

ORIGINAL ARTICLE

Sirtuin 1 activator SRT1720 suppresses inflammation in an ovalbumin-induced mouse model of asthma

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ABSTRACT

Background and objective: In asthma, reduced histone deacetylase activity and enhanced histone acetyltransferase activity in the lungs have been reported. However, the precise function of Sirtuin 1 (Sirt1), a class III histone deacetylase, and the effect of the Sirt1 activator SRT1720 on allergic inflammation have not been fully elucidated.

Methods: The effect of SRT1720, a synthetic activator of Sirt1, in an ovalbumin (OVA)-induced asthma mouse model was investigated. The effect of SRT1720 and resveratrol on OVA stimulation in splenocytes from OVA-sensitized and challenged mice was also examined.

Results: In OVA-sensitized and challenged mice (OVA mice) compared with saline-sensitized and challenged mice (control mice), Sirt1 messenger RNA expression in the lungs was decreased ($P = 0.02$), while cellular infiltration, airway eosinophilia and bronchoalveolar lavage (BAL) fluid levels of interleukin (IL)-4, IL-5 and IL-13 were increased ($P < 0.01$). In OVA mice, SRT1720 treatment decreased total and eosinophil cell counts and IL-5 and IL-13 levels in the BAL fluid compared with the vehicle treatment ($P < 0.05$). In OVA mice, SRT1720 treatment also decreased inflammatory cell lung infiltrates histologically ($P = 0.002$). Both SRT1720 and resveratrol suppressed OVA-induced cell proliferation and IL-6 ($P < 0.05$) and tumour necrosis factor- α (TNF- α) ($P < 0.05$) production in splenocytes ($P < 0.01$).

Conclusions: The Sirt1 activator SRT1720 suppressed inflammatory cell infiltration and cytokine production in an OVA-induced mouse model of asthma. SRT1720 and resveratrol suppressed OVA-induced splenocyte proliferation and TNF- α and IL-6 production. Sirt1

SUMMARY AT A GLANCE

We investigated the effect of Sirt1 activator SRT1720 on an inflammatory model of asthma. SRT1720 suppressed inflammatory cell infiltration and cytokine production in an OVA-challenged mouse model. SRT1720 and resveratrol also directly suppressed OVA-induced splenocyte proliferation and TNF- α and IL-6 production. SRT1720 has potential as a therapeutic drug for asthma.

activators might have beneficial effects in asthmatics by suppressing inflammation.

Key words: cytokine, eosinophil, inflammation, Sirt1.

Abbreviations: BAL, bronchoalveolar lavage; IL, interleukin; OVA, ovalbumin; Sirt1, Sirtuin (silent mating type information regulation 2 homolog) 1; TNF- α , tumour necrosis factor- α .

INTRODUCTION

Asthma is a complex chronic inflammatory disorder of the airway that involves many cell types and cytokines.¹ Asthma affects 300 million people worldwide.¹ The high asthma prevalence were found in Scotland (18.4%), New Zealand (15.1%) and Australia (14.7%).¹ The current prevalence of asthma among Japanese adults is reported to be 4.2%.² While most asthmatics respond well to currently available treatments, 5–10% of patients exhibit severe disease.³ The effects of current therapies on airway remodeling remain to be elucidated.⁴ Although inhaled therapy is the cornerstone of asthma treatment, orally administered medications yield better rates of adherence,^{5,6} and therefore, could contribute to better asthma control.

Protein acetylation is a post-translational modification and an important transcriptional regulatory

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mechanism.⁷ It is controlled by the histone acetyltransferases and histone deacetylases. Reduced histone deacetylase and enhanced histone acetyltransferase activity has been reported in asthmatic patients.^{8,9} Two asthma drugs, glucocorticoids and theophylline, have been shown to downregulate the inflammatory response by modulating histone acetyltransferase and histone deacetylase activity.⁹

Sirtuin 1 (Sirt1) is a member of the Sirtuins which are class III histone deacetylases.¹⁰ The therapeutic potential of Sirt1 activators has been explored in several diseases such as type 2 diabetes,^{11–13} chronic obstructive pulmonary disease,^{14–16} cardiovascular disease¹⁷ and neurodegeneration.^{18,19} Resveratrol, a polyphenolic Sirt1 activator, suppresses asthmatic reaction in mouse model.²⁰ SRT1720 is a small molecule activator of Sirt1 that is more potent than resveratrol.¹¹ The effect of SRT1720 on allergic inflammation has not been fully elucidated.

The objective of this study was to investigate the effect of the oral administration of SRT1720 on airway inflammation in a mouse asthma model. We also studied the direct effects of SRT1720 and resveratrol *ex vivo*.

METHODS

Ovalbumin mouse model and SRT1720 treatment

To investigate the effects of SRT1720, we administered it before and during the challenge by oral gavage. Six- to eight-week-old female BALB/c mice (Sankyo Labo Service, Tokyo, Japan) were immunized with an intraperitoneal injection of 0.1 mg of OVA (albumin from chicken egg white, Grade V; Sigma-Aldrich, St. Louis, MO, USA) and 1 mg of aluminum hydroxide (alum; Wako Pure Chemical Industries, Osaka, Japan) on days 1 and 8. The control mice received saline instead of the OVA/alum solution. The mice were anaesthetized with sevoflurane (Maruishi Pharmaceutical, Osaka, Japan) and intranasally challenged with OVA (50 µg in 40 µL) or saline on days 24, 26 and 28. SRT1720 was synthesized according to a previously described protocol.^{11,21} The mice were treated with 100 mg/kg SRT1720 or water (vehicle) by oral gavage once daily from days 23 to 28. The mice were sacrificed on day 29, at which point samples were obtained. The experimental protocols were approved by the Ethical Committee for Animal Use and Care of the University of Toyama.

Reverse transcription and real-time quantitative polymerase chain reaction analysis

The mice were challenged with OVA or saline, as described earlier. The lungs were removed after bronchoalveolar lavage (BAL) and perfusion with phosphate buffer saline (–). Total RNA was isolated from whole lung samples using RNeasy Mini Kits (Qiagen, Tokyo, Japan). Total RNA was reverse-transcribed to complementary DNA using the PrimeScript RT

Reagent Kit (Takara Bio, Inc, Shiga, Japan). The real-time quantitative polymerase chain reaction analysis was performed using SYBR Premix Ex Taq II (Takara Bio, Inc) and Mx3000P (Stratagene, La Jolla, CA, USA). The oligonucleotide primers for glyceraldehyde 3-phosphate dehydrogenase were as follows: forward, 5'-TGAAGCAGGCATCTGAGGG-3' and reverse, 5'-CGAAGGTGGAAGAGTGGGAG-3'.²² The oligonucleotide primers for Sirt1 were as follows: forward, 5'-TTCCAGCCGTCTCTGTGTCA-3' and reverse, 5'-ATTCCTGC AACCTGCTCCAA-3'. The primers were constructed from the corresponding GenBank sequences using Primer3 and BLAST.

Bronchoalveolar lavage

The mice were euthanized and the trachea was cannulated. The lungs were lavaged with 1 mL of saline twice. The BAL samples were then centrifuged at 500 × g for 10 min. The number of cells was counted using a Burker-Turk haemocytometer. The remaining cells were centrifuged onto glass slides using a Cytospin 4 (Thermo Scientific, Kanagawa, Japan) and stained using Wright-Giemsa stain. At least 300 cells were counted in each sample.

Measurement of cytokine concentrations

We measured the concentrations of interleukin (IL)-4, IL-5 and IL-13 in the BAL as T-helper-type 2 cytokine levels and the interferon-γ levels as a T-helper-type 1 cytokine. We measured IL-6 and tumour necrosis factor-α (TNF-α) because Sirt1 has been reported to inhibit the production of IL-6 and TNF-α.^{21,23,24}

The concentrations of IL-4, IL-5, IL-6, TNF-α and interferon-γ were determined using Flowcytometer (Bender Medsystem, Vienna, Austria) and a FACS-Canto II (Nippon Becton, Dickinson and Company, Tokyo, Japan). The IL-13 enzyme-linked immunosorbent assay kits were obtained from Invitrogen (Carlsbad, CA, USA).

Lung histology

After BAL and perfusion with 10 mL of phosphate buffer saline (–) and 10 mL of 4% paraformaldehyde via the left ventricle, the lungs were removed and fixed in phosphate-buffered 4% paraformaldehyde. The paraffin-embedded lung tissues were then stained with haematoxylin and eosin or periodic acid-Schiff (PAS).

We assessed the level of inflammatory cell infiltration and goblet cell hyperplasia. A semi-quantitative scoring system was used to grade the extent of lung infiltration in the haematoxylin and eosin-stained sections, with +5 signifying widespread infiltrates around the majority of the vessels and bronchioles and +1 signifying a small number of inflammatory foci.²⁵ The mean of the results obtained by two investigators (T.I. and S.I.) was considered the final score. The goblet cell hyperplasia of the bronchus in the periodic acid-Schiff-stained sections was graded (0, less than 5% goblet cells in the airway epithelium; 1, 5–25% goblet cells; 2, 25–50% goblet cells; 3, 50–75%

goblet cells; 4, equal to or greater than 75% goblet cells) as previously described.²⁶

***In vitro* stimulation and treatment with Sirt1 activators**

The spleen was collected from OVA-sensitized and challenged mice. The spleens were homogenized and the red blood cells lysed using VersaLyse (Beckman Coulter/Immunotech, Marseille, France). The splenocytes were then cultured in a 96-well plate in 10% FCS-RPMI1620 at 37°C and 5% CO₂. The SRT1720 and resveratrol were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide was less than 0.1%. The splenocytes were stimulated with 100 µg/mL OVA and treated with SRT1720, resveratrol or the vehicle. Because the maximum plasma concentration of SRT1720 was reported to be approximately 1 µmol/L,¹¹ SRT1720 was used at a concentration equal to or less than 1 µmol/L. Resveratrol exerts biological effects at a wide range of concentrations (0.3 to 30 µmol/L) *in vivo*.²⁷

We investigated the effects of SRT1720 and resveratrol on cell proliferation and cytokine production in an *ex vivo* system. We used the bromodeoxyuridine proliferation test (2×10^5 cells per well). We added the bromodeoxyuridine (Roche, Basel, Swiss Confederation) during the last 24 h of a 72-h incubation period. Chemiluminescence was detected using GENios (Tecan Japan, Kanagawa, Japan). For the cytokine assays (2×10^5 cells per well), we collected supernatants after 72 h. The cytokine concentrations were evaluated as described in the *in vivo* study.

Statistical analysis

The results are expressed as the mean $\pm$ standard deviation. The between-group differences were determined using a two-way analysis of variance or repeated measures analysis of variance. We performed an unpaired *t*-test or a paired *t*-test to compare each group. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Lower Sirt1 levels are found in the lung homogenates of OVA-sensitized and challenged mice

The expression of Sirt1 messenger RNA was examined in the lungs of saline- or OVA-sensitized and challenged mice using real-time quantitative polymerase chain reaction analysis. The expression of Sirt1 messenger RNA was lower in the lung homogenates of the OVA-sensitized and challenged mice (OVA group) than the saline-sensitized and challenged mice (saline group; Fig. 1, *P* = 0.02).

SRT1720 treatment decreases the OVA-induced total and eosinophil cell infiltration and the IL-5 and IL-13 levels in the BAL fluid

The cell counts in the BAL fluid of the saline mice were not significantly different from the cell counts

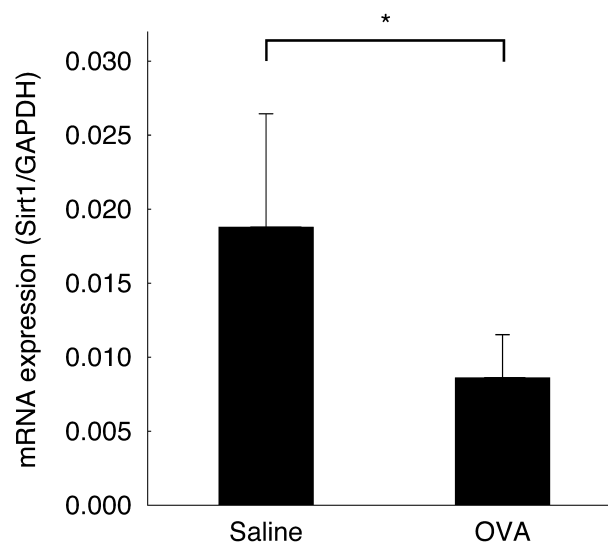


Figure 1 Sirtuin 1 (Sirt1) messenger RNA (mRNA) expression in the lung following ovalbumin (OVA) sensitization and challenge. The expression of Sirt1 mRNA in the total lung homogenates of saline-sensitized and challenged mice (saline) and OVA-sensitized and challenged mice (OVA) is shown. The Sirt1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels were measured using the real-time quantitative polymerase chain reaction. The Sirt1 expression levels were normalized to the GAPDH expression levels. Values are expressed as the mean $\pm$ standard deviation. **P* < 0.05 .

from the animals that were treated with SRT1720 (Saline + SRT1720) or vehicle alone (Saline + Vehicle; Fig. 2 and Table S1 in the online supporting information). OVA sensitization and challenge significantly affected the cell counts in the BAL, with the exception of neutrophils (lymphocytes, *P* < 0.05 ; all other cells, *P* < 0.01). SRT1720 treatment significantly affected the total number of cells and eosinophils (*P* < 0.01). The OVA mice treated with SRT1720 exhibited significantly fewer cells and eosinophils in the BAL than the OVA mice that were treated with vehicle alone (Fig. 2; OVA +SRT1720 vs OVA +Saline; *P* = 0.034 and *P* = 0.020, respectively).

We measured the concentrations of IL-4, IL-5 and IL-13 in the BAL (Fig. 3). OVA and SRT1720 significantly affected the concentrations of these cytokines (*P* < 0.01). The levels of IL-4, IL-5 and IL-13 in the BAL of OVA mice (OVA + Vehicle and OVA + SRT1720) were significantly higher than those in the saline mice treated with vehicle alone (Saline + Vehicle, *P* < 0.01 , Fig. 3). The OVA mice that were treated with SRT1720 exhibited significantly lower levels of IL-5 and IL-13 in the BAL compared with the OVA mice that were treated with vehicle alone (OVA + Vehicle vs OVA + SRT1720; *P* = 0.026, *P* = 0.001, respectively; Fig. 3b,c). We did not observe a significant difference in the IL-4 levels in the BAL of SRT1720-treated mice (OVA + SRT1720) and vehicle-treated mice (OVA + Saline). The interferon- γ levels were not significantly different between the groups (data not shown). We were unable to detect TNF- α and IL-6 in the BAL.

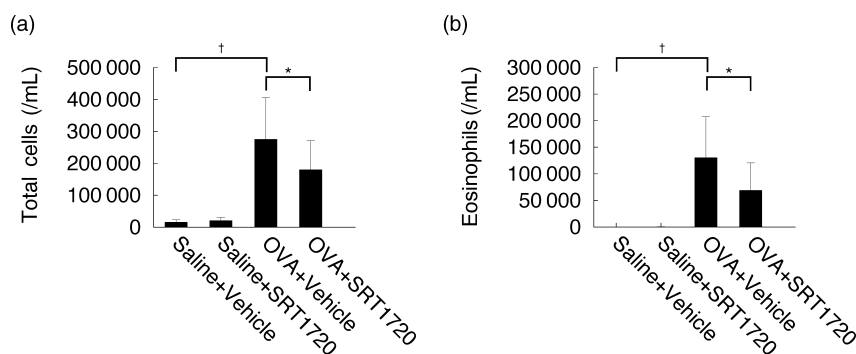


Figure 2 The total number of cells and eosinophils in the bronchoalveolar lavage (BAL) fluid. Mice were sensitized and challenged with ovalbumin (OVA) or saline and treated with SRT1720 or vehicle. The total number of (a) cells and (b) eosinophils in the BAL was determined. The number of neutrophils, macrophages and lymphocytes is shown in Table S1 in the online supporting information. Values are expressed as the mean $\pm$ standard deviation. Saline + Vehicle, saline-sensitized and challenged group receiving vehicle ($n = 6$); Saline + SRT1720, saline-sensitized and challenged group receiving SRT1720 ($n = 6$); OVA + Vehicle, OVA-sensitized and challenged group receiving vehicle ($n = 12$); OVA + SRT1720, OVA-sensitized and challenged group receiving SRT1720 ($n = 12$). The two-way analysis of variance indicates that OVA challenge ($P < 0.05$) and SRT1720 treatment ($P < 0.01$) significantly affected the total number of cells and eosinophils. The interaction was significant for the total number of cells ($P < 0.01$). Using Student's *t*-test, * $P < 0.05$ and † $P < 0.01$.

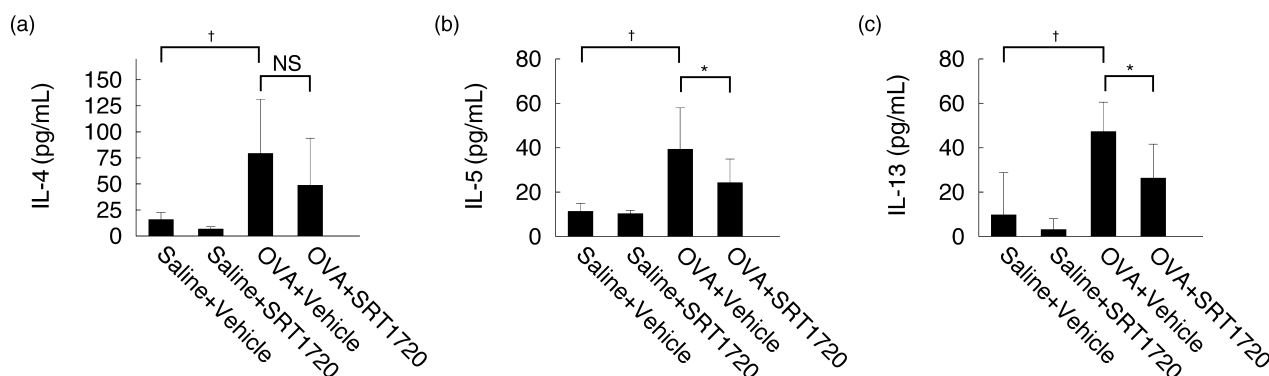


Figure 3 The interleukin (IL)-4, IL-5 and IL-13 levels in the bronchoalveolar lavage (BAL). The mice were sensitized and challenged with ovalbumin (OVA) or saline and treated with SRT1720 or vehicle. The (a) IL-4, (b) IL-5 and (c) IL-13 concentrations in the BAL samples were measured using a multiplexed fluorescent bead-based immunoassay and enzyme-linked immunosorbent assay. Values are expressed as the mean $\pm$ standard deviation. Saline + Vehicle, saline-sensitized and challenged group receiving vehicle ($n = 6$); Saline + SRT1720, saline-sensitized and challenged group receiving SRT1720 ($n = 6$); OVA + Vehicle, OVA-sensitized and challenged group receiving vehicle ($n = 12$); OVA + SRT1720, OVA-sensitized and challenged group receiving SRT1720 ($n = 12$). A two-way analysis of variance indicated that OVA challenge, SRT1720 treatment and their interaction did not significantly affect the IL-4 levels. OVA challenge ($P < 0.05$) and SRT1720 treatment ($P < 0.05$) had significant effects on the IL-5 and IL-13 levels. The interaction between OVA challenge and SRT1720 treatment did not significantly affect the IL-5 and IL-13 levels. Using Student's *t*-test, * $P < 0.05$ and † $P < 0.01$. NS, no significant difference.

SRT1720 treatment suppresses inflammatory cell infiltration into the lungs

OVA sensitization and challenge significantly increased the inflammatory infiltration and goblet cell hyperplasia in histological examination (Fig. 4g,h; two-way analysis of variance, $P < 0.01$). Treating the saline mice with SRT1720 did not cause inflammatory cell infiltration and goblet cell hyperplasia. SRT1720 treatment significantly reduced the inflammatory infiltration (two-way analysis of variance, $P < 0.01$). The OVA mice that were treated with SRT1720 exhibited suppressed inflammatory cell infiltration compared with the OVA mice treated with vehicle alone (OVA + Saline vs OVA + SRT1720; $P = 0.002$; Fig. 4j).

SRT1720 treatment did not significantly affect the extent of goblet cell hyperplasia (OVA + Saline vs OVA + SRT1720; Fig. 4h).

The effect of SRT1720 and resveratrol on OVA-stimulated splenocytes from OVA-sensitized and challenged mice

We investigated the effects of SRT1720 and resveratrol on cell proliferation and cytokine production in an *ex vivo* system. SRT1720 and resveratrol suppressed the OVA-induced proliferation of splenocytes (Fig. 5).

SRT1720 did not suppress the *ex vivo* production of IL-4 or IL-5 (Fig. 6a,b), and the interferon- γ levels did

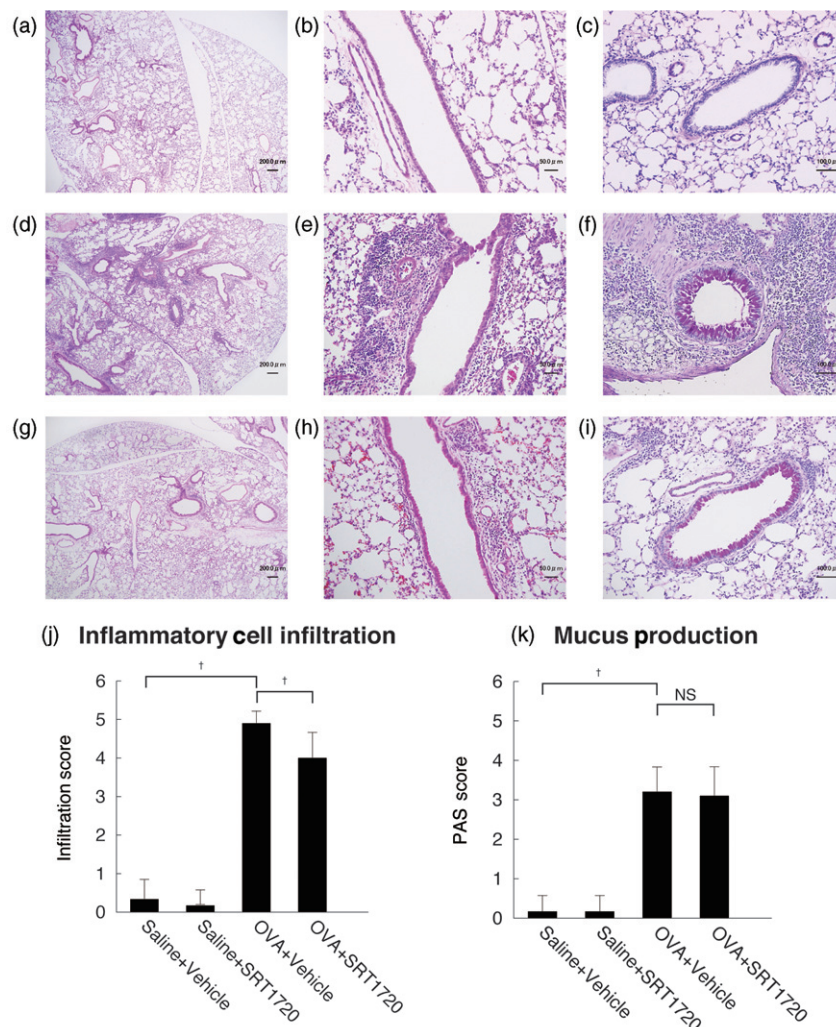


Figure 4 Histological analysis of lung sections. The mice were sensitized and challenged with either ovalbumin (OVA) or saline and treated with SRT1720 or vehicle alone. Representative findings from the (a–c) saline-sensitized and challenged mice receiving vehicle (Saline + Vehicle) and the (d–f) OVA-sensitized and challenged mice receiving vehicle (OVA + Vehicle) or (g–i) SRT1720 (OVA + SRT1720) are shown. Haematoxylin and eosin (HE)-stained sections are shown in (a), (b), (d), (e), (g) and (h). Periodic acid-Schiff (PAS)-stained sections are shown in (c), (f) and (i). A lower magnification (20×) is depicted in (a), (d) and (g) and a higher magnification (100×) is depicted in (b), (c), (e), (f), (h) and (i). The scale bars are 200 μm in (a), (d) and (g); 50 μm in (b), (e) and (h); and 100 μm in (c), (f) and (i), respectively. To evaluate the infiltration and mucus production, HE- and PAS-stained sections were semi-quantitatively scored as described in the Methods section (j,k). The most severe changes were +5 in the inflammation evaluation and +4 in the goblet cell hyperplasia evaluation. Saline + Vehicle, saline-sensitized and challenged group receiving vehicle ($n = 6$); Saline + SRT1720, saline-sensitized and challenged group receiving SRT1720 ($n = 6$); OVA + Vehicle, OVA-sensitized and challenged group receiving vehicle ($n = 10$); OVA + SRT1720, OVA-sensitized and challenged group receiving SRT1720 ($n = 10$). Values are expressed as the mean $\pm$ standard deviation. The two-way analysis of variance indicates that OVA challenge and SRT1720 treatment and their interaction had a significant effect on cellular infiltration ($P < 0.01$). OVA challenge, SRT1720 treatment and their interaction did not have a significant effect on goblet cell hyperplasia. Using Student's *t*-test, † $P < 0.01$. NS, no significant difference.

not differ significantly between the treatments (data not shown). However, SRT1720 and resveratrol did suppress the production of IL-6 and TNF- α by splenocytes (Fig. 6c,d).

DISCUSSION

We demonstrated that the Sirt1 activator SRT1720 suppressed the production of IL-5 and IL-13, but not IL-4, in an OVA-induced mouse model of asthma.

SRT1720 also decreased the eosinophilic inflammatory cell infiltration, but did not decrease the extent of goblet cell hyperplasia. Consistent with our results, Lee *et al.* reported that resveratrol reversed the increased levels of T-helper-type 2 cytokines, such as IL-4 and IL-5, found in the plasma and BAL fluid of a mouse model of asthma.²⁰

The decreased IL-5 and IL-13 levels found in our study may explain the inhibition of eosinophilic inflammatory cell infiltration. A recent study found that anti-IL-5 or anti-IL-13 therapy benefits some

asthmatic patients.^{28,29} Those patients receiving the IL-5 antibody reslizumab showed reductions in sputum eosinophils and improvements in airway function. Treatment with lebrikizumab, a monoclonal antibody to IL-13, was associated with improved lung function. Airway remodeling was not addressed in either study. A surprising result was that while SRT1720 suppress IL-5 and IL-13 levels, it does not

decrease goblet cell hyperplasia. A possible explanation is that the suppression of cytokines was not sufficient to decrease the extent of goblet cell hyperplasia. Although goblet cell hyperplasia was not significantly different in the different treatment groups, the epithelial cell height might be smaller in the SRT1720-treated mice than the saline-treated mice (Fig. 4f,i). The absence of IL-4 suppression might be another reason for goblet cell hyperplasia. IL-4, IL-5 and IL-9 reportedly contribute to goblet cell hyperplasia and eosinophilic inflammation, while IL-13 is primarily responsible for airway hypersensitivity, airway smooth muscle hyperplasia.³⁰ The mechanism underlying the T-helper-type 2 cytokine-mediated induction of mucus production or goblet cell hyperplasia is poorly understood.³¹

We investigated the effects of Sirt1 activators on cell proliferation and cytokine production *ex vivo*. SRT1720 and resveratrol directly suppressed the OVA-induced TNF- α and IL-6 production and proliferation observed in splenocytes. SRT1720 is reportedly more potent than resveratrol in the concentration of compound required to increase enzyme activity.¹¹ In our study, the suppressive effect of SRT1720 in the OVA-induced TNF- α and IL-6 production and proliferation observed in splenocytes was at best equal to resveratrol.

Sirt1 activation is known to suppress IL-6 and TNF- α production through nuclear factor kappa-light-chain enhancer of activated B cells inhibition. Nuclear factor kappa-light-chain enhancer of activated B cells is regulated by acetylation both *in vivo* and *in vitro*.^{7,15,23,24} Sirt1 deacetylates the p65 subunit of nuclear factor kappa-light-chain enhancer of activated B cells at lysine 310,¹⁹ attenuating nuclear factor kappa-light-chain enhancer of activated B cells transcriptional activation. Resveratrol has been shown to suppress the cigarette smoke extract-mediated release of TNF- α in a human macrophage cell line.²³ In lipopolysaccharide (LPS)-activated macrophages, resveratrol inhibited both TNF- α and IL-6 secretion.³²

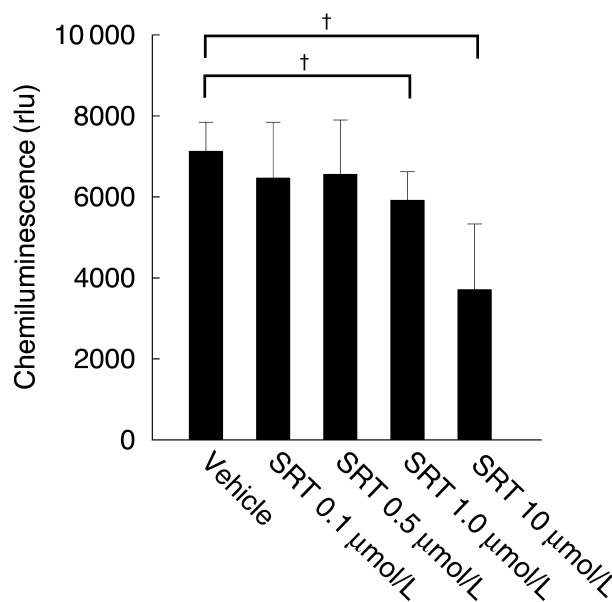


Figure 5 The effect of Sirtuin 1 (Sirt1) activators on ovalbumin (OVA)-induced splenocyte proliferation. Splenocytes from OVA-sensitized and challenged mice were stimulated with OVA *in vitro* and incubated for 72 h ($n = 6$). The cell proliferation was measured by bromodeoxyuridine uptake. Values are expressed as the mean $\pm$ standard deviation. Using repeated measures analysis of variance, $P < 0.01$; using Student's *t*-test, $^{\dagger}P < 0.01$ compared with treatment with vehicle. Res, resveratrol. rlu, relative light unit; SRT, SRT1720.

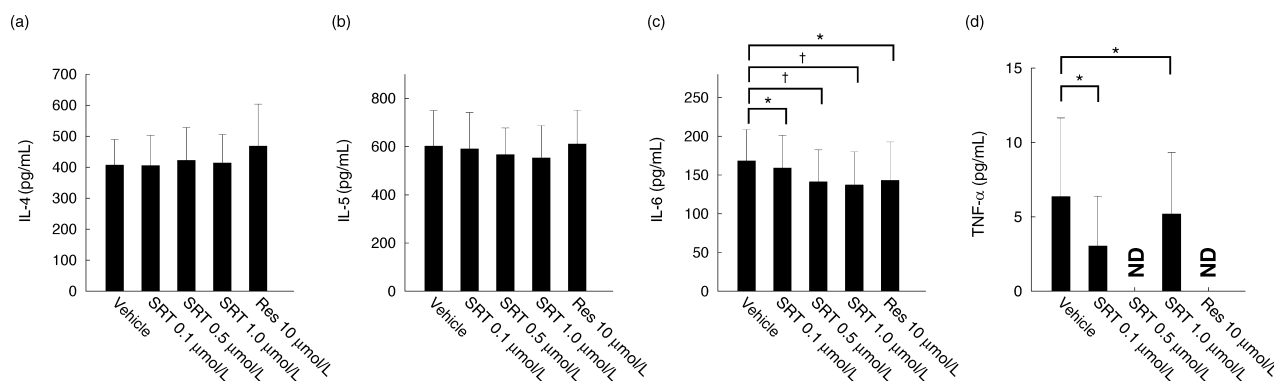


Figure 6 The effect of Sirtuin 1 activators on ovalbumin (OVA)-induced splenocyte cytokine production. Splenocytes from OVA-sensitized and challenged mice were stimulated with OVA *in vitro* and incubated for 72 h ($n = 6$). The cytokine levels in the supernatants were quantified using a multiplexed fluorescent bead-based immunoassay. The (a) interleukin (IL)-4, (b) IL-5, (c) IL-6 and (d) tumour necrosis factor α (TNF- α) concentrations are shown separately. Values are expressed as the mean $\pm$ standard deviation. Res, resveratrol; SRT, SRT1720. Using repeated measures analysis of variance, $P < 0.05$ in IL-6 and TNF- α . Using Student's *t*-test, $^*P < 0.05$ and $^{\dagger}P < 0.01$ compared with treatment with vehicle. ND, not detected.

TNF- α is a pro-inflammatory cytokine that has been implicated in many aspects of asthma airway pathology.^{33,34} TNF- α recruits eosinophils into the lungs³⁵ and regulates the growth and function of T cells.³⁶ Inhaled TNF- α increases airway responsiveness and the infiltration of eosinophils into the sputum.³⁷ Some clinical trials have demonstrated a reduction in exacerbation frequency in asthmatic patients treated with anti-TNF- α therapy.³⁴ IL-6 is another key regulator of allergic airway inflammation.^{33,38} We were unable to evaluate the IL-6 and TNF- α levels in the airway in this study. Whether the inhibition of IL-6 and TNF- α production plays an important role in the airway remains to be elucidated.

We showed that SRT1720 inhibits OVA-induced splenocyte proliferation. Sirt1 plays an important role in lymphocyte activation and T-cell tolerance.^{39,40} Sirt1-deficient mice exhibit abnormal T-cell activation and a breakdown of CD4⁺ T-cell tolerance.³⁹ Human immunodeficiency virus type 1 promoted T-cell hyperactivation by blocking Sirt1.⁴⁰ Therefore, Sirt1 activators might regulate T-cell activation and inhibit the proliferation of T cells in our study.

In this study, the expression of Sirt1 messenger RNA in the lungs of OVA-sensitized and challenged mice was lower than that of saline-sensitized and challenged mice. We could not find a report on Sirt1 expression specifically in asthmatic patients. Precise descriptions of the mechanisms controlling Sirt1 expression and activity are provided elsewhere.⁴¹

In conclusion, the specific Sirt1 activator SRT1720 exerted anti-inflammatory effects in a mouse model of asthma. Sirt1 activators might mediate beneficial effects in asthmatics by suppressing inflammation.

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REFERENCES

- Global Initiative for Asthma (GINA). 2011. The global strategy for asthma management and prevention, Global Initiative for Asthma (GINA) 2011. December. [Accessed 14 Jan 2012.] Available from URL: <http://www.ginasthma.org/>
- Fukutomi Y, Nakamura H, Kobayashi F *et al.* Nationwide cross-sectional population-based study on the prevalences of asthma and asthma symptoms among Japanese adults. *Int. Arch. Allergy Immunol.* 2010; **153**: 280–7.
- Adcock IM, Caramori G, Chung KF. New targets for drug development in asthma. *Lancet* 2008; **372**(9643): 1073–87.
- Bergeron C, Al-Ramli W, Hamid Q. Remodeling in asthma. *Proc. Am. Thorac. Soc.* 2009; **6**(3): 301–5.
- Dahlén SE, Caramori G, Drazen JM. Asthma treatment guidelines meet the real world. *N. Engl. J. Med.* 2011; **364**: 1769–70.
- Price D, Musgrave SD, Shephstone L *et al.* Leukotriene antagonists as first-line or add-on asthma-controller therapy. *N. Engl. J. Med.* 2011; **364**: 1695–707.
- Glozak MA, Sengupta N, Zhang X *et al.* Acetylation and deacetylation of non-histone proteins. *Gene* 2005; **363**: 15–23.
- Ito K, Caramori G, Lim S *et al.* Expression and activity of histone deacetylases in human asthmatic airways. *Am. J. Respir. Crit. Care Med.* 2002; **166**(3): 392–6.
- Cosio BG, Mann B, Ito K *et al.* Histone acetylase and deacetylase activity in alveolar macrophages and blood monocytes in asthma. *Am. J. Respir. Crit. Care Med.* 2004; **170**: 141–7.
- Milne JC, Denu JM. The Sirtuin family: therapeutic targets to treat diseases of aging. *Curr. Opin. Chem. Biol.* 2008; **12**: 11–7.
- Milne JC, Lambert PD, Schenk S *et al.* Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* 2007; **450**(7170): 712–6.
- Baur JA, Pearson KJ, Price NL *et al.* Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006; **444**(7117): 337–42.
- Lagouge M, Argmann C, Gerhart-Hines Z *et al.* Resveratrol improves mitochondrial function and protects against metabolic disease by activating sirt1 and PGC-1 α . *Cell* 2006; **127**: 1109–22.
- Nakamaru Y, Vuppusetty C, Wada H *et al.* A protein deacetylase SIRT1 is a negative regulator of metalloproteinase-9. *FASEB J.* 2009; **23**: 2810–9.
- Rajendrasozhan S, Yang SR, Kinnula VL *et al.* SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2008; **177**: 861–70.
- Yang SR, Wright J, Bauter M *et al.* Sirtuin regulates cigarette smoke-induced proinflammatory mediator release via RelA/p65 NF- κ B in macrophages *in vitro* and in rat lungs *in vivo*: implications for chronic inflammation and aging. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2007; **292**: L567–76.
- Alcendor RR, Gao S, Zhai P. Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ. Res.* 2007; **100**: 1512–21.
- Araki T, Sasaki Y, Milbrandt J. Increased nuclear NAD biosynthesis and Sirt1 activation prevent increased nuclear NAD biosynthesis and Sirt1 activation prevent axonal degeneration. *Science* 2004; **305**(5686): 1010–3.
- Chen J, Zhou Y, Mueller-Stieber S *et al.* Sirt1 protects against microglia-dependent amyloid-beta toxicity through inhibiting NF- κ B signaling. *J. Biol. Chem.* 2005; **280**(48): 40364–74.
- Lee M, Kim S, Kwon OK *et al.* Anti-inflammatory and anti-asthmatic effects of resveratrol, a polyphenolic stilbene, in a mouse model of allergic asthma. *Int. Immunopharmacol.* 2009; **9**: 418–24.
- Yamazaki Y, Usui I, Kanatani Y *et al.* Treatment with SRT1720, a SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice. *Am. J. Physiol. Endocrinol. Metab.* 2009; **297**(5): E1179–1186.
- Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* 2007; **117**: 175–84.
- Yang SR, Wright J, Bauter M *et al.* Sirtuin regulates cigarette smoke-induced proinflammatory mediator release via RelA/p65 NF- κ B in macrophages *in vitro* and in rat lungs *in vivo*: implications for chronic inflammation and aging. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2007; **292**(2): L567–76.
- Chen LF, Greene WC. Regulation of distinct biological activities of the NF- κ B transcription factor complex by acetylation. *J. Mol. Med.* 2003; **81**: 549–57.
- Terawaki K, Yokomizo T, Nagase T *et al.* Absence of leukotriene B₄ receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses. *J. Immunol.* 2005; **175**(7): 4217–25.
- McMillan SJ, Bishop B, Townsend MJ *et al.* The absence of interleukin 9 does not affect the development of allergen-induced pulmonary inflammation nor airway hyperreactivity. *J. Exp. Med.* 2002; **195**: 51–7.

- 27 Csiszar A, Labinskyy N, Podlutzky A *et al.* Vasoprotective effects of resveratrol and SIRT1: attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. *Am. J. Physiol. Heart Circ. Physiol.* 2008; **294**: 2721–35.
- 28 Castro M, Mathur S, Hargreave F *et al.* Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am. J. Respir. Crit. Care Med.* 2011; **184**: 1125–32.
- 29 Corren J, Lemanske RF, Hanania NA *et al.* Lebrikizumab treatment in adults with asthma. *N. Engl. J. Med.* 2011; **365**: 1088–98.
- 30 Nath P, Leung SY, Williams AS *et al.* Complete inhibition of allergic airway inflammation and remodelling in quadruple IL-4/5/9/13–/–mice. *Clin. Exp. Allergy* 2007; **37**: 1427–35.
- 31 Izuhara K, Ohta S, Shiraishi H *et al.* The mechanism of mucus production in bronchial asthma. *Curr. Med. Chem.* 2009; **16**: 2867–75.
- 32 Sharma S, Chopra K, Kulkarni SK. Resveratrol and curcumin suppress immune response through CD28/CTLA-4 and CD80 co-stimulatory pathway. *Clin. Exp. Immunol.* 2007; **147**: 155–63.
- 33 Virchow JC Jr, Kroegel C, Walker C *et al.* T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am. J. Respir. Crit. Care Med.* 1995; **151**(4): 960–8.
- 34 Brightling C, Berry M, Amrani Y. Targeting TNF- α : a novel therapeutic approach for asthma. *J. Allergy Clin. Immunol.* 2008; **121**: 5–10; quiz 11–2.
- 35 Lukacs NW, Strieter RM, Kunkel SL. TNF- α mediates recruitment of neutrophils and eosinophils during airway inflammation. *J. Immunol.* 1995; **154**: 5411–7.
- 36 Scheurich P, Thoma B, Ucer U *et al.* Immunoregulatory activity of recombinant human tumor necrosis factor (TNF)- α : induction of TNF receptors on human T cells and TNF- α -mediated enhancement of T cell responses. *J. Immunol.* 1987; **138**: 1786–90.
- 37 Thomas PS, Heywood G. Effects of inhaled tumour necrosis factor α in subjects with mild asthma. *Thorax* 2002; **57**: 774–8.
- 38 Neveu WA, Allard JB, Dienz O *et al.* IL-6 is required for airway mucus production induced by inhaled fungal allergens. *J. Immunol.* 2009; **183**(3): 1732–8.
- 39 Zhang J, Lee SM, Shannon S *et al.* The type III histone deacetylase Sirt1 is essential for maintenance of T cell tolerance in mice. *J. Clin. Invest.* 2009; **119**(10): 3048–58.
- 40 Kwon HS, Brent MM, Getachew R *et al.* Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation. *Cell Host Microbe.* 2008; **3**: 158–67.
- 41 Kwon HS, Ott M. The ups and downs of SIRT1. *Trends Biochem. Sci.* 2008; **33**: 517–25.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Cell counts in bronchoalveolar lavage fluid

TABLE S1 Online Supporting Information

Table S1. Cell counts in bronchoalveolar lavage fluid

	Saline +Vehicle (n = 6)	Saline +SRT1720 (n = 6)	OVA +Vehicle (n = 14)	OVA +SRT1720 (n = 14)
Total Number of Cells ($\times 10^3$ [/mL])	15.3 $\pm$ 7.9	20.8 $\pm$ 10.2 ^{NS}	275 $\pm$ 130 [†]	180 $\pm$ 90.7 ^{*†}
Macrophages ($\times 10^3$ [/mL])	14.6 $\pm$ 7.8	19.6 $\pm$ 9.8 ^{NS}	77.0 $\pm$ 33.8 [†]	68.7 $\pm$ 32.5 [†]
Lymphocytes ($\times 10^3$ [/mL])	0.3 $\pm$ 0.2	0.4 $\pm$ 0.4 ^{NS}	35.6 $\pm$ 23.6 [†]	22.4 $\pm$ 20.9 [†]
Eosinophils ($\times 10^3$ [/mL])	0.1 $\pm$ 0.1	0.2 $\pm$ 0.2 ^{NS}	130 $\pm$ 76.9 [†]	68.7 $\pm$ 51.8 ^{*†}
Neutrophils ($\times 10^3$ [/mL])	0.4 $\pm$ 0.2	0.5 $\pm$ 0.5 ^{NS}	32.2 $\pm$ 29.3 [†]	20.1 $\pm$ 17.3 [†]

Values are expressed as the mean $\pm$ SD. * P< 0.05 significant difference compared with the OVA-sensitized and challenged mouse group treated with vehicle (OVA +Vehicle). † P< 0.01, significant difference compared with the saline-sensitized and challenged group treated with vehicle (Saline +Vehicle). NS, no significant difference compared with the Saline +Vehicle group.