



Exposure to intermittent hypoxia inhibits allergic airway inflammation in a murine model of asthma

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Abstract

Purpose Obesity increases the severity of asthma, and patients with severe asthma are often complicated with obstructive sleep apnea syndrome (OSAS), a concomitant disease of obesity. We investigated whether intermittent hypoxia (IH), which is a physiological feature of OSAS, modifies allergic airway inflammation in a murine model of asthma.

Methods Balb/c mice were sensitized by ovalbumin (OVA) intraperitoneally twice (days 1 and 14) and challenged with intranasal OVA three times (days 21, 22, and 23). The mice were exposed to IH either from days 1 to 24 (long exposure) or only from days 21 to 24 (short exposure). The impact of IH exposure to allergic airway inflammation was investigated using these mice models by histologic, morphometric, and molecular techniques. Additionally, the airway responsiveness to acetylcholine was also assessed.

Results OVA-sensitized and OVA-challenged mice exposed to room air (RA) showed increased total cell and eosinophil numbers in the BALF. The levels of interleukin (IL)-5 and IL-13 in the BALF also increased and goblet cell metaplasia was induced. In contrast, both long and short exposure to IH inhibited the increased total cell and eosinophil numbers. The levels of IL-5 and IL-13 in the BALF also decreased on exposure to IH. Moreover, the goblet cell hyperplasia and airway hyperresponsiveness were significantly reduced in mice exposed to IH compared to those exposed to RA.

Conclusions These results suggest that IH may not deteriorate the asthmatic condition in a murine model of asthma.

Keywords Allergic airway inflammation • Animal model • Asthma • Intermittent hypoxia • Sleep apnea syndrome

Introduction

Sleep apnea syndrome (SAS) is a disorder that causes intermittent hypoxia (IH) and apnea while the patient is sleeping. Complete or partial upper airway obstruction is one of the mechanisms for SAS, which is classified as obstructive SAS (OSAS). This type of SAS occurs when the throat muscles intermittently relax and block the airway during sleep [1]. Obesity is considered as a major risk factor for the development and progression of OSAS, because of thickened or large neck and smaller airways in the nose, throat, and mouth associated with this condition [2, 3].

Obese patients have more severe bronchial asthma than non-obese patients [4]. Mosen et al. reported that obese patients with asthma were more likely than those with normal BMI to have a poor asthma-specific quality of life, poor asthma control, and a history of asthma-related hospitalizations [5]. Additionally, obese patients with asthma are difficult to treat, resulting in a worse quality of life [6, 7]. These findings suggest that obesity contributes to the aggravation of the severity of bronchial asthma.

The interaction between obesity and bronchial asthma is unclear. One proposed mechanism is OSAS, which causes intermittent hypoxia and apnea [4]. Julian et al. have shown increased severity of the condition of asthmatics with OSAS compared to those without OSAS [8]. Additionally, OSAS is a risk factor for poor control of asthma, independent of other known asthma aggravators [9, 10]. Continuous positive airway pressure (CPAP) for the treatment for OSAS in asthmatic patients reportedly improves not only OSAS but also asthma, at least in part, through modification of the quality of life [11–14].

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OSAS is associated with increased cardiovascular morbidity and mortality [15–19]. We have previously shown that moderate to severe OSAS also leads to increased cerebrovascular morbidity [20]. The impact of OSAS on cardiovascular morbidity may result mainly from IH-related inflammation [21], including increased serum levels of pro-inflammatory factors involved in the development of atherosclerosis [22–24]. We have reported that OSAS patients have increased carotid intima-media thickness and increased serum levels of C-reactive protein (CRP), IL-6, and IL-18 [25]. Arnaud et al. reported that IH exposure in mice induced systemic inflammation and pre-atherosclerotic remodeling involving, regulated upon activation, normal T cell expressed and secreted/C-C chemokine ligand 5 (RANTES/CCL5), a key chemokine in atherogenesis [26]. Arachidonic acid-derived metabolites have been shown to contribute to chronic inflammation and atherogenesis in OSAS patients [27, 28]. Additionally, activation of the COX-1-dependent pathway of arachidonic acid-derived metabolites contributes to the inflammation and vascular remodeling associated with IH exposure in a mouse model [29]. These findings suggest that OSAS plays a role in the induction of systemic inflammation, which is involved in the progression of atherosclerosis. As bronchial asthma is also a systemic inflammatory disease, the inflammation caused by OSAS may also affect allergic inflammation in asthma.

Although OSAS has been shown to contribute to the exacerbation of asthma, the direct contribution of intermittent hypoxia and apnea in airway allergic inflammation remains unknown. The purpose of this study was to investigate the impact of IH on allergic airway inflammation in mice.

Materials and methods

Animals

BALB/c mice (female, 6–8 weeks old, weight range 19–24 g) were obtained from Saitama Experimental Animal Supply Co., Ltd. (Saitama, Japan). All animal experiments were approved by the Animal Care and Use Committee of the Showa University.

Establishment of airway allergic inflammation and IH exposure

The mice were sensitized with OVA (Sigma-Aldrich, St. Louis, MO, USA) emulsified in alum (50 µg/mouse) intraperitoneally on days 1 and 14. Then, they were challenged by intranasal administration of OVA (10 µg/mouse) on days 21, 22, and 23. The control mice received only normal saline (Ns).

We performed three patterns of experimental protocol in this study (Fig. 1a–c). In the first experiment, the mice were exposed to either room air (RA) or IH from days 1 to 24 (long

exposure; Fig. 1a). The second experiment was done by exposing mice to either RA or IH from days 21 to 24 (short exposure; Fig. 1b). The third pattern was to expose the mice to IH or RA during the OVA sensitization from days 1 to 21 in order to investigate the effect of IH in during the sensitization phase by means of cell culture in vitro, which was described as the “exposure to IH in the sensitization phase” (Fig. 1c). The exposure to RA and to IH was performed by a gas control delivery system as previously described [30]. In the IH group, the oxygen concentration was reduced from 20.9 to 5.0–6.0% over a 30-s period and immediately elevated to room air levels, i.e., 20.9%, and with an IH exposure for 30 s, for 12 h during the light phase from 8:00 to 20:00.

Cellular analysis and measurement of cytokines in BALF

The mice were sacrificed 24 h after the last challenge. BAL was performed three times per mouse (0.35 mL/time) with saline. The BAL fluid (BALF) was centrifuged and its supernatant was collected. The cells were washed and re-suspended in saline. The total number of the cells was counted by hemocytometer with Turk solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Differential cell counts were performed on cytopspin cell preparations (Cytopspin 3; Shandon Inc., Pittsburgh, PA, USA) and stained with Diff-Quick (Dade Behring Inc., Newark, DE, USA). A differential count of 400 cells was performed using standard morphological criteria.

The levels of cytokines including IL-4, IL-5, IL-13, and IFN-γ in the BALF were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions.

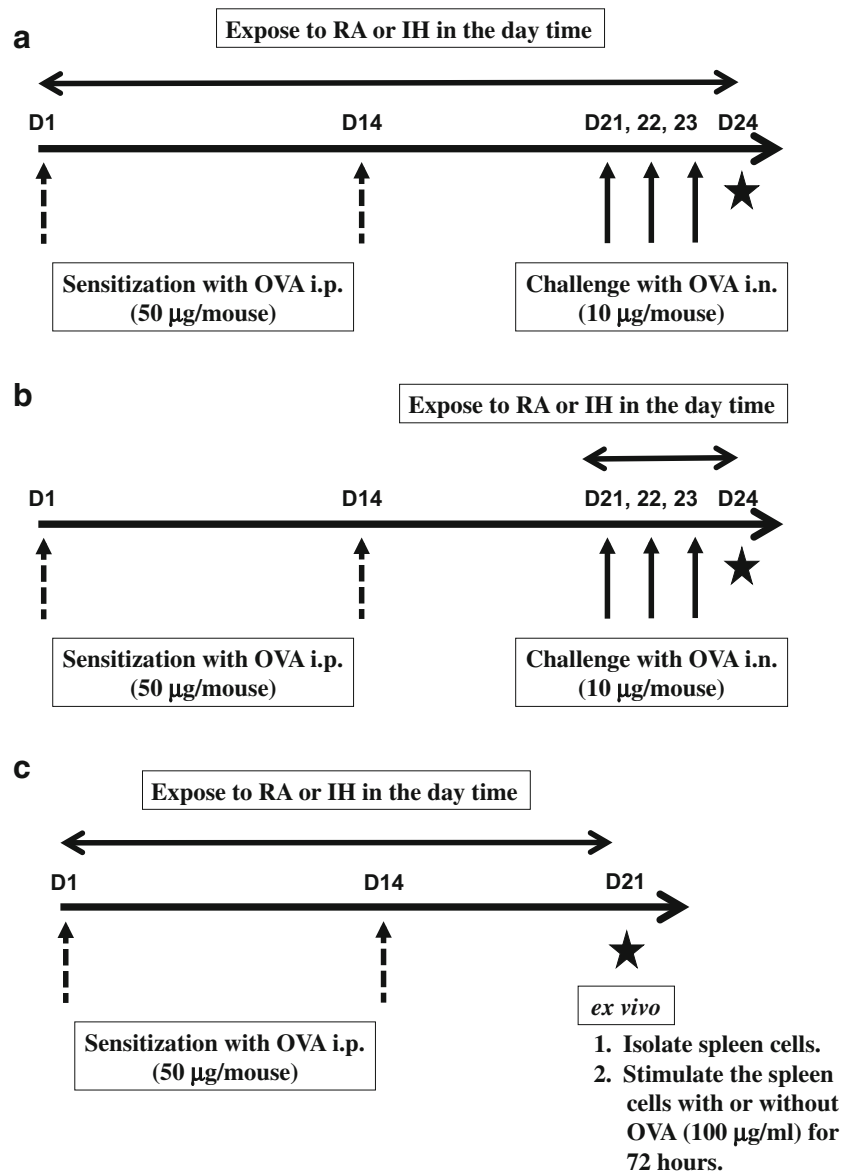
Analysis of lung histopathology

After performing BAL, the lungs were removed from the chest and inflated with 500 µL of 10% formalin in PBS (pH 7.4), followed by continuous inflation for 24 h at 10 cm H₂O pressure. The lung tissues were dehydrated in ethanol, embedded in paraffin, sectioned (5 µm), and stained with Periodic Acid-Schiff (PAS; Sigma-Aldrich). For analysis of goblet cells, PAS-positive cells in the lungs were classified on a 0–4+ score according to the PAS grading method [31]. The scores were determined as follows: 0, < 0.5% PAS-positive cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, > 75%.

Measurement of airway responsiveness

Airway responsiveness to acetylcholine was measured as previously described in short exposure group [32, 33]. Briefly, the mice were tracheostomized and connected to a Harvard ventilator, under general anesthesia and volume control mandatory ventilation after receiving pentobarbital and xylazine.

Fig. 1 Protocol for murine model of OVA-induced allergic airway inflammation and IH exposure. Long exposure (a), short exposure (b), and exposure to IH in the sensitization phase (c). OVA, ovalbumin; RA, room air; IH, intermittent hypoxia



Then, they were placed in a whole-body plethysmograph (Buxco Electronics Inc., Troy, NY, USA) to measure airway resistance (Raw). Five milligrams per milliliter of acetylcholine was administered for 3 min using an in-line aerosol system. Data are expressed as percent Raw, which is defined by the formula (Raw after inhalation of acetylcholine/Raw before inhalation) $\times$ 100 (%).

Exposure to IH during the sensitization phase

To investigate the effect of IH in the sensitization phase, the mice were exposed to IH or RA during the OVA sensitization from days 1 to 21. After the exposure, spleen cells were collected and stimulated with OVA *in vitro* (Fig. 1c).

The mice were sensitized with OVA emulsified in alum (50 µg/mouse) administered intraperitoneally on days 1 and

14. From days 1 to 21, the mice were exposed to either RA or IH. On day 21, the mice were sacrificed, and their spleens and serum were collected. The spleen cells were isolated and suspended in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% fetal bovine serum and antibiotics (penicillin, 100 mg/mL; streptomycin, 100 U/mL) at a concentration of 4.0×10^6 cells/mL. The cells were stimulated with OVA (100 µg/mL) in 48-well plates at 37 °C in 5% CO₂. After 72 h of OVA stimulation, supernatants were collected to analyze cytokine production (IL-4, IL-5, IL-13, and IFN- γ) using ELISA kits (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Differences between two groups were investigated by unpaired *t* tests. The significance of the differences between

the three groups was determined by analysis of variance (ANOVA) with Bonferroni correction. All experiments were performed at least twice with 4 or more animals per group. Data are expressed as means $\pm$ standard errors of the means (SEMs), and $P < 0.05$ was considered to indicate significance.

Results

Long exposure to IH inhibits allergic airway inflammation

We investigated the effect of IH on allergic airway inflammation. The OVA-sensitized and OVA-challenged mice showed significant increased total cell, eosinophil, and macrophage numbers in the BALF compared to those in the control mice (Fig. 2a). In contrast, the numbers of total cells, eosinophils, and macrophages in the BALF significantly decreased with long exposure to IH ($n = 6$ for all groups; Fig. 2a, b). There was no difference in the numbers of lymphocytes between groups. We also measured the levels of Th₂- (IL-4, IL-5, and IL-13) and Th₁- (IFN- γ) type cytokines in the BALF. The levels of IL-5 and IL-13 were significantly decreased in the OVA-sensitized and OVA-challenged mice with long exposure to IH compared to those exposed to RA ($n = 6$ for all groups; Fig. 2c). These results indicated that long exposure to IH inhibited the OVA-induced allergic airway inflammation in the mouse model. However, there was no difference in the levels of the other cytokines such as IL-4 and IFN- γ following exposure to RA and IH in OVA-sensitized and OVA-challenged mice.

Short exposure to IH inhibits allergic airway inflammation

To investigate the effect of IH on OVA challenge, we exposed some mice to RA or IH only during the challenge phase (short exposure). The OVA-sensitized and OVA-challenged mice with short exposure to RA showed increased total cell and eosinophil numbers in the BALF compared to those in the control mice (Fig. 3a). The total cell and eosinophil numbers in the BALF significantly decreased by short exposure to IH ($n = 4-5$; Fig. 3a, b). The levels of IL-5 and IL-13 in the BALF were significantly higher in the OVA-sensitized and OVA-challenged mice with short exposure to RA compared to those in the control mice. The short exposure to IH significantly reduced the levels of IL-5 and IL-13 in comparison with exposure to RA ($n = 4-5$; Fig. 3c). However, there was no significant difference between short exposure to RA and IH in the levels of IL-4 and IFN- γ . These results may suggest that short exposure to IH only during the challenge phase also inhibited the OVA-induced allergic airway inflammation.

Short exposure to IH inhibits goblet cell hyperplasia and prevents AHR

Goblet cell hyperplasia was evaluated by the PAS grading method. The levels of PAS-positive cells were significantly increased in OVA-sensitized and OVA-challenged mice with short exposure to RA. In short exposure to IH in the OVA-sensitized and OVA-challenged mice, the levels of PAS-positive cells were significantly reduced compared to the levels following exposure to RA ($n = 10-12$; Fig. 4).

Airway responsiveness to acetylcholine was assessed in our murine model of allergic airway inflammation with short exposure to RA or IH. No Raw differences were observed between non-OVA RA- and non-OVA IH-exposed mice (Fig. 5). The OVA-sensitized and OVA-challenged mice showed significantly higher AHR to acetylcholine compared to that in the control mice, i.e., non-OVA RA-exposed mice and non-OVA IH-exposed mice (Fig. 5). Short exposure to IH in OVA-sensitized and OVA-challenged mice significantly inhibited the AHR to acetylcholine induced by OVA sensitization and challenge ($n = 6$ for all groups; Fig. 5).

The effect of IH exposure during the sensitization phase

To investigate the effect of IH on OVA sensitization, mice were exposed to RA or IH only during the sensitization phase (days 1 to 21). Then, spleen cells were isolated and cultured with or without OVA stimulation. After 72 h of OVA stimulation, supernatants were harvested and cytokines were measured. There was a significant increase in the levels of Th₂-type cytokines, such as IL-4, IL-5, and IL-13, in OVA-stimulated spleen cells obtained from OVA-sensitized mice compared with those of the OVA-stimulated spleen cells obtained from the control mice ($n = 6$ for all groups; Fig. 6). The levels of these cytokines (IL-4, IL-5, and IL-13) from OVA-stimulated spleen cells obtained from OVA-sensitized and exposed to IH mice was significantly inhibited compared with those of the OVA-stimulated spleen cells without IH exposure. There was no significant difference in the levels of IFN- γ among the all three groups with OVA stimulation (Fig. 6).

Discussion

In the present study, we have reported that IH inhibits allergic airway inflammation in an OVA-induced murine model of asthma. IH is one of the major features in OSAS patients. And most of the OSAS patients have obesity, which leads to IH due to obstruction of the upper airways during sleep. Obesity is known as a factor that exacerbates asthma symptoms in patients with asthma [4–7]. Also, OSAS is thought to contribute to the exacerbation of asthma control in obese

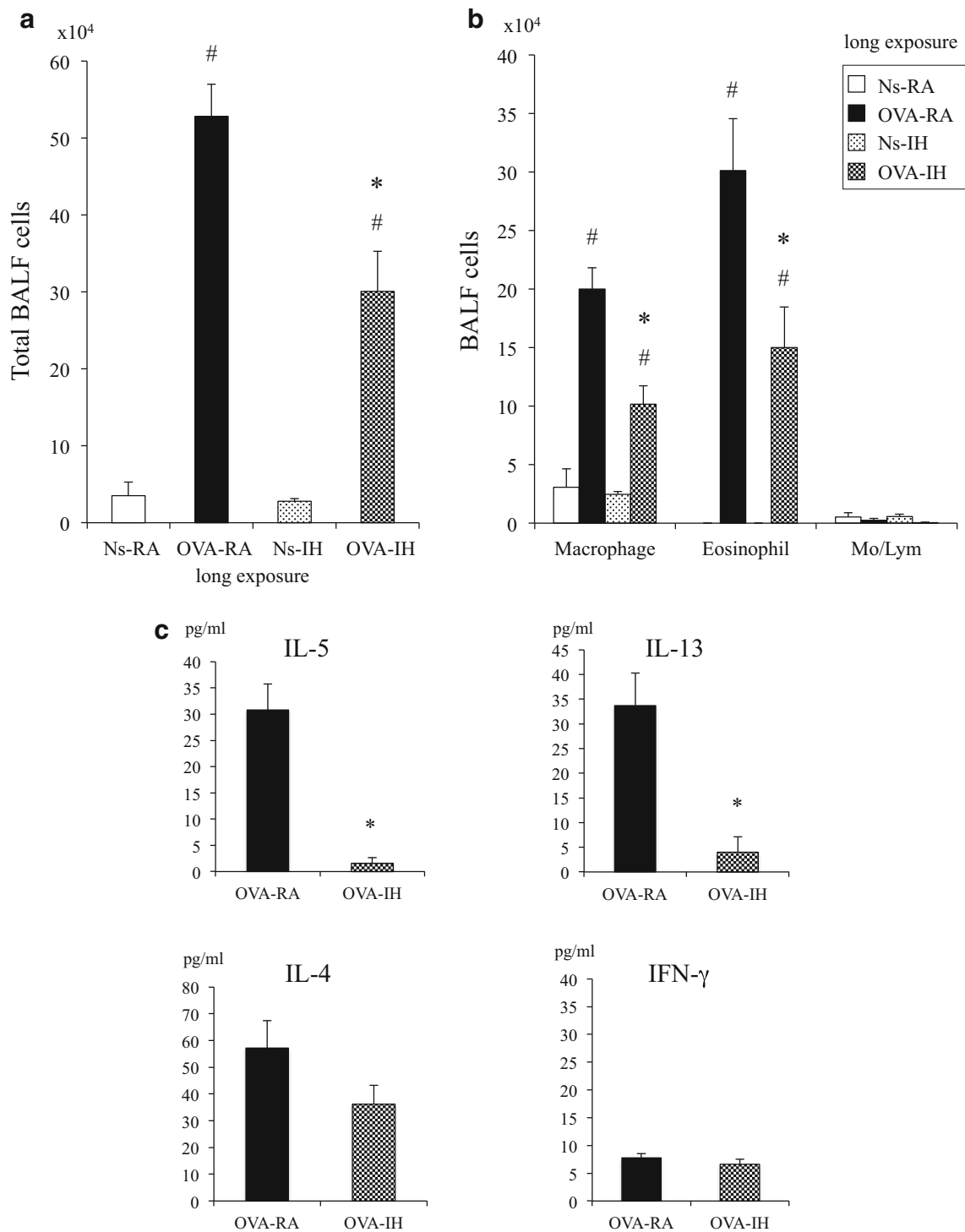


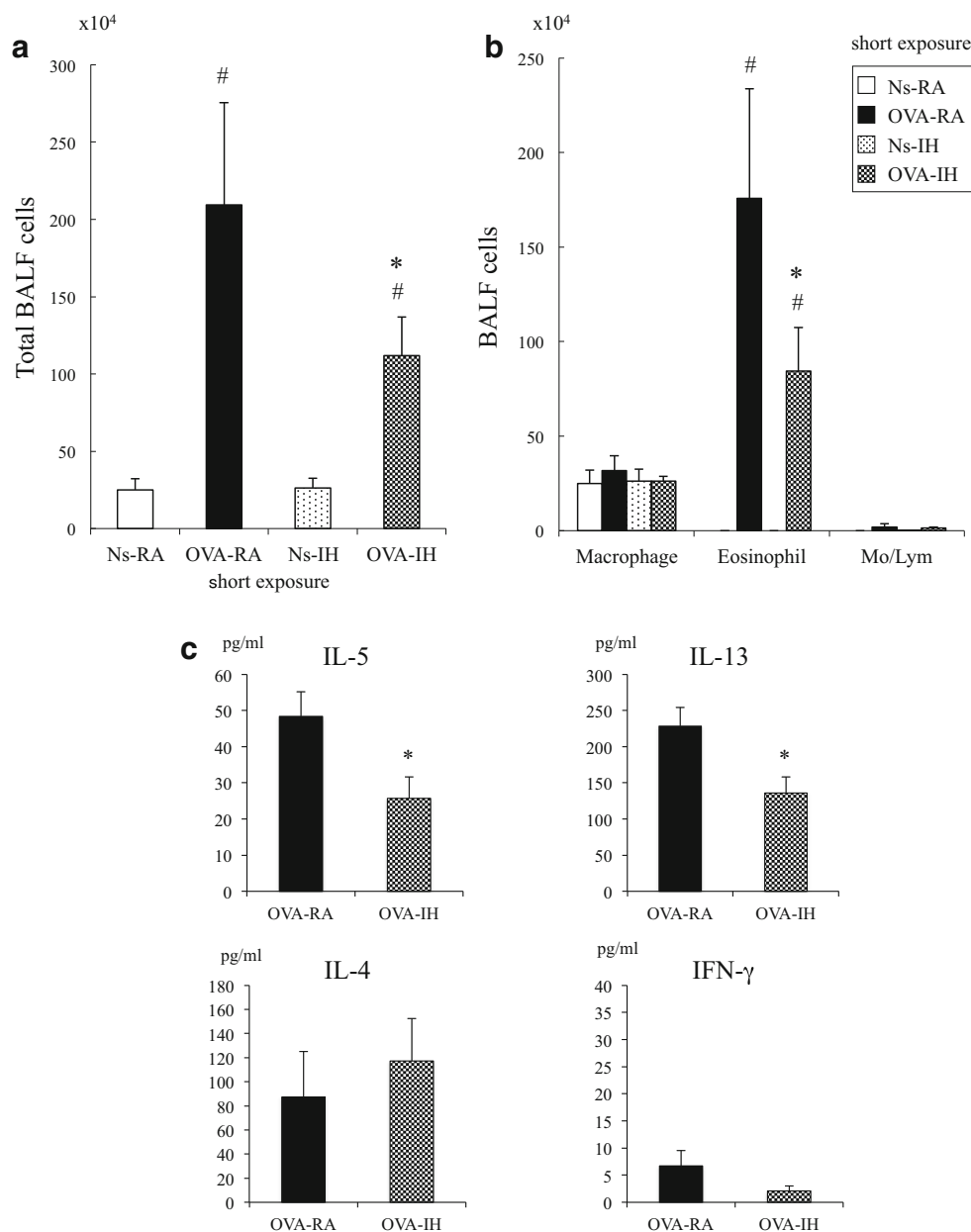
Fig. 2 OVA-induced allergic airway inflammation with long exposure to RA or IH. Total number of inflammatory cells (**a**) and differential count of the inflammatory cells (**b**) in the BALF. Cytokine levels in the BALF (**c**). Results are expressed as mean $\pm$ SEM ($n = 6$ for all groups). * $P < 0.05$

compared with OVA RA, [#] $P < 0.05$ compared with Ns-RA. Ns, normal saline; OVA, ovalbumin; RA, long exposure to room air; IH, long exposure to intermittent hypoxia; BALF, bronchoalveolar lavage fluid

patients with asthma [8]. Moreover, CPAP treatment may improve asthma quality of life in patients with asthma suffering from OSAS [12]. Allkhalil et al. also reported that CPAP treatment might modify airway smooth muscle function and

asthma control in asthma patients with OSAS [11]. However, only few studies have assessed the association between IH and asthma. Thus, we investigated the effects of IH on allergic airway inflammation using a murine model of asthma.

Fig. 3 OVA-induced allergic airway inflammation with short exposure to RA or IH. Total number of inflammatory cells (**a**) and differential count of the inflammatory cells (**b**) in the BALF. Cytokine levels in the BALF (**c**). Results are expressed as mean $\pm$ SEM ($n = 4-5$). * $P < 0.05$ compared with OVA-RA, # $P < 0.05$ compared with Ns-RA. Ns, normal saline; OVA, ovalbumin; RA, short exposure to room air; IH, short exposure to intermittent hypoxia; BALF, bronchoalveolar lavage fluid



First, we exposed mice to IH in both the OVA sensitization and challenge phases (long exposure). The long exposure to IH inhibited the increase in total cell and eosinophil numbers in the BALF in our murine model of asthma. This result may suggest that long exposure to IH inhibits the eosinophilic inflammation in the airway, which is one of the characteristic features of asthma. We also measured the levels of Th₂- and Th₁-type cytokines such as IL-4, IL-5, IL-13, and IFN- γ in the BALF. The levels of IL-5 and IL-13 were reduced with long exposure to IH in the murine model of asthma. There was no significant difference in the levels of IFN- γ , a Th₁-type cytokine. These results suggest that long exposure to IH could inhibit OVA-induced allergic airway inflammation through the reduction of Th₂-type cytokine production.

In a previous study, Broymann et al. investigated the effect of chronic IH (CIH) exposure in a rat model of OVA-induced airway inflammation [34]. This study shows that the CIH altered immune response to allergen toward Th₁ predominance, which induced collagen deposition and matrix degradation and led to airflow limitation. In this study, there was no difference in eosinophilia between two OVA-induced asthma model groups having received OVA sensitization and challenge, i.e., with or without IH exposure. However interestingly, in wild-type rats with OVA sensitization and vehicle inhalation without OVA challenge, the number of eosinophils in BALF was significantly decreased in the IH-exposed group compared to the non-exposed group. These results support our results that IH inhibited the eosinophilia in asthma model of

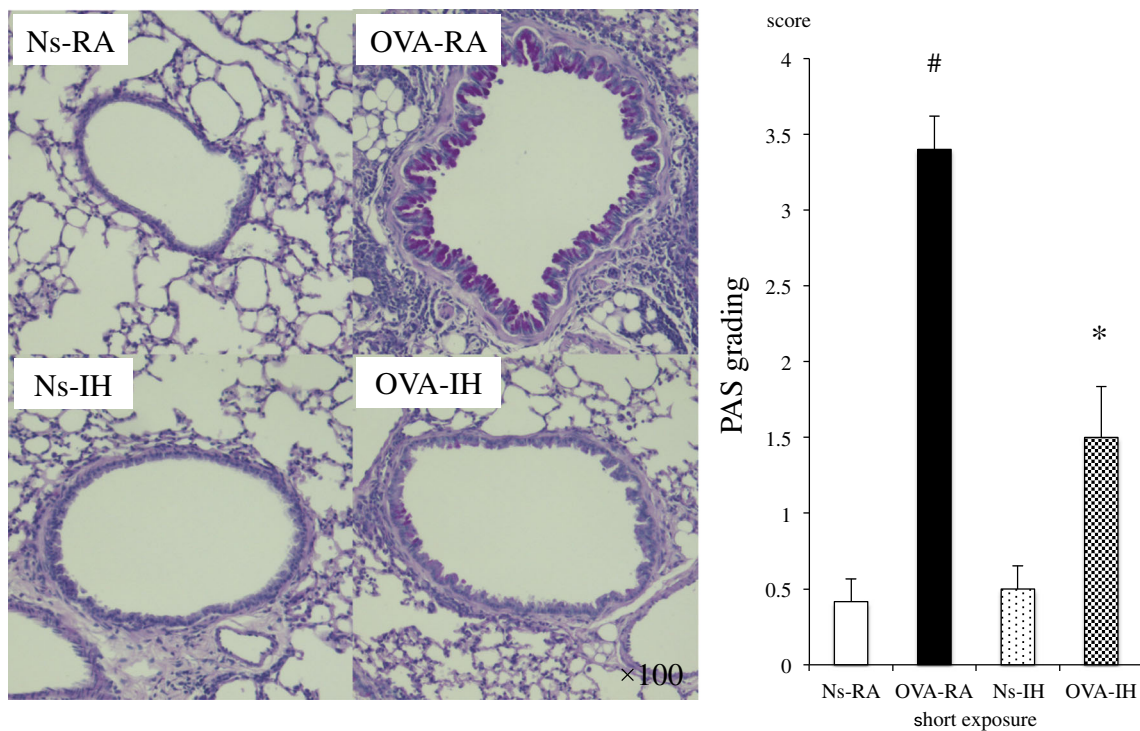


Fig. 4 OVA-induced allergic airway inflammation with short exposure to RA or IH. Representative lung sections showing PAS staining and statistic analysis for PAS grading method (0–4+ scale). Results are expressed as mean $\pm$ SEM ($n = 10$ – 12). * $P < 0.01$ compared with OVA

RA, # $P < 0.05$ compared with Ns-RA. PAS, Periodic Acid-Schiff; Ns, normal saline; OVA, ovalbumin; RA, short exposure to room air; IH, short exposure to intermittent hypoxia

mice toward Th₁ predominance. One reason why IH only reduced the eosinophilia in the wild-type rat (OVA sensitization and no OVA challenge) but not in the asthma model might be the allergic inflammation in this rat model was much

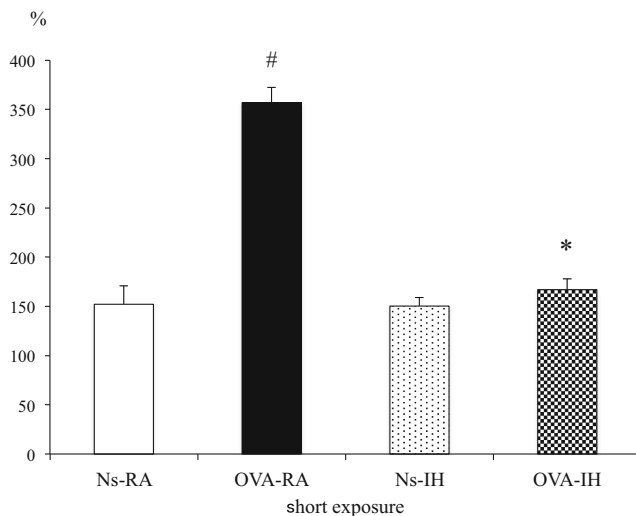


Fig. 5 Airway responsiveness to acetylcholine in OVA-induced allergic airway inflammation with short exposure to RA or IH. Results are expressed as mean $\pm$ SEM ($n = 6$ for all groups). * $P < 0.01$ compared with OVA RA, # $P < 0.05$ compared with Ns-RA. Ns, normal saline; OVA, ovalbumin; RA, short exposure to room air; IH, short exposure to intermittent hypoxia

stronger than our mouse model of asthma, and IH exposure was considered to be not sufficient to suppress the eosinophilia in their rat asthma model.

Also, Arnaud et al. showed that the production of RANTES/CCL5, Interleukin Adhesion Molecule (ICAM)-1, and IFN- γ were induced by exposure to IH in mice, which leads to enhanced Th₁-type inflammation [26]. In the present study, as only Th₂-type cytokines were inhibited by IH exposure and not Th₁-type cytokines, IH exposure appears to induce Th₁-type inflammation; however, the Th₁/Th₂ paradigm might have been collapsed and the Th₁ predominance led to inhibition of the Th₂-type inflammation in the murine asthma model. Additionally, the hypoxic stress acts on the neuroendocrine response, which may modify the airway inflammation. To clarify the mechanisms of our observation, the involvement of a pathway via the neuroendocrine responses should be considered.

First, we found that allergic airway inflammation was induced by long exposure to IH in murine model of asthma. In this model, there are two phases, OVA sensitization phase and challenge phase. So as the next step, in order to identify which phase is the phase when IH could show the inhibitory effects on the allergic inflammation, we investigated the inhibitory effects of exposure to IH by separating the IH exposure into two occasions, i.e., OVA sensitization and challenge phases. Similar to the long exposure to IH, analyses of the BALF

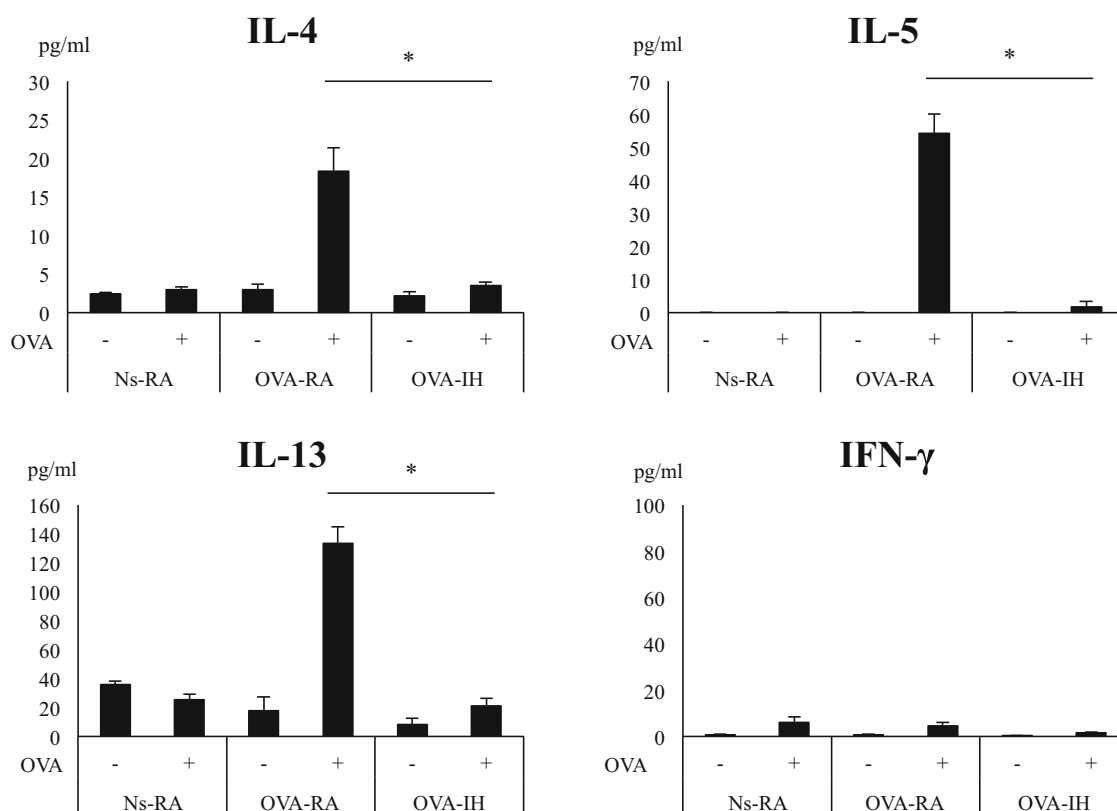


Fig. 6 Analysis of cytokines in the supernatant after stimulation of spleen cells with or without OVA. Murine spleen cells were collected after OVA sensitization and exposed to either RA or IH. The cells were stimulated for 72 h then supernatants were collected and analyzed for IL-4, IL-5, IL-

13, and IFN- γ . Results are expressed as mean $\pm$ SEM ($n = 6$ for all groups). * $P < 0.05$. Ns, normal saline; OVA, ovalbumin; RA, exposure to room air during the sensitization phase; IH, exposure to intermittent hypoxia during the sensitization phase

revealed that the short exposure to IH during the challenge phase can inhibit eosinophilic airway inflammation and reduce Th₂-type cytokine production. These results indicate that the exposure to IH only during the challenge phase could inhibit the allergic airway inflammation even in the mice already sensitized to OVA.

In both long and short exposure to IH significantly inhibited the levels of IL-5 and IL-13 but not IL-4 in murine model of asthma. However, when the exposure to IH during the sensitization phase and stimulate the spleen cells with OVA in vitro, the levels of IL-4 in addition to IL-5 and IL-13 were inhibited. These results indicate that the ability to produce IL-4 is suppressed only in spleen cells, which are mainly consisting of T cells, whereas IL-5 and IL-13 are also suppressed in other inflammatory cells, e.g., eosinophils, mast cells, and macrophages. However, intimate mechanisms of this remain unclear.

Histological analysis also showed that short exposure to IH had an inhibitory effect on goblet cell hyperplasia, one of the histological characteristics of allergic airway inflammation. In order to investigate the functional effect of short exposure to IH in the asthma model, we measured the airway responsiveness to acetylcholine. OVA sensitization and challenge induced AHR. The short exposure to IH inhibited the OVA-

induced AHR, suggesting that IH exposure could inhibit the functional changes elicited by allergic airway inflammation.

IH reportedly induces the intravascular inflammation and accelerates the vascular remodeling, which causes atherosclerosis [35–37]. However, intriguingly, we showed that pathological changes in the airway and induction of AHR caused by airway allergic inflammation were inhibited by IH exposure. The exact mechanisms of this observation require further investigation in this model.

We also investigated the impact of IH on allergic airway inflammation during the allergen sensitization phase. For that purpose, the mice were exposed to RA or IH during the OVA sensitization phase. Then, their spleen cells were isolated and stimulated with OVA in vitro. We found that the spleen cells produced Th₂-type cytokines such as IL-4, IL-5, and IL-13, only when exposed to RA and not to IH. This result suggests that IH exposure inhibited the OVA sensitization. Therefore, there may be an additional anti-allergic inflammatory effect with exposure to IH during the sensitization phase, independent from its effect during the challenge phase.

The results of the current study show that exposure to IH inhibits the allergic inflammation in both the sensitization and challenge phases, which suggests that IH exposure is not an exacerbating factor of asthma. These findings were opposite

of those of a previous report, which showed that OSAS patients have more severe asthma than non-OSAS patients do [8]. However, based on the finding of the current study, the exacerbation of asthma in patients with OSAS might not be attributable to IH.

It has been reported that 4 weeks of IH exposure to mice did not affect malondialdehyde (MDA) levels in the aorta and heart, whereas the liver appeared to be affected as increase in MDA levels, a marker of increased reactive oxygen species (ROS) generation [38]. In addition, in a study that examined the effect of IH exposure in rats, when the IH was exposed 15 s or 90 s for 10 days, only 15 s of IH exposure increased the generation of ROS in dorsal and ventral medulla [39]. From these study, the effect of oxidative stress caused by IH exposure seems to be depends on each organs or/and the exposure time. The discrepancy between our study and others might be due to these matters.

One limitation of this study was the relatively short exposure to IH, which was only either during the OVA challenge or sensitization phase and for a short duration compared to human OSAS patients. In addition to short-term exposure to IH, our asthma model in this study employed only OVA as an allergen, while in the real world multiple allergens or epitopes are involved in the allergic response. Thus, the results may differ with longer-term exposure to IH in a chronic model of asthma or with using other allergens to establish asthma model. Moreover, in this study, the hypoxia was not induced by airway obstruction and the mice were not obese, which may have affected the results.

In conclusion, on the contrary to our prediction, the exposure of IH inhibited the allergic airway inflammation in our murine model of asthma. Our findings suggest that the deterioration of asthma with OSAS is not simply caused by IH based on OSAS but it may have other factors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

- Partinen M, Telakivi T (1992) Epidemiology of obstructive sleep apnea syndrome. *Sleep* 15:S1–S4
- Durán J, Esnaola S, Rubio R, Iztueta A (2001) Obstructive sleep apnea-hypopnea and related clinical features in a population-based sample of subjects aged 30 to 70 yr. *Am J Respir Crit Care Med* 163(3 Pt 1):685–689
- Peppard PE, Young T, Palta M, Dempsey J, Skatrud J (2000) Longitudinal study of moderate weight change and sleep-disordered breathing. *JAMA* 284(23):3015–3021
- Farah CS, Salome CM (2010) Asthma and obesity: a known association but unknown mechanism. *Respirology* 17:412–421
- Mosen DM, Schatz M, Magid DJ, Camargo CA Jr (2008) The relationship between obesity and asthma severity and control in adults. *J Allergy Clin Immunol* 122:507–511
- Taylor B, Mannino D, Brown C, Crocker D, Twum-Baah N, Holguin F (2008) Body mass index and asthma severity in the National Asthma Survey. *Thorax* 63:14–20
- Vortmann M, Eisner MD (2008) BMI and health status among adults with asthma. *Obesity* 16:146–152
- Julien JY, Martin JG, Ernst P, Olivenstein R, Hamid Q, Lemièrre C, Pepe C, Naor N, Olha A, Kimoff RJ (2009) Prevalence of obstructive sleep apnea-hypopnea in severe versus moderate asthma. *J Allergy Clin Immunol* 124:371–376
- Teodorescu M, Polomis DA, Hall SV, Teodorescu MC, Gangnon RE, Peterson AG, Xie A, Sorkness CA, Jarjour NN (2010) Association of obstructive sleep apnea risk with asthma control in adults. *Chest* 138:543–550
- Alkhalil M, Schulman E, Getsy J (2009) Obstructive sleep apnea syndrome and asthma: what are the links? *J Clin Sleep Med* 5(1): 71–79
- Alkhalil M, Schulman ES, Getsy J (2008) Obstructive sleep apnea syndrome and asthma: the role of continuous positive airway pressure treatment. *Ann Allergy Asthma Immunol* 101:350–357
- Lafond C, Sériè F, Lemièrre C (2007) Impact of CPAP on asthmatic patients with obstructive sleep apnea. *Eur Respir J* 29:307–311
- Ciftci TU, Ciftci B, Guven SF, Kokturk O, Turktaş H (2005) Effect of nasal continuous positive airway pressure in uncontrolled nocturnal asthmatic patients with obstructive sleep apnea syndrome. *Respir Med* 99(5):529–534
- Chan CS, Woolcock AJ, Sullivan CE (1988) Nocturnal asthma: role of snoring and obstructive sleep apnea. *Am Rev Respir Dis* 137: 1502–1504
- Martin JM, Carrizo SJ, Vicente E, Agustí AG (2005) Long-term cardiovascular outcomes in me with obstructive sleep apnea-hypopnea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 365:1046–1053
- Arzt M, Young T, Finn L, Skatrud JB, Bradley TD (2005) Association of sleep-disordered breathing and the occurrence of stroke. *Am J Respir Crit Care Med* 172:1447–1451
- Yaggi HK, Concato J, Keman WN, Lichtman JH, Brass LM, Mohsenin V (2005) Obstructive sleep apnea as a risk factor for stroke and death. *N Engl J Med* 353:2034–2041
- Peker Y, Hedner J, Norum J, Kraiczi H, Carlson J (2002) Increased incidence of cardiovascular disease in middle-aged men with obstructive sleep apnea: a 7-year follow up. *Am J Respir Crit Care Med* 166:159–165
- Shahar E, Whitney CW, Redline S, Lee ET, Newman AB, Javier NF, O'Connor GT, Boland LL, Schwartz JE, Samet JM (2001) Sleep-disordered breathing and cardiovascular disease: cross-sectional results of the Sleep Heart Health study. *Am J Respir Crit Care Med* 163:19–25
- Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Oda N, Tanaka A, Yamamoto Y, Ohta S, O'Donnell CP, Adachi M (2007) Silent brain infarction and platelet activation in obstructive sleep apnea. *Am J Respir Crit Care Med* 175:612–617
- Ryan S, Taylor CT, McNicholas WT (2005) Selective activation of inflammatory pathways by intermittent hypoxia in obstructive sleep apnea syndrome. *Circulation* 112:2660–2667

22. Hansson GK, Libby P (2006) The immune response in atherosclerosis: a double-edged sword. *Nat Rev Immunol* 6:508–519
23. Tedgui A, Mallat Z (2006) Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* 86:515–581
24. Nadeem R, Molnar J, Madbouly EM, Nida M, Aggarwal S, Sajid H, Naseem J, Loomba R (2013) Serum inflammatory markers in obstructive sleep apnea: a meta-analysis. *J Clin Sleep Med* 9(10):1003–1012
25. Minoguchi K, Yokoe T, Tazaki T, Minoguchi H, Tanaka A, Oda N, Okada S, Ohta S, Naito H, Adachi M (2005) Increased carotid intima-media thickness and serum inflammatory markers in obstructive sleep apnea. *Am J Respir Crit Care Med* 172:625–630
26. Arnaud C, Beguin PC, Lantuejoul S, Pépin JL, Guillemet C, Pelli G, Burger F, Buatois V, Ribuot C, Baguet JP, Mach F, Lévy P, Dematteis M (2011) The inflammatory preatherosclerotic remodeling induced by intermittent hypoxia is attenuated by RANTES/CCL5 inhibition. *Am J Respir Crit Care Med* 184:724–731
27. Stanke-Labesque F, Bäck M, Lefebvre B, Tamisier R, Baguet JP, Arnol N, Lévy P, Pépin JL (2009) Increased urinary leukotriene B4 excretion in obstructive sleep apnea: effects of obesity and hypoxia. *J Allergy Clin Immunol* 124:364–370
28. Lefebvre B, Pépin JL, Baguet JP, Roustit M, Riedweg K, Bessard G, Lévy P, Stanke-Labesque F (2008) Leukotriene B4: early mediator of atherosclerosis in obstructive sleep apnea? *Eur Respir J* 32:113–120
29. Gautier-Veyret E, Arnoud C, Bäck M, Pépin JL, Petri MH, Baguet JP, Tamisier R, Lévy P, Stanke-Labesque F (2013) Intermittent hypoxia-activated cyclooxygenase pathway: role in atherosclerosis. *Eur Respir J* 42:404–413
30. Yokoe T, Alonso LC, Romano LC, Rosa TC, O'Doherty RM, Garcia-Ocana A, Minoguchi K, O'Donnell CP (2008) Intermittent hypoxia reverses the diurnal glucose rhythm and causes pancreatic β -cell replication in mice. *J Physiol* 586:899–911
31. Myou S, Leff AR, Myo S, Boetticher E, Tong J, Meliton AY, Liu J, Munoz NM, Zhu X (2003) Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase-TAT. *J Exp Med* 198(10):1573–1582
32. Ohta S, Oda N, Yokoe T, Yamamoto Y, Watanabe Y, Minoguchi K, Ohnishi T, Hirose T, Nagase H, Ohta K, Adachi M (2010) Effect of tiotropium bromide on airway inflammation and remodeling in a mouse model of asthma. *Clin Exp Allergy* 40:1266–1275
33. Yamashita N, Sekine K, Miyasaka T, Kawashima R, Nakajima Y, Nakano J, Yamamoto T, Horiuchi T, Hirai K, Ohta K (2001) Platelet-derived growth factor is involved in the augmentation of airway responsiveness through remodeling of airways in diesel exhaust particulate-treated mice. *J Allergy Clin Immunol* 107:135–142
34. Broymann O, Braun RK, Morgan BJ, Pegelow DF, Hsu PN, Mei LS, Koya AK, Eldridge M, Teodorescu M (2015) Effects of chronic intermittent hypoxia on allergen-induced airway inflammation in rats. *Am J Respir Cell Mol Biol* 52(2):162–170
35. Drager LF, Yao Q, Hernandez KL, Shin MK, Bevans-Fonti S, Gay J (2013) Chronic intermittent hypoxia induces atherosclerosis via activation of adipose-angiopoietin-like 4. *Am J Respir Crit Care Med* 188(2):240–248
36. Dumitrascu R, Heitmann J, Seeger W, Weissmann N, Schulz R (2013) Obstructive sleep apnea, oxidative stress and cardiovascular disease: lessons from animal studies. *Eur Respir J* 42(2):404–413
37. Quercioli A, Mach F, Montecucco F (2010) Inflammation accelerates atherosclerotic processes in obstructive sleep apnea syndrome (OSAS). *Sleep Breath* 14(3):261–269
38. Jun J, Savransky V, Nanayakkara A, Bevans S, Li J, Smith PL, Polotsky VY (2008) Intermittent hypoxia has organ-specific effects on oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 295(4):1274–1281
39. Raghuraman G, Rai V, Peng YJ, Prabhakar NR, Kumar GK (2009) Pattern-specific sustained activation of tyrosine hydroxylase by intermittent hypoxia: role of reactive oxygen species-dependent downregulation of protein phosphatase 2A and upregulation of protein kinases. *Antioxid Redox Signal* 11(8):1777–1789

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