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Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

High and low temperatures aggravate airway inflammation of asthma: Evidence in a mouse model[☆]

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ARTICLE INFO

Article history:

Received 9 August 2019

Received in revised form

16 October 2019

Accepted 17 October 2019

Available online xxx

Keywords:

High temperature

Low temperature

Mouse asthma

Airway inflammation

TRP

ABSTRACT

Epidemiology suggests ambient temperature is the triggers and potential activator of asthma. The role of high and low temperatures on airway inflammation of asthma, and the underlying molecular mechanism are not yet understood. A mouse model of asthma was adopted in our experiment. The BALB/c mice were exposed at different temperature for 4 h (2 h in the morning and 2 h in the afternoon) on weekday. The exposure temperatures were 10 °C, 24 °C and 40 °C. Ovalbumin (OVA) was used to sensitize the mice on days 14, 18, 22, 26, and 30, followed by an aerosol challenge for 30 min from day 32–38. After the final OVA challenge, lung function, serum protein and pulmonary inflammation were assessed. Comparing the OVA with the saline group at 24 °C, we saw a significant increase in: serum Total-IgE ($p < 0.05$); OVA-sIgE ($p < 0.01$); IL-4 ($p < 0.05$); IL-1 β ($p < 0.01$); IL-6 ($p < 0.01$); TNF- α ($p < 0.01$); and the ratio of IL-4/IFN- γ ($p < 0.01$). At the same time, there was a significant decrease in IFN- γ ($p < 0.01$). As the temperature increase, there is a U shape for immune proteins and pro-inflammatory factors with a peak value at 24 °C, exception for IFN- γ (inverted U-shape). After the high and low temperature exposure, the Ri and Re increased significantly, while Cldyn decreased significantly compared with the 24 °C group. Histopathological analysis of the OVA groups showed airway remodeling, airway wall thickening and deforming, and subepithelial fibrosis. More obvious changes were found in the high and low temperature exposure groups. The immunohistochemistry suggested that TRPs changed with temperatures. High and low temperatures can aggravate airway inflammation in a mouse model of asthma. TRPs play an important role in temperature aggravation of allergic asthma. The results suggest that asthmatics should avoid exposure to high and low temperatures for too long time.

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1. Introduction

Climate change is probably the biggest global health threat of

the 21st century. There will be likely 2 °C increase in the earth's average surface temperature over the course of this century, which exceeds the preindustrial average temperatures (Costello et al., 2009). Temperature is an important climate factor that has a significant effect on human health, particularly for high and low temperatures (Seltenrich, 2015). Exposure to excessive heat or cold can result in temperature-related death, like heat stroke (Bouchama and Knochel, 2002) or sepsis-related hypothermia (Tanaka and Tokudome, 1991). Deaths attributable to exposure to cold or heat accounted for 63% and 31% of all temperature-related

[☆] This paper has been recommended for acceptance by Christian Sonne.

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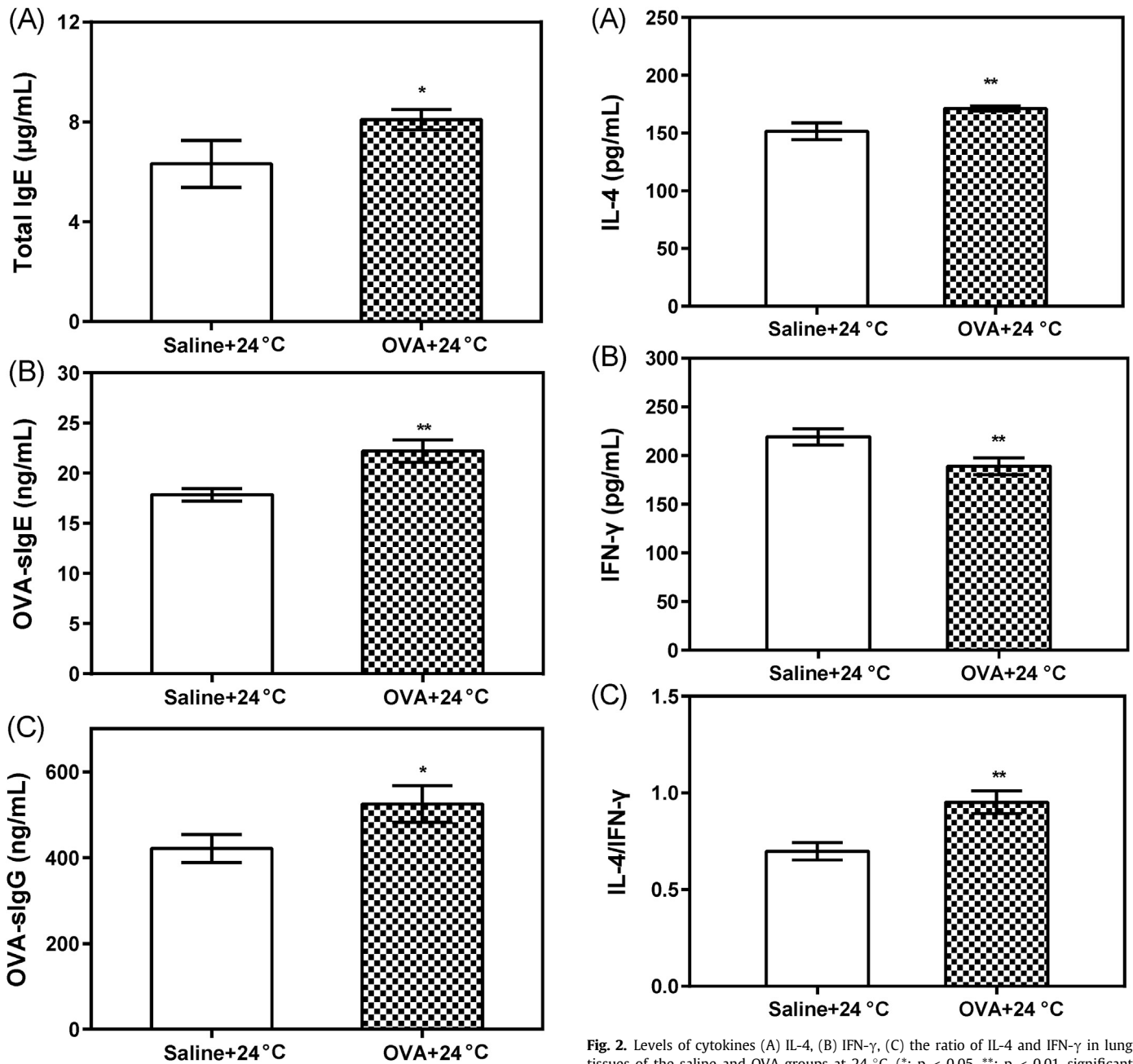


Fig. 2. Levels of cytokines (A) IL-4, (B) IFN-γ, (C) the ratio of IL-4 and IFN-γ in lung tissues of the saline and OVA groups at 24 °C. (*: $p < 0.05$, **: $p < 0.01$, significant difference compared with the saline group, t -test, $n = 7$).

Fig. 1. Serum concentration of T-IgE (A), OVA-sIgE(B) and OVA-sIgG(C) in the saline and the OVA groups at 24 °C. (*: $p < 0.05$, **: $p < 0.01$, significant difference compared with the saline group, t -test, $n = 7$).

deaths between 2006 and 2010 (Berko et al., 2014). Results indicate that both hot and cold temperatures increase mortality due to cardiovascular diseases (Basu and Ostro, 2008; Guo et al., 2013). Since temperatures are going to become more extreme with global warming, more attention needs to be given to the temperature.

People frequently experience high and low temperatures as a result of occupational exposure, leisure activities (such as skiing) and summer or winter outdoor temperatures exposure. Temperatures above or below “comfortable” range levels for humans, can induce various respiratory symptoms. High and low temperatures act as a stimulus to the airway, which may trigger bronchoconstriction and be a risk factor for asthma (Hales et al., 1998; Li et al.,

2014; Xu et al., 2013). Asthma is a consequence of complex gene–environment interactions (Cabieres et al., 2014), with heterogeneity in clinical presentation, like the type and intensity of airway inflammation and remodeling (Papi et al., 2018). Asthma is the most common chronic disease, and is of great public health concern, since it could lead to a heavy economic burden on the family, and poor quality of the life (Eder et al., 2006). The incidence and prevalence of childhood asthma increased markedly during the 20th century, especially in developing countries (Wong et al., 2002; Zhang et al., 2013). This substantial increase of asthma prevalence implies a major public health problem in a country like China.

There is growing evidence that exposure to allergens and irritants (cat dander, cockroaches, house dust, mites, pollen) exacerbate asthma (Deng et al., 2016). In addition to allergens and

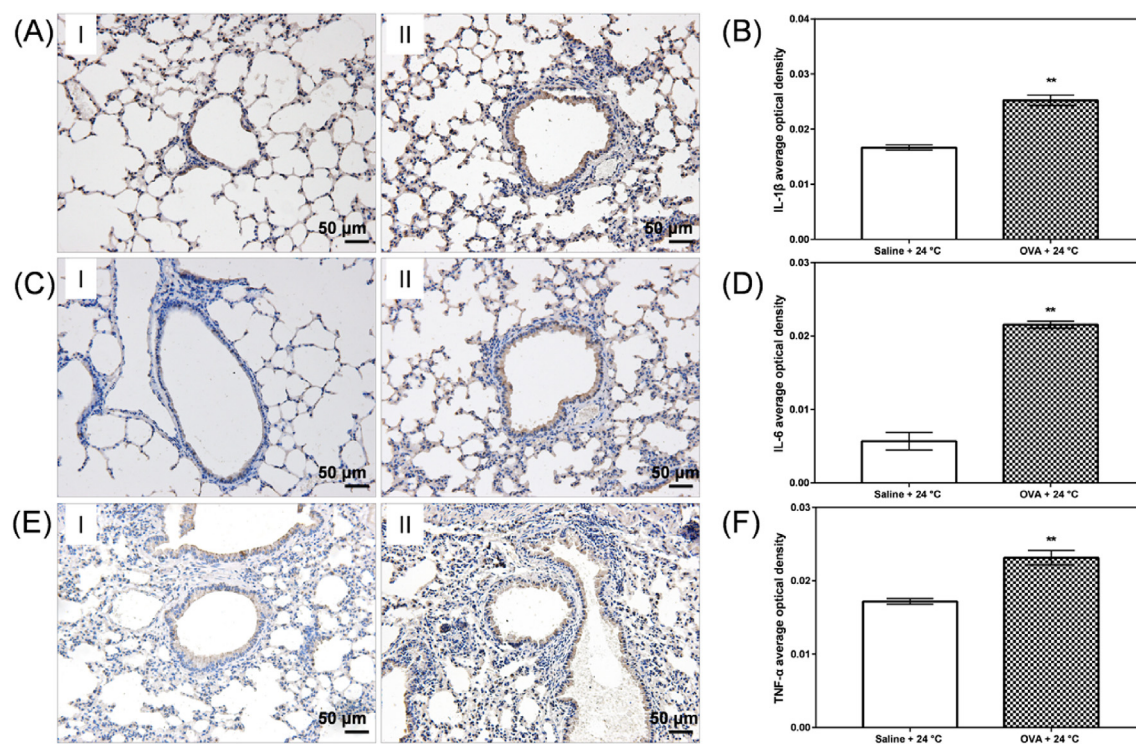


Fig. 3. The levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α). (A), (C) and (E) show the immunohistochemistry of IL-1 β , IL-6 and TNF- α , respectively. The average optical density assay of IL-1 β , IL-6 and TNF- α are (B), (D) and (F) respectively. I: saline+24 °C; II: OVA +24 °C; (n = 7 per group). (*: p < 0.05, **: p < 0.01, significant difference compared with the saline+24 °C group; ANOVA, n = 7).

irritants, evidence also indicate that air pollution is a risk factor resulting in asthma (Guarnieri and Balmes, 2014), especially during pregnancy and in early life (Deng et al., 2018; Deng et al., 2015). A potential mechanism explaining this outcome may be that air pollutants can cause oxidative injury, inflammation, and increase the risk of sensitization (Guarnieri and Balmes, 2014). It is possible that the transient receptor potential (TRP) may participate in the process of temperature aggravated asthma, however the underlying molecular mechanism is unknown.

The body has a number of mechanisms to cope with potentially harmful hot or cold objects. TRP channels are found in a variety of sensory organs, including in the upper and lower respiratory tract (TRPV1, TRPM8, TRPA1) (Corey et al., 2004). Serving as a polymodal detector, TRPA1 is activated by temperatures below 18 °C, and TRPV1 by temperature above 42 °C, while the apparent threshold for TRPM8 activation is 25 °C (Caterina, 2007). Studies (Caterina et al., 1997; Dhaka et al., 2007; Fajardo et al., 2008; Gavva et al., 2007; Story et al., 2003) have proven that TRPA1, TRPV1 and TRPM8 channels are important in the transduction of temperature signals, and that activation of the TRPV1 and TRPA1 channels could cause airway inflammation and hyperresponsiveness (Andr  et al., 2008; Lin et al., 2010; Reilly et al., 2003; Sadosky et al., 2012; Seki et al., 2007; Zhang et al., 2008). This means that the TRPA1, TRPM8 and TRPV1 channels react to temperature. However, the role of TRPA1, TRPM8 and TRPV1 in temperature aggravated asthma is not clear.

Few toxicological studies have focused on the relationship between asthma and temperature and the role of the TRPA1 and TRPV1 channels in the process. In our study, we hypothesize that temperature acts as an environmental adjuvant to aggravate allergic asthma, and that TRP channels are probably involved in temperature induced airway symptoms but via different pathways.

To test this hypothesis, we examined the effect of temperature on asthma airway inflammation using a mouse model of asthma. We also tried to elucidate the underlying mechanism.

2. Materials and methods

2.1. Ethics statement

The experimental procedures were approved by the Office of Scientific Research Management of Central South University. According to the ethical requirements 15 mice were used in each group to ensure the validity of experimental data.

2.2. Animals and study protocol

SPF male BALB/c mice were purchased from Liaoning Changsheng Biotechnology Co. Ltd. (Liaoning, China). The mice were housed in pathogen-free cages at 20–25 °C and 40–60% relative humidity for 1 week to acclimatize, and were fed OVA (ovalbumin)-free food and tap water *ad libitum*. Mice were randomly divided into 4 experimental groups with 15 mice per group. The protocol of this study is as follows: 1) saline +24 °C group; 2) OVA +24 °C group; 3) OVA +10 °C group; 4) OVA +40 °C group. According to the high and low temperature human occupation model (GB 938–1989), all groups were exposed at their different temperatures for 4 h every weekday (2 h in the morning and 2 h in the evening).

The control group was exposed at 24 °C receiving only saline treatment, while the other groups were exposed at one of 10 °C, 24 °C, and 40 °C temperatures, with OVA-treatment in an artificial intelligence climate warehouse (AI) (PRx-250D). The AI was used to controlling the temperature, humidity (40 ± 5%) and illumination. Group 2, 3 and 4 received OVA sensitization via intraperitoneal

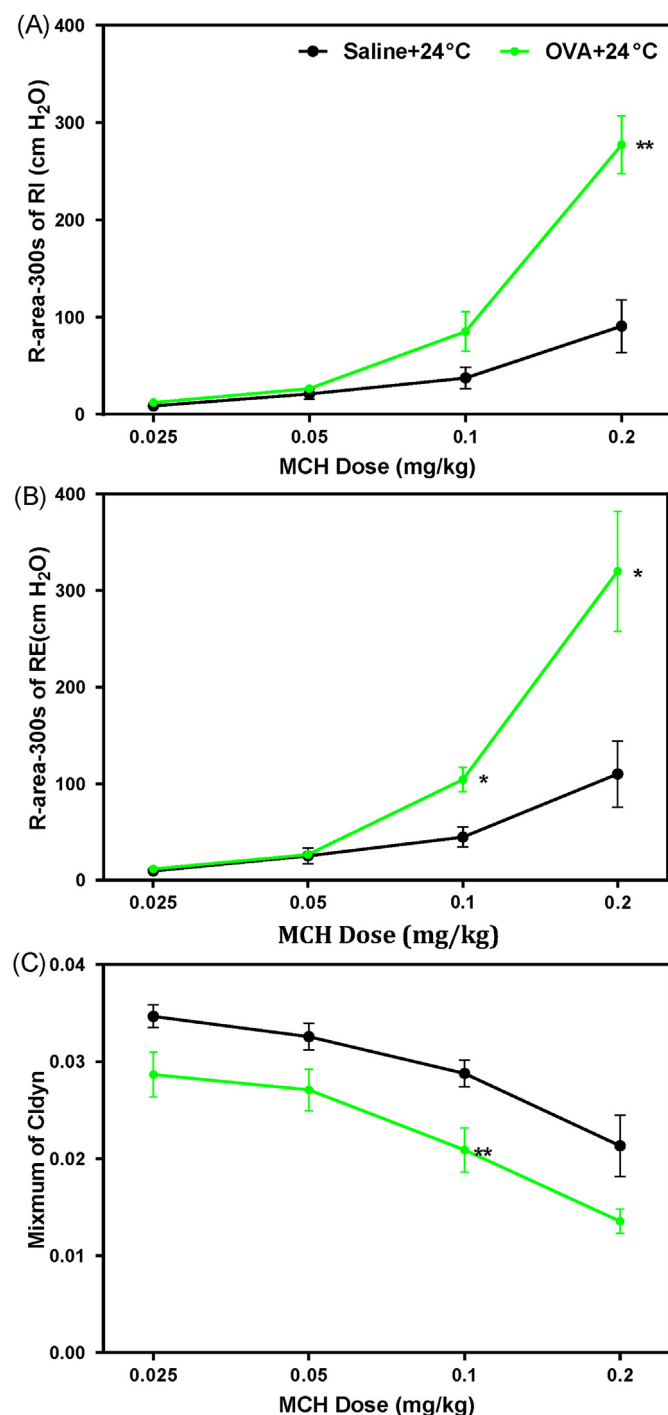


Fig. 4. AHR assessments. The result of Ri (A), Re (B) and Cldyn (C) in the saline and OVA groups at 24 °C. (*: $p < 0.05$; **: $p < 0.01$, significant difference compared with the saline + 24 °C group, t -test, $n = 6$).

injection of 1 mL of 25 mg/mL OVA solution containing 1.75 mg gelatinous Al(OH)₃ (Sigma, USA) as an adjuvant on days 14, 18, 22, 26 and 30. From days 32–38, these mice received an aerosol challenge with 1% OVA for 30 min using an ultrasonic nebulizer (Yuyue, version 402AI, China). A similar protocol was followed for the saline group in which OVA was replaced with saline. The exposure period was 32 d (day 1–32). The mice were sacrificed on day 39 for further biochemical and histological analysis. The protocol is shown in Fig. S1.

2.3. Airway hyper-responsiveness (AHR)

wAHR was conducted within 24 h after the final aerosol challenge, using an AniRes 2005 lung function system (Beijing Bestlab High-Tech Co., Ltd., Beijing, China). Mice were anaesthetized with pentobarbital sodium (Urchem, China) at a dose of 100 mg/kg by intraperitoneal injection. The respiratory rate was pre-set to be 90 breaths/min and the expiration/inspiration ratio was set to be 1.5:1. Methacholine (MCH, Sigma-Aldrich) was injected into the jugular vein at doses of 0.025, 0.05, 0.1 and 0.2 mg/kg successively every 5 min. After each injection, inspiratory resistance (Ri), expiratory resistance (Re) (the graphic area between the peak value and baseline) and the minimum value of lung dynamic compliance (Cldyn) were recorded for later analysis.

2.4. Total serum immunoglobulin (IgE, OVA-IgE and OVA-IgG) detection

Venous blood from the heart was collected by cardiac puncture, and kept at room temperature for 30 min to separate the serum. Serum samples were collected after centrifugation (3000 rpm, 25 °C, 10 min) and stored at –80 °C. The concentration of total serum IgE, OVA-IgE and OVA-IgG were determined using ELISA kits (Biolegend, USA). All operations were conducted according to the manufacturer's instructions.

2.5. The interleukin-4 (IL-4) and interferon-gamma (IFN- γ) in the lung

Lung tissue was excised and homogenized in a 1% phosphate buffer saline solution, and the supernatant was obtained by centrifugation (2500 rpm, 4 °C, 10 min). The IL-4 and IFN- γ content in the lung homogenate were measured using ELISA kits (eBioscience, USA) according to the manufacturer's instructions.

2.6. Histological analysis

Lung tissue was excised and fixed with a 4% neutral buffered polyformaldehyde and kept overnight at room temperature. The tissue samples were then embedded in paraffin, sectioned and stained according to standard protocols: (1) haematoxylin and eosin (H&E) to assess airway remodeling; (2) periodic acid schiff (PAS) to examine mucus production; (3) Masson's trichrome (MT) to determine collagen deposition in the airway.

After dewaxing, rehydration and antigen retrieval, the sections were incubated with 0.3% hydrogen peroxide and blocked by appropriate normal serum. Sections were subsequently incubated overnight at 4 °C with the monoclonal antibodies: anti-IL-1 β ; anti-IL-6; anti-TNF- α ; anti-TRPA1, anti-TRPM8 and anti-TRPV1. For the specific procedure followed, refer to (Costinean et al., 2006). All tissue slices were examined using a BX53 microscope (Olympus).

2.7. Statistical analysis

Analysis of variance (ANOVA) and a T-test were performed for statistical analysis using Graphpad Prism 7 software. Tissue slices were analyzed by Image-Pro Plus 6.0 to measure the average optical density of the IL-1 β ; IL-6; TNF- α ; TRPA1, TRPM8 and TRPV1 stained slices. The data were presented as the mean $\pm$ standard error (SEM). A p -value less than 0.05 was considered to be a significant difference. The 15 mice were divided into two parts for test, one for biochemical indicators test ($n = 8$), the other is for airway hyper-responsiveness test (AHR) ($n = 7$). We exclude outliers of each group, hence, there are 7 or 8 mice used in the final results for biochemical indicators. About the number of mice in the AHR test,

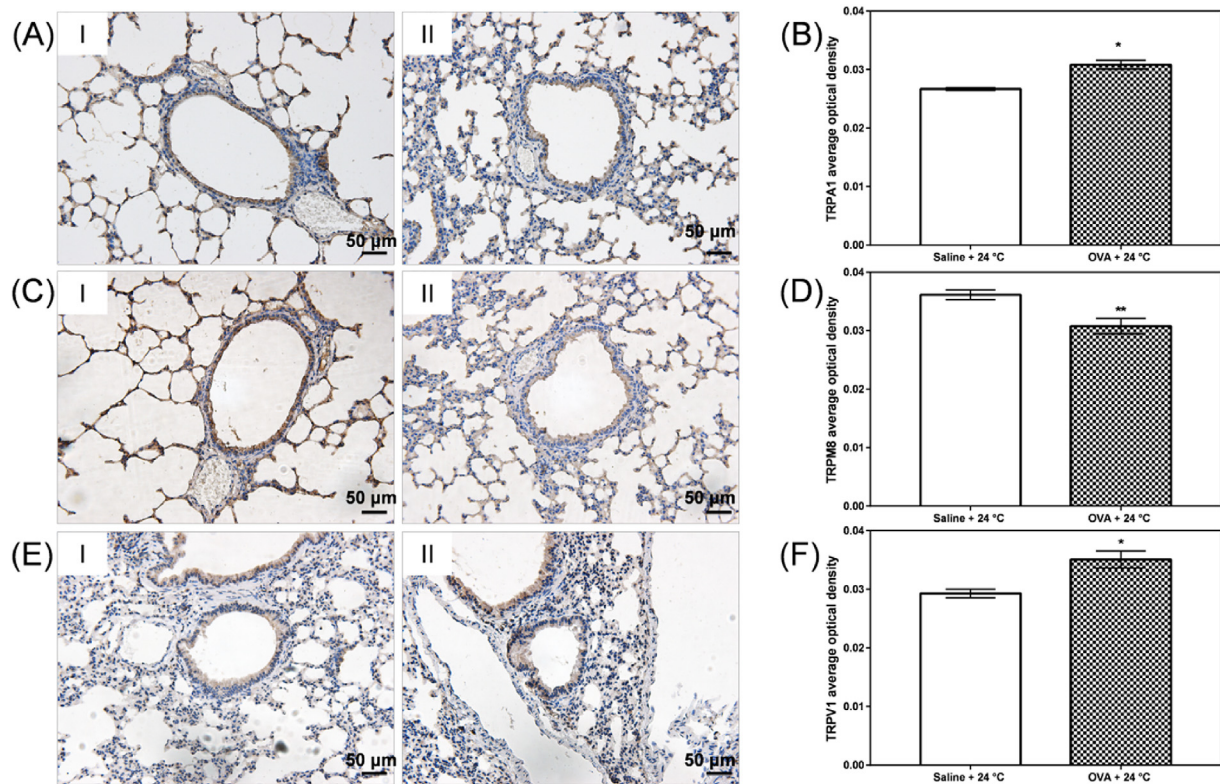


Fig. 5. The levels of TRPA1, TRPM8 and TRPV1. (A), (C) and (E) give the immunohistochemistry of TRPA1, TRPM8 and TRPV1, respectively. The average optical density assay of TRPA1, TRPM8 and TRPV1 are shown in (B), (D) and (F) respectively. I: Saline + 24 °C; II: OVA + 24 °C; (n = 8 per group). (*: $p < 0.05$, **: $p < 0.01$, significant difference compared with the saline + 24 °C group; ANOVA, $n = 8$).

as there was one mouse in 40 °C group was died during the experiment because of bite by other mice, we chose 6 mice in AHR test to keep the same number in every group." in the revised manuscript. Hence there are 6 data for every indicator ($N = 6$).

3. Results

The serum concentration of T-IgE, OVA-sIgE and OVA-sIgG in the OVA + 24 °C group are apparently higher than those of the saline + 24 °C group, as shown in Fig. 1 and there is significant difference for T-IgE ($p < 0.05$), OVA-sIgE ($p < 0.01$) and OVA-sIgG ($p < 0.05$).

Fig. 2 gives the concentration of cytokines in the lung tissue. Comparing the OVA group with the saline group, a significant increase is found for IL-4 ($p < 0.01$), and a significant decrease is for IFN- γ ($p < 0.01$). There is a significant increase in the ratio of IL-4 and IFN- γ , which reflects the immune imbalance of TH1/TH2.

The pro-inflammatory factors of IL-1 β , IL-6 and TNF- α in lung tissue is displayed in Fig. 3. The immunohistochemical images (A, C and E) demonstrate significant differences between saline + 24 °C and OVA + 24 °C. The average optical density of IL-1 β (B), IL-6 (D) and TNF- α (F) in also OVA + 24 °C showed significantly difference from saline + 24 °C.

The changes in AHR between the OVA and the saline groups are revealed in Fig. 4. Ri and Re increase with increasing doses of MCH, and that the minimum Cldyn decrease with dose of MCH. Specifically, the Ri at MCH (0.2 mg/kg) changed significantly ($p < 0.01$) in the OVA group, when compared with the saline group, and Re at MCH (0.1 mg/kg and 0.2 mg/kg) and Cldyn at MCH (0.1 mg/kg) also changed significantly.

The concentrations of T-IgE, OVA-sIgE and OVA-sIgG in the 10 °C or 40 °C groups are significantly higher than those of the 24 °C group (Fig. 6). The concentrations of T-IgE, OVA-sIgE and OVA-sIgG follow a U-shaped trend with increasing temperature, with the minimum value being at 24 °C.

Fig. 7 shows the concentration of cytokines in lung tissue. The concentration of IL-4 has a U-shaped trend with increasing temperature, with the minimum value being at 24 °C. IFN- γ has an inverted U-shaped trend with increasing temperature. The ratio of IL-4 and IFN- γ also shows a U-shaped trend with increasing temperature, which indicates destruction of the TH1/TH2 immune balance.

The pro-inflammatory factors of IL-1 β , IL-6 and TNF- α in lung tissue are displayed in Fig. 8. The immunohistochemical images (A, C and E) demonstrate significant differences among the different temperature treated groups. The average optical density of IL-1 β (B), IL-6 (D) and TNF- α (F) changed with increasing temperature, showing a U-shaped trend with the minimum value at 24 °C. In the OVA treatment groups, the pro-inflammatory factors of the 10 °C and 40 °C groups showed a significant difference compared with the 24 °C group ($p < 0.01$).

Fig. 9 shows the airway responses of the mice for the different temperature and OVA treatment groups. The Ri increased with an increasing dose of MCH (Fig. 9A), and this increase was more significant at higher MCH doses. Re (Fig. 9B) follows the same trend as Ri, the increase being more significant at MCH of 0.05 (mg/kg). The minimum Cldyn decrease with increasing dose of MCH, which were more significant at lower doses between 10 °C or 40 °C groups and OVA groups (Fig. 9C).

The histopathological changes in the lung tissue are revealed in

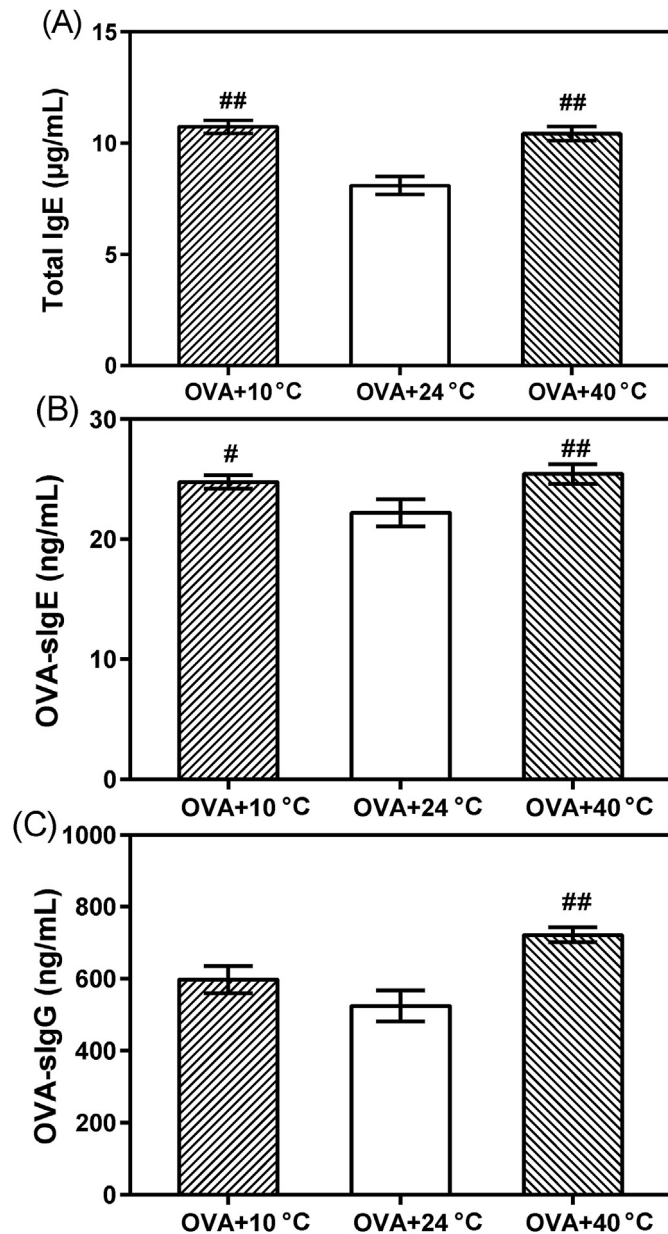


Fig. 6. Serum T-IgE(A), OVA-sIgE(B) and OVA-sIgG(C) after treatment with OVA and exposure to different temperatures. (#: $p < 0.05$, ##: $p < 0.01$, significant difference compared with the OVA+24 °C group, ANOVA, $n = 7$).

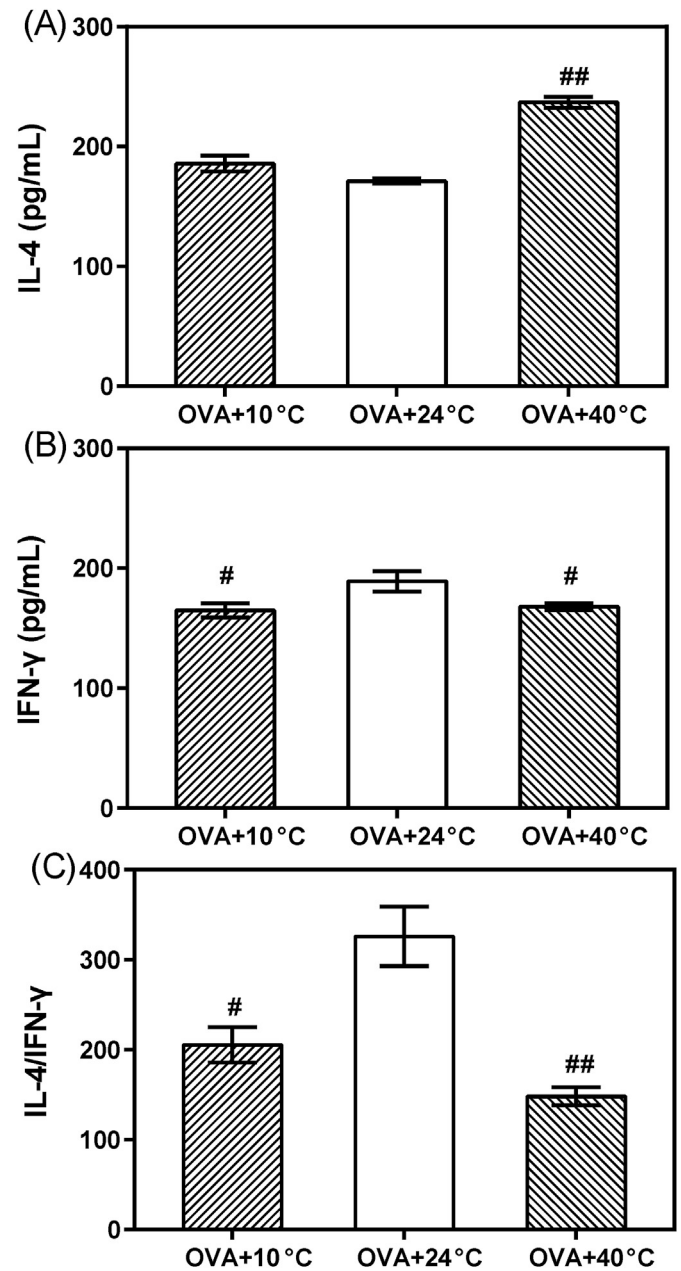


Fig. 7. The level of IL-4 (A), IFN-γ (B) and IL-4/IFN-γ (C) in mouse lung tissue after treatment with OVA and exposure to different temperatures. (##: $p < 0.01$, significant difference compared with the OVA+24 °C group, ANOVA, $n = 7$).

Fig. 10. Compared to the saline-24 °C group, there is a remarkable difference in the bronchial epithelium thickness, inward folding and inflammatory cell infiltration around the airways in the OVA + different temperature groups (H&E staining; Fig. 10A). The PAS-positive mucus cell staining (Fig. 10B, pale pink color) is not very obvious, while there is a significant difference among different temperature treated groups. Subepithelial collagen deposition was increased (MT staining; Fig. 10C, blue color) in all the OVA + different temperature groups.

The levels of TRPA1, TRPM8 and TRPV1 in lung tissue are described in Fig. 11. There are marked differences for the immunohistochemistry of TRPA1, TRPM8 and TRPV1 among the groups. Similarly, there is a remarkable difference between the saline and OVA groups for the three proteins (TRPA1, TRPM8 and TRPV1) (Fig. 5). The average optical density for the OVA (10 °C) group is

significantly higher than the OVA (24 °C) or the saline (24 °C) groups. Our results are consistent with previous studies that showed that TRPA1 acts as a major sensor for noxious cold. TRPM8 are obviously high in the 24 °C groups, and extremely significant differences were found between the OVA (24 °C) group and the OVA (10 °C) or OVA (40 °C) groups. Consistently, TRPV1 expression in the OVA (40 °C) group is significantly higher than the OVA (24 °C).

4. Discussion

Animal models of asthma should mimic, as closely as possible, the real expression of the human disease. In our experiment, we observed the major indicators associated with asthma (AHR,

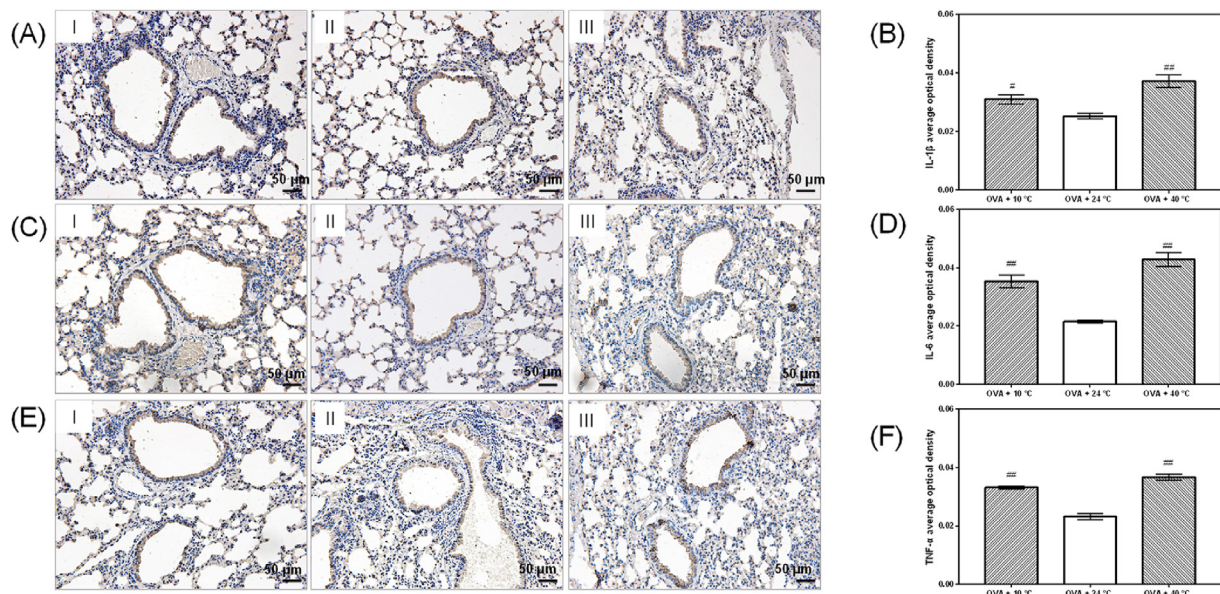


Fig. 8. The levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α). (A), (C) and (E) show the immunohistochemistry of IL-1 β , IL-6 and TNF- α , respectively. The average optical density assay of IL-1 β , IL-6 and TNF- α are (B), (D) and (F) respectively. I: OVA + 10 °C; II: OVA + 24 °C; III: OVA + 40 °C; (n = 7 per group). (#: $p < 0.05$, ##: $p < 0.01$, significant difference compared with the OVA+24 °C group, ANOVA, $n = 7$).

inflammatory index, histological analysis) to evaluate the biochemical and airway structure changes caused by temperature and OVA. The obvious changes in several indicators implied that our mouse model of asthma was successful.

AHR is a very characteristic feature of asthma, and is always related to chronic inflammation which leads to wheezing, shortness of breath, chest tightness, and coughing (Busse and Jr, 2001; Papi et al., 2018). Our results are consistent with other studies, which observed decline in lung function of asthma (O'Byrne et al., 2009). We found that lung function changed significantly (see Figs. 4 and 9), and this was induced by OVA sensitization and was aggravated by exposure at high and low temperatures in the presence of OVA (Fig. 9).

Another biomarker of asthma is the inflammatory index. In a clinical diagnosis of allergic asthma, both an increase of IgE levels, and an imbalance between the Th1 and Th2 immune responses, are very important indicators (Busse and Jr, 2001). Our results revealed a remarkable increase in IgE, OVA-sIgE and other pro-inflammatory factors in the OVA groups compared with the saline group, which are consistent with previous studies (Out et al., 2010; You et al., 2014). In animal asthma models, IL-4 is primarily produced by Th2 cells, and is the cytokine that supports IgE production (Oettgen and Geha, 1999). After OVA exposure, we see a large increase in the levels of IL-4 and a sharp decrease in the levels of IFN- γ . This large increase in the ratio of IL-4 to IFN- γ , exacerbated the Th2 response, which then aggravated the Th2/Th1 balance (Hansen et al., 2007). The resulting imbalance of Th2/Th1 is the pathological basis of asthma, causing airway inflammation. From Figs. 6, Figs. 7 and 8, we can see that there is a significant difference among the different temperature exposure groups, showing that low and high temperatures could exacerbate airway inflammation. As the temperature increased, different indicators followed a U-shaped or inverse-U-shaped curve with a peak at 24 °C, which is consistent with the conclusion that mortality and temperature are in a nonlinear U-shaped relationship (Anderson and Bell, 2009; Curriero et al., 2002; McMichael et al., 2006).

One feature of allergic airway inflammation is the alteration of

airway structure. The pulmonary histological assay (Fig. 10) verified the inflammation in the different groups. Thickening of the airway wall, subepithelial fibrosis, airway mucus, goblet cell hyperplasia, and hyperplasia and hypertrophy of airway smooth muscle cells have been defined as characteristic remodeling manifestations of asthma (Elias et al., 1999; Kohei, 2006). According to the above characteristics, we observed obvious remodeling in the OVA groups at different temperatures. There was apparent airway wall thickening and deforming (Fig. 10 A) and subepithelial fibrosis (Fig. 10 C) in the OVA groups compared with the saline group, especially for 10 °C and 40 °C exposure. While the observed goblet cell hyperplasia (Fig. 10 B, pale pink color) is not extensive, there is a distinct difference among the different groups. These results are consistent with previous research (Du et al., 2019). We showed that in the presence of OVA sensitization, exposure to low or high temperatures aggravated airway wall remodeling of the asthma model.

Temperature plays a significant role in increasing the prevalence of asthma (Li et al., 2014). Temperature is not as severe an environmental adjuvant inducing allergic asthma as some other environmental pollutants (formaldehyde, automobile exhaust and EST) (Deng et al., 2016), but it is still very important for public health.

As we all know, cold air acts as a stimulant to our airways. However, the relationship between low or high temperatures and allergic asthma, has not received much attention. Epidemiological studies have shown that cold air can increase symptoms of asthma in an asthmatic person (Ehara et al., 2000; YÜKSEL et al., 1996). Cold weather aggravates the asthma symptoms in children and adults (Hyrkäs et al., 2014; Nastos et al., 2008). High ambient temperatures (>28 °C) are also a risk factor for children with asthma (Li et al., 2014). Our results showed that both low and high temperatures aggravated the airway inflammation of asthma, which is consistent with previous epidemiological studies.

Du et al. (2019) found that repeated exposure to temperature variations (10/26 °C) exacerbated experimental 'asthma'. It is one of only a few toxicology studies about the relationship between temperature and airway inflammation. Our research is similar in certain respects, such as in the low temperature exposures and

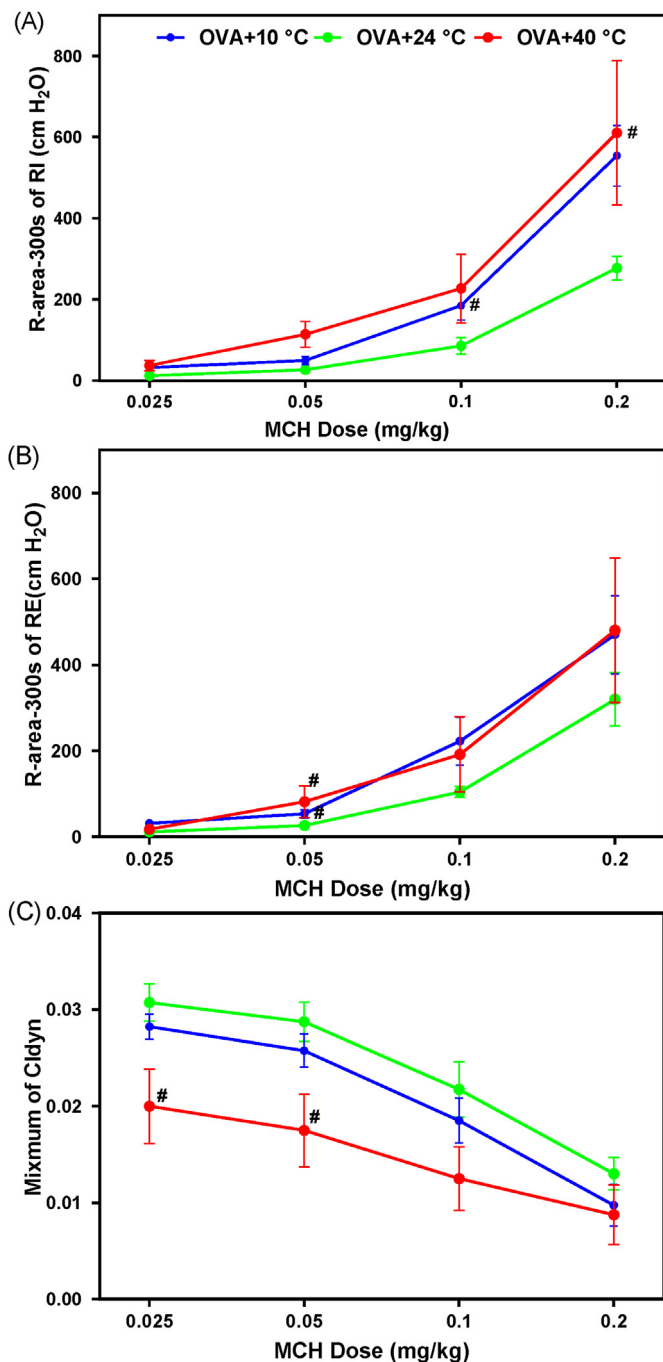


Fig. 9. AHR assessments. The result of Ri (A), Re (B), and Cldyn (C) after treatment with OVA and exposure to different temperature. (#: $p < 0.05$, significant difference compared with the OVA+24 °C group, ANOVA, $n = 6$).

airway injury at low temperatures. There was also two major differences between the two studies. Firstly, high temperature exposure was also included in our experiment. A core temperature of 40 °C (104 °F) is the threshold for heat stroke (Bouchama and Knochel, 2002). Heatstroke can rapidly become life threatening and there is little time to seek treatment in emergency room or hospitals (Kovats et al., 2004). Hence, we considered that there is a need to study the health effect of high temperature. Secondly, we chose an exposure of 4 h/day on weekdays, according to typical

occupational exposure (e.g. as defined in GB 935–1989).

Different TRP proteins behaved differently at high or low temperature. TRPA1 expression was higher at 10 °C than at 24 °C or 40 °C, while TRPM8 was expressed more at 24 °C and TRPV1 at 40 °C. Figs. 5 and 11 clearly illustrated this trend. Our results suggested that TRPs sensed the temperature and participated in temperature regulation. We hypothesized that different TRPs were activated either by temperature within their threshold, or by endogenous cytokines produced due to temperature-induced oxidative stress and airway inflammation in asthma. Many studies have found that thermo TRPs were activated at different temperatures (Caterina, 2007; Caterina et al., 1997; Corey et al., 2004; McKemy et al., 2002), but none of these studies include an asthma model. Andersson et al. found that episodes of oxidative stress can activate TRPA1 expression in sensory neurons (Andersson et al., 2008). A number of studies reported that activating TRPV1 could release pro-inflammatory cytokines (Reilly et al., 2003; Yu et al., 2012; Zhang et al., 2008). It has been suggested that TRPA1-induced inflammation could contribute to the progression of chronic obstructive pulmonary disease (COPD) (Andr  et al., 2008). Similar to TRPA1, TRPV1 expression was found to be higher in the lung tissue of patients with COPD (Baxter et al., 2014). In cases of asthma, it has been proved that expression of TRPA1 and TRPV1 was markedly increased in the asthma airway, which was induced by chemical irritation (Caceres et al., 2009; Deering-Rice et al., 2011; McGarvey et al., 2014; Zhao et al., 2013). Some researchers have hypothesized that cold-induced asthmatic bronchoconstriction is induced by dehydration of the airway mucosa, as well as dry air inhalation (Anderson, 2006; Millqvist, 2015). What the potential molecular mechanisms for heat-induced asthma would be is not very clear. No single nucleotide polymorphisms of TRPM8 have been reported to be associated with asthma or COPD (Grace et al., 2014), however, TRPM8 has been associated with coughing and airway remodeling (Peier et al., 2002; Xing et al., 2008). Very little research exists concerning temperature as a stimulus in an asthma model. Ours is the first to study whether temperature (cold and hot) is an adjuvant in aggravating airway inflammation of an asthma model *in vivo*. An understanding of how temperature affects asthma will enable the development of new drugs targeting temperature-induced asthma, which in turn will greatly improve health and quality of life.

Our result has showed that our mouse model of asthma was successful. The low and high temperature both could aggravate the airway inflammation of asthma. The asthmatics should avoid exposure to high and low temperatures for too long time.

The main limitations of this work are as follows: We evaluated TRP by immunohistochemistry without using an antagonist. We don't know whether the low or high temperature is a direct or indirect activator of TRPs, and more research is needed to explore the underlying mechanism. In future work, we plan to study what happens when an antagonist is added; study the effects of low or high temperature at different humidity; and look at changing exposure times.

5. Conclusion

Our data *in vivo* confirm previous reports that high and low temperature can aggravate the airway inflammation of asthma. Moreover, we found that TRPs take part in the temperature-induced asthma, and that TRPs behave differently at different temperatures. Our new data may be useful for human health and drug research to treat asthma.

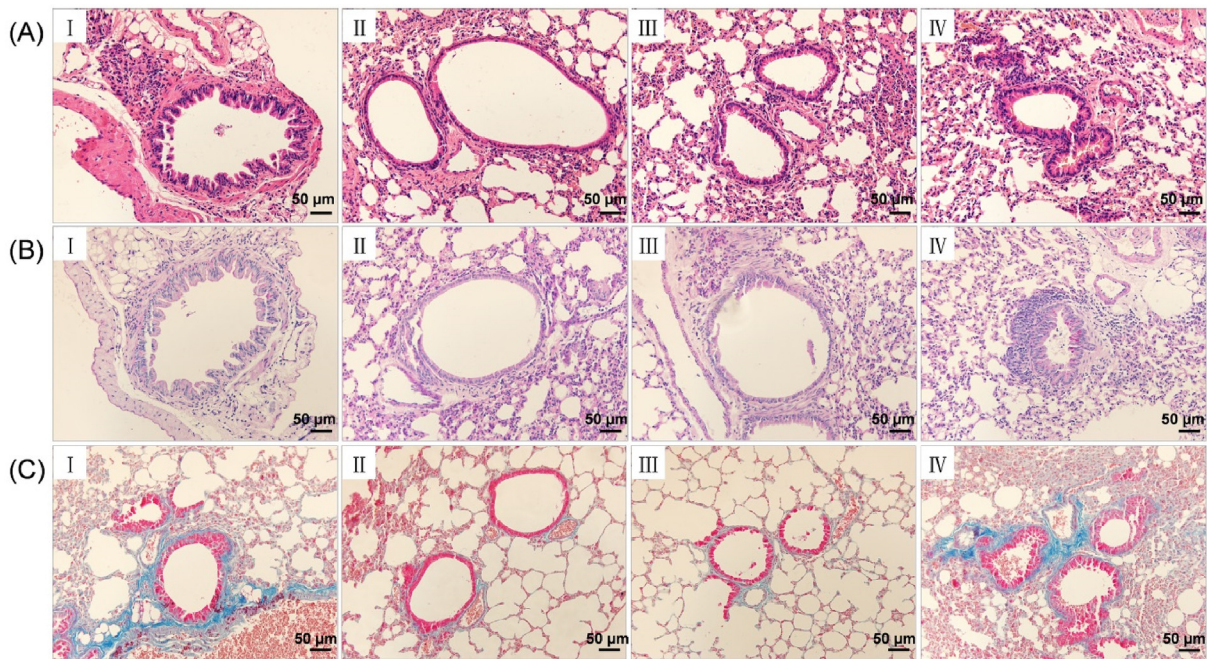


Fig. 10. Histopathological analysis. (A) H&E staining showing infiltration of inflammatory cells and hypertrophy of structural cells; (B) PAS staining showing the mucous-producing goblet cells (pale pink color); and (C) MT staining showing subepithelial collagen deposition (blue color). I: OVA+10 °C; II: Saline +24 °C; III: OVA+ 24 °C; IV: OVA+ 40 °C; (n = 8 per group). H&E, haematoxylin and eosin; PAS, Periodic Acid-Schiff; MT, Masson's Trichrome; OVA, ovalbumin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

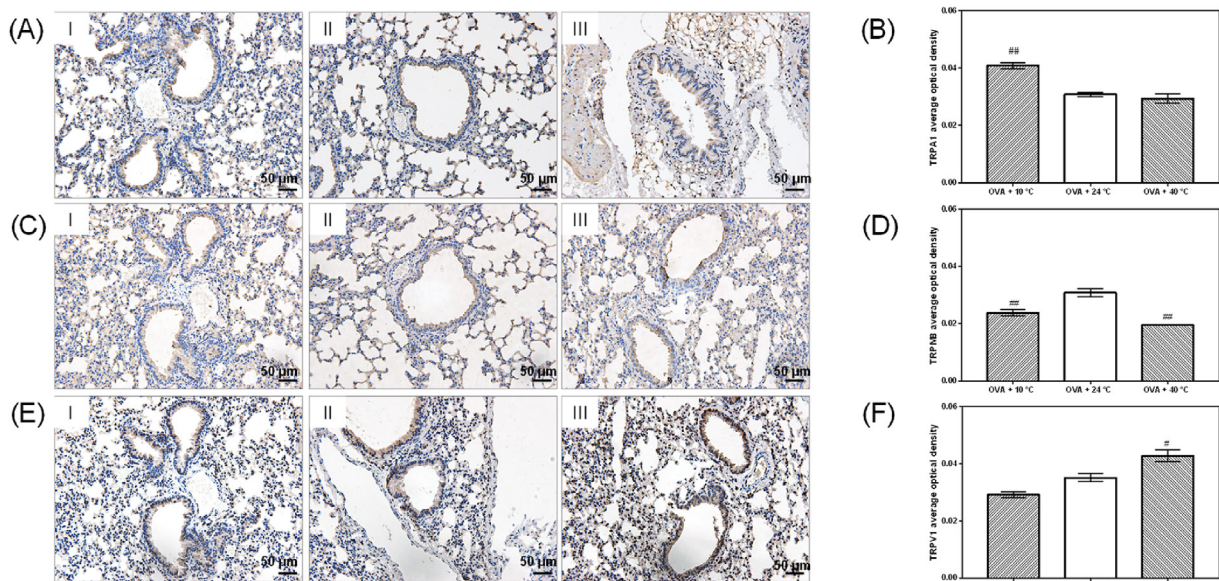


Fig. 11. The levels of TRPA1, TRPM8 and TRPV1. (A), (C) and (E) give the immunohistochemistry of TRPA1, TRPM8 and TRPV1, respectively. The average optical density assay of TRPA1, TRPM8 and TRPV1 are shown in (B), (D) and (F) respectively. I: OVA+10 °C; II: OVA+ 24 °C; III: OVA+ 40 °C; (n = 8 per group). (#: $p < 0.05$, ##: $p < 0.01$, significant difference compared with the OVA+24 °C group, ANOVA, $n = 8$).

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (51576214 and 21777193) and the Key

Research and Development Program of Hunan Province, China (2017SK2091).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2019.113433>.

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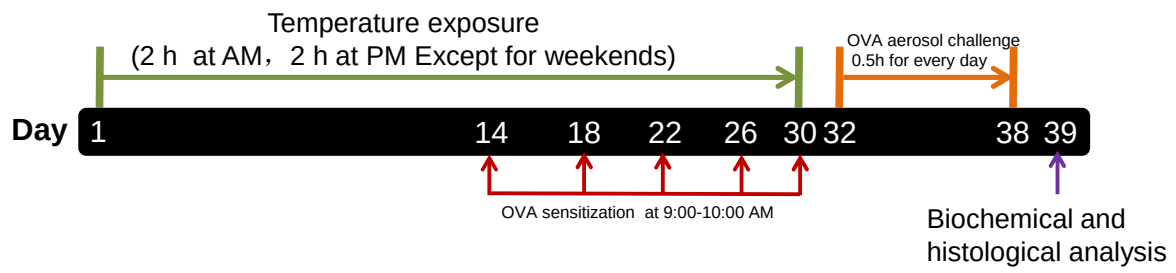


Figure S1 The protocol of the experimental