dual oxidase 1; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; FBS: Fetal bovine serum; FEV₁: Forced expiratory volume in one second; FVC: Forced vital capacity; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GOLD: The global initiative for chronic obstructive lung disease; GSH: Glutathione; H₂O₂: Hydrogen peroxide; HBECs: Human bronchial epithelial cells; HBSS: HANK's balanced salt solution; IgE: Immunoglobulin E; IL-33: Interleukin-33; JNK: c-jun N-terminal kinase; LDH: Lactate dehydrogenase; MAPK: Mitogen-activated protein kinase; MOI: Multiplicity of infection; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide; NAC: N-acetylcysteine; NADPH: Nicotinamide adenine dinucleotide phosphate; NF-κB: Nuclear factor-kappa B; PCR: Polymerase chain reaction; poly (I:C): Polyinosinic-polycytidylic acid; PVDF: PolyVinylidene DiFluoride; ROS: Reactive oxygen species; RV14: Type 14 rhinovirus; SDS-PAGE: Sodium dodecyl sulfate- polyacrylamide gels; SEM: Standard error of the mean; Th2: T helper 2 cell; TLRs: Toll-like receptors; TNF-α: Tumor necrosis factor alpha; VA: Alveolar volume

Acknowledgements

We thank Mr. Brent Bell for reading this manuscript.

Funding

This work was supported by Grant-in-Aid for Scientific Research (C) from Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number 16 K09569), Research Grant 2015 from Terumo Foundation for Life Sciences and Arts, and Research Grant 2016 from Novartis Pharma K.K.

Availability of data and materials

Source data and material will be made available upon reasonable request.

Authors' contributions

HA, AK, YS, SY and MY analyzed the data and HA and AK drafted the manuscript. HA, AK, HS and MI contributed to the conception and design of the original study and contributed substantially to the manuscript. YS, TN and MN assisted with the recruitment of patients and obtaining their informed consent. MY, TI, NF and YO assisted with technical advice and the interpretation of results. All authors approved the final version for publication.

Ethics approval and consent to participate

All experiments in the study were approved by ethics committee of Tohoku University Graduate School of Medicine and written informed consent was obtained from all participating patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing of interests.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details

¹Department of Respiratory Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan. ²Department of Advanced Preventive Medicine for Infectious Disease, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan. ³Department of Thoracic Surgery, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan.

Received: 30 November 2017 Accepted: 19 March 2018

Published online: 27 March 2018

References

- Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. *Lancet*. 2012;380:2095–128.
- Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax*. 2002;57:847–52.
- Kanner RE, Anthonisen NR, Connett JE. Lower respiratory illnesses promote FEV(1) decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease: results from the lung health study. *Am J Respir Crit Care Med*. 2001;164:358–64.
- Miravittles M, Murio C, Guerrero T, Gisbert R, et al. Pharmacoeconomic evaluation of acute exacerbations of chronic bronchitis and COPD. *Chest*. 2002;121:1449–55.
- Connors AF Jr, Dawson NV, Thomas C, Harrell FE Jr, Desbiens N, Fulkerson WJ, et al. Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (study to understand prognoses and preferences for outcomes and risks of treatments). *Am J Respir Crit Care Med*. 1996;154:959–67.
- Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol*. 2016;16:676–89.
- Molofsky AB, Savage AK, Locksley RM. Interleukin-33 in tissue homeostasis, injury, and inflammation. *Immunity*. 2015;42:1005–19.
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. 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.
- Préfontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol*. 2010;125:752–4.
- Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X, et al. Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. *J Clin Invest*. 2013;123:3967–82.
- Kearley J, Silver JS, Sanden C, Liu Z, Berlin AA, White N, et al. Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity*. 2015;42:566–79.
- Donovan C, Bourke JE, Vlahos R. Targeting the IL-33/IL-13 Axis for respiratory viral infections. *Trends Pharmacol Sci*. 2016;37:252–61.
- MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2005;2:50–60.
- Barnes PJ. Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev*. 2004;56:515–48.
- Ichinose M, Sugiura H, Yamagata S, Koarai A, Shirato K. Increase in reactive nitrogen species production in chronic obstructive pulmonary disease airways. *Am J Respir Crit Care Med*. 2000;162:701–6.
- Komaki Y, Sugiura H, Koarai A, Tomaki M, Ogawa H, Akita T, et al. Cytokine-mediated xanthine oxidase upregulation in chronic obstructive pulmonary disease's airways. *Pulm Pharmacol Ther*. 2005;18:297–302.
- Kostikas K, Papatheodorou G, Psathakis K, Panagou P, Loukides S. Oxidative stress in expired breath condensate of patients with COPD. *Chest*. 2003;124:1373–80.
- Rahman I. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. *Cell Biochem Biophys*. 2005;43:167–88.
- Dekhuijzen PN, Aben KK, Dekker I, Aarts LP, Wielders PL, van Herwaarden CL, et al. Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1996;154:813–6.
- Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti A, et al. Oxidative stress and airway inflammation in severe exacerbations of COPD. *Thorax*. 2005;60:293–300.
- DeForge LE, Preston AM, Takeuchi E, Kenney J, Boxer LA, Remick DG. Regulation of interleukin 8 gene expression by oxidant stress. *J Biol Chem*. 1993;268:25568–76.
- Yanagisawa S, Koarai A, Sugiura H, Ichikawa T, Kanda M, Tanaka R, et al. Oxidative stress augments toll-like receptor 8 mediated neutrophilic responses in healthy subjects. *Respir Res*. 2009;10:50.
- Koarai A, Sugiura H, Yanagisawa S, Ichikawa T, Minakata Y, Matsunaga K, et al. Oxidative stress enhances toll-like receptor 3 response to double-stranded RNA in airway epithelial cells. *Am J Respir Cell Mol Biol*. 2010;42:651–60.
- Uchida M, Anderson EL, Squillace DL, Patil N, Maniak PJ, Iijima K, et al. Oxidative stress serves as a key checkpoint for IL-33 release by airway epithelium. *Allergy*. 2017;72:1521–31.
- Global Initiative for Chronic Obstructive Lung Disease. 2017 Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease. <http://goldcopd.org/gold-2017-global-strategy-diagnosis-management-prevention-copd/>.
- Shishikura Y, Koarai A, Aizawa H, Yamaya M, Sugiura H, Watanabe M, et al. Extracellular ATP is involved in dsRNA-induced MUC5AC production via P2Y2R in human airway epithelium. *Respir Res*. 2016;17:121.
- Kanai K, Koarai A, Shishikura Y, Sugiura H, Ichikawa T, Kikuchi T, et al. Cigarette smoke augments MUC5AC production via the TLR3-EGFR pathway in airway epithelial cells. *Respir Investig*. 2015;53:137–48.
- Koarai A, Yanagisawa S, Sugiura H, Ichikawa T, Kikuchi T, Furukawa K, et al. 25-hydroxycholesterol enhances cytokine release and toll-like receptor 3 response in airway epithelial cells. *Respir Res*. 2012;13:63.
- Lusamba Kalonji N, Nomura K, Kawase T, Ota C, Kubo H, Sato T, et al. The non-antibiotic macrolide EM900 inhibits rhinovirus infection and cytokine production in human airway epithelial cells. *Physiol Rep*. 2015;3:e12557.
- Hewson CA, Haas JJ, Bartlett NW, Message SD, Laza-Stanca V, Kebabdzic T, et al. Rhinovirus induces MUC5AC in a human infection model and in vitro via NF-kappaB and EGFR pathways. *Eur Respir J*. 2010;36:1425–35.
- Unger BL, Ganesan S, Comstock AT, Faris AN, Hershenson MB, Sajjan US. Nod-like receptor X-1 is required for rhinovirus-induced barrier dysfunction in airway epithelial cells. *J Virol*. 2014;88:3705–18.
- Takeyama K, Dabbagh K, Jeong Shim J, Dao-Pick T, Ueki IF, Nadel JA. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. *J Immunol*. 2000;164:1546–52.
- Richter A, O'Donnell RA, Powell RM, Sanders MW, Holgate ST, Djukanović R, et al. Autocrine ligands for the epidermal growth factor receptor mediate interleukin-8 release from bronchial epithelial cells in response to cigarette smoke. *Am J Respir Cell Mol Biol*. 2002;27:85–90.
- Jang MK, Kim SH, Lee KY, Kim TB, Moon KA, Park CS, et al. The tyrosine phosphatase, SHP-1, is involved in bronchial mucin production during oxidative stress. *Biochem Biophys Res Commun*. 2010;393:137–43.
- Wilkinson TM, Hurst JR, Perera WR, Wilks M, Donaldson GC, Wedzicha JA. Effect of interactions between lower airway bacterial and rhinoviral infection in exacerbations of COPD. *Chest*. 2006;129:317–24.
- Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med*. 2006;173:1114–21.
- Pace E, Di Sano C, Sciarriano S, Scafidi V, Ferraro M, Chiappara G, et al. Cigarette smoke alters IL-33 expression and release in airway epithelial cells. *Biochim Biophys Acta*. 2014;1842:1630–7.
- Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect*. 1985;64:111–26.
- Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxyacetaldehyde, and peroxyacetyl nitrate. *Ann N Y Acad Sci*. 1993;686:12–27.
- Richards CD, Izakelian L, Dubey A, Zhang G, Wong S, Kwofie K, et al. Regulation of IL-33 by Oncostatin M in mouse lung epithelial cells. *Mediat Inflamm*. 2016;2016:9858374.
- Qi F, Bai S, Wang D, Xu L, Hu H, Zeng S, et al. Macrophages produce IL-33 by activating MAPK signaling pathway during RSV infection. *Mol Immunol*. 2017;87:284–92.
- Hsu CL, Bryce PJ. Inducible IL-33 expression by mast cells is regulated by a calcium-dependent pathway. *J Immunol*. 2012;189:3421–9.
- Zhang L, Lu R, Zhao G, Pflugfelder SC, Li DQ. TLR-mediated induction of pro-allergic cytokine IL-33 in ocular mucosal epithelium. *Int J Biochem Cell Biol*. 2011;43:1383–91.
- Demyanets S, Kaun C, Pentz R, Krychtiuk KA, Rauscher S, Pfaffenberger S, et al. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. *J Mol Cell Cardiol*. 2013;60:16–26.
- Su Z, Lin J, Lu F, Zhang X, Zhang L, Gandhi NB, et al. Potential autocrine regulation of interleukin-33/ST2 signaling of dendritic cells in allergic inflammation. *Mucosal Immunol*. 2013;6:921–30.

46. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, et al. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med*. 2014;190:1373–82.
47. Calvén J, Akbarshahi H, Menzel M, Ayata CK, Idzko M, Bjermer L, et al. Rhinoviral stimuli, epithelial factors and ATP signalling contribute to bronchial smooth muscle production of IL-33. *J Transl Med*. 2015;13:281.
48. Leigh R, Oyelusi W, Wiehler S, Koetzler R, Zaheer RS, Newton R, et al. Human rhinovirus infection enhances airway epithelial cell production of growth factors involved in airway remodeling. *J Allergy Clin Immunol*. 2008;121:1238–45.
49. Zaheer RS, Koetzler R, Holden NS, Wiehler S, Proud D. Selective transcriptional down-regulation of human rhinovirus-induced production of CXCL10 from airway epithelial cells via the MEK1 pathway. *J Immunol*. 2009;182:4854–64.
50. Hristova M, Habibovic A, Veith C, Janssen-Heininger YM, Dixon AE, Geiszt M, et al. Airway epithelial dual oxidase 1 mediates allergen-induced IL-33 secretion and activation of type 2 immune responses. *J Allergy Clin Immunol*. 2016;137:1545–56.
51. Numakura T, Sugiura H, Akaike T, Ida T, Fujii S, Koarai A, et al. Production of reactive persulfide species in chronic obstructive pulmonary disease. *Thorax*. 2017;72:1074–83.
52. Lin TH, Cheng CC, Su HH, Huang NC, Chen JJ, Kang HY, et al. Lipopolysaccharide attenuates induction of Proallergic cytokines, Thymic stromal Lymphopoietin, and interleukin 33 in respiratory epithelial cells stimulated with PolyI:C and human Parechovirus. *Front Immunol*. 2016;7:440.
53. Xiang Y, Evers F, Herbert C, Tay HL, Foster PS, Yang M. MicroRNA-487b is a negative regulator of macrophage activation by targeting IL-33 production. *J Immunol*. 2016;196:3421–8.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

