

Dexmedetomidine reduces IL-4 and IgE expression through downregulation of the TLR4/NF- κ B signaling pathway to alleviate airway hyperresponsiveness in OVA mice

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ABSTRACT

Background: Airway hyperresponsiveness (AHR) is a clinical manifestation of airflow limitation due to abnormal tracheal and bronchial sensitivity and is the main basis for the diagnosis of asthma. Patients with AHR are at high risk of perioperative tracheal and bronchospasm, which can lead to hypoxaemia and haemodynamic instability and, in severe cases, to a life-threatening 'silent lung'. It is therefore important to reduce the incidence or intensity of AHR episodes in the perioperative period. The inflammatory response is key to the development and progression of AHR.

Hypothesis/purpose: Based on the modulatory role of dexmedetomidine (DEX) in the inflammatory response, we hypothesised that dexmedetomidine (DEX) attenuates inflammatory properties by inhibiting the toll-like receptor 4 (TLR4)/nuclear factor (NF- κ B) signalling pathway and can reduce the respiratory parameters of mechanical ventilation in ovalbumin-induced allergic airway hyperresponsiveness.

Study design: BALB/C mice were divided into control and OVA groups (ovalbumin-induced allergy). Ten mice in all OVA models were randomly selected for in vivo invasive lung function monitoring to analyse airway resistance parameters and demonstrate successful model establishment. The remaining OVA mice were treated with dexmedetomidine 25 μ g/kg for 5 days (OVA + DEX group) or dexmedetomidine 25 μ g/kg + yohimbine 1 mg/kg for 5 days (OVA + DEX + yohimbine). After treatment, bronchoalveolar lavage fluid (BAL) and peripheral blood (ELISA) and lung tissue (H&E and PAS) were collected for analysis of inflammatory factors, and lung tissue was verified by PCR for genes and proteins that do correlate with inflammatory mediators.

Results: All airway resistance parameters were increased in OVA mice by invasive lung function monitoring. Proximal airway resistance (parameter Rn) and total respiratory resistance (parameter Rrs) were attenuated after dexmedetomidine intervention treatment. Dexmedetomidine reduced total inflammatory cell count and inflammatory infiltration of lung tissue in BALF and down-regulated IL-4 and IgE levels in BALF and peripheral blood, as shown by Giemsa, H&E, PAS staining and ELISA; this mechanism of action was found to be related to the TLR4/NF κ B pathway, but not to TLR4/NF κ B, as measured by PCR.

Conclusion: Dexmedetomidine reduces hyperresponsiveness and airway inflammatory responses. This mechanism of action may be related to the TLR4/NF κ B signalling pathway. Overall conclusions are presented in.

1. Introduction

Asthma is a heterogeneous respiratory disease and a serious global health problem [6]. It is estimated that asthma affects over 300 million people worldwide, up to 10% of adults and 30% of children [1], with

380,000 deaths each year [2]. The Lancet reported in 2015 that the prevalence of asthma in China was 4.2% [3]. According to the Global Initiative for Asthma 2020 (GINA), the prevalence of asthma is still rising in many countries [4]. It is now generally accepted that airway hyperreactivity is the main pathological feature and diagnostic indicator

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of asthma. Airway HyperResponsiveness (AHR) is caused by chemical or physical factors (internal or external) that can trigger excessive and premature contraction of airway smooth muscle and increase mucus secretion and microvascular leakage [5]. In addition, many clinical diseases (including asthma, chronic obstructive pulmonary disease, allergic eutyroidism, novel coronavirus pneumonia, gastroesophageal reflux, etc.) and environmental factors (irritants, PM_{2.5}) can cause neuron sensitization, airway inflammation [3,9] thus airway hyperactivity. Some clinical patients can have increased airway pressure when exposed to certain anaesthetics and fluids due to underlying allergies. The behaviour of AHR is often insidious and unpredictable. AHR is not only seen in asthma (i.e. major clinical manifestation of asthma) but also seen in other diseases. Asthma patients are at high risk of perioperative tracheal and bronchospasm due to AHR, which can lead to hypoxaemia and haemodynamic instability, and in severe cases life-threatening “silent lung”. It is therefore important to prevent the development of AHR or to reduce the intensity of HAR in the perioperative period. In general, preoperative use of β_2 agonists can treat or prevent asthma patients, but there are no good preventive measures for patients with potential AHR due to other diseases during the perioperative period. Thus, it is important to underlying the mechanism for AHR and to develop effective preventive and therapeutic therapies for patients’ airway protection and, even for saving lives during the perioperative period. Fig. 1

Dexmedetomidine (DEX) is a highly selective α_2 -adrenergic agonist with sedative, analgesic and anxiolytic effects. It is commonly used in anaesthesia. The protective effect of DEX on lung injury has been studied extensively [7,8]. In addition, the immunomodulatory effects of dexmedetomidine have been noted [9]. Studies have shown that DEX affects immunity by modulating specific Th2 helper cells [10,11], which are

major players in asthma, mediating inflammatory responses and AHR through the production of type 2 cytokines (IL-4, IL-13, thymic stromal lymphopoietin (TSLP), etc.) [11]. Dexmedetomidine reduces inflammatory factor secretion by attenuating NF κ B signalling pathway phosphorylation in monocytes and macrophages [12]. Meanwhile, Hammad [11] noted that most allergens and many air pollutants trigger the NF- κ B signalling pathway to promote Th2 helper cells development by activating Toll-like receptors (TLRs), thus, among others, activating innate immune cells and subsequently producing many mediators that contribute to airway inflammation. Manson [13] found that IL-13 and IL-4 induced AHR rather than IL-5 and IL17A. Therefore, we hypothesise that dexmedetomidine prevents the incidence or intensity of perioperative AHR by downregulating IL-4 or (and) IL-13 in OVA mice through downregulation of TLR4 (or TLR2)/NF- κ B signalling pathways.

2. Methods

2.1. Study approval

All animal care and experiments were performed by following per under guidelines established by the Research Animal Care Subcommittee of the Plastic Surgery Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences. These experiments were approved by the Institutional Animal Care and Use Committee (20181205) of the Plastic Surgery Hospital, Peking Union Medical College. All methods are reported in accordance with ARRIVE guidelines (Animal Research: Reporting In Vivo Experiments Guidelines). Laboratory Animal Practitioner Certificate Number: 1119121800169.

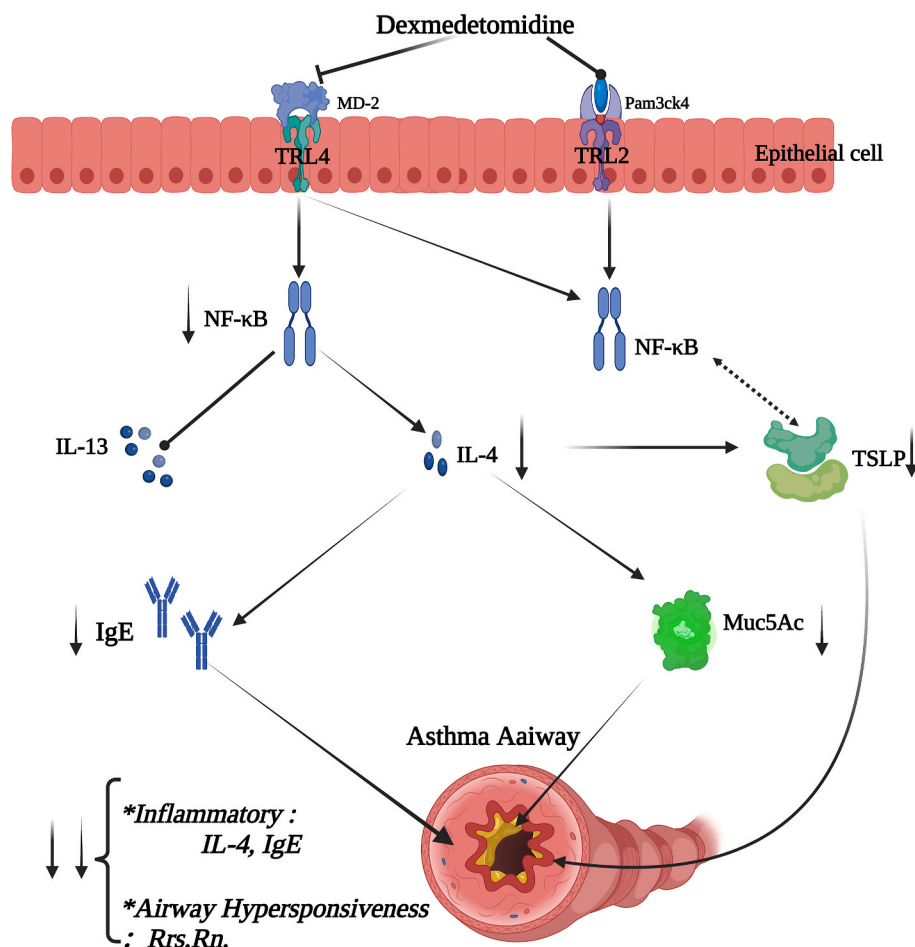


Fig. 1. Summary Chart. 1. Dexmedetomidine down-regulates IL-4 via inhibition of the TLR4/NF- κ B signaling pathway in epithelial cells; independent of the TLR2/NF- κ B signaling pathway. 2. Down-regulation of IL-4 caused a decrease in IgE, Mac5Ac and TSLP1 expression. 3. By down-regulating IL-4, IgE, Mac5Ac and TSLP1, it reduces the inflammatory response, decreases sputum secretion and moderates AHR.

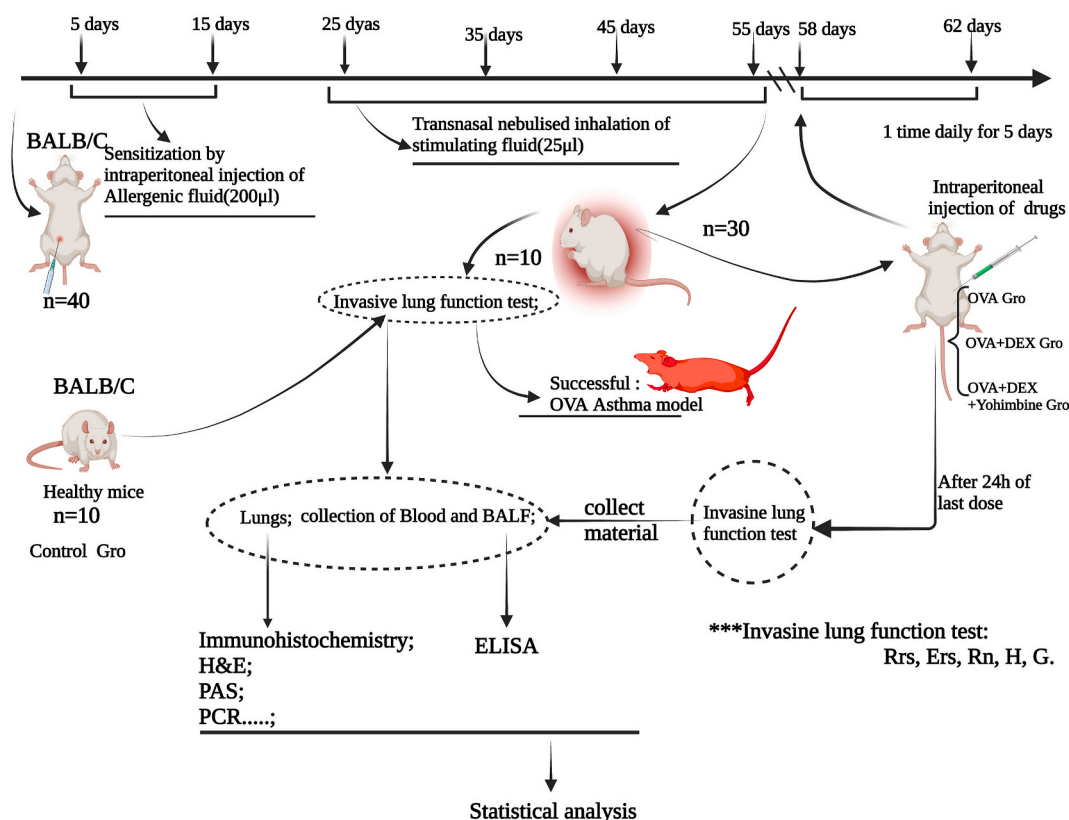


Fig. 2. Experimental flow chart.

2.2. Mice

BALB/C mice were purchased from Plastic Surgery Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences. The mice were fed with sterilized chow with drinking water and housed in a 12 light/12 dark environment. An asthma model was made from these mice after one week of arrival.

2.3. Experimental groups and interventions

A total of sixty BALB/C mice were used. Among them, ten were randomly selected as the control group ($n = 10$) and the remaining fifty were used to establish the asthma model (see below). Among the fifty asthma mice, ten were randomly selected for invasive lung function tests to verify the success of establishing the asthma model after administering the last dose of stimulating fluid, and the remaining forty were further divided into four groups according to the interventions: Group OVA ($n = 10$), same dose saline injection; Group OVA + DEX ($n = 10$), a single intraperitoneal injection of dexmedetomidine (Jiangsu Hengrui Pharmaceutical Co.) 25 µg/kg for 5 days (As reported in previous studies [19]); Group OVA + Yohimbine ($n = 10$), intraperitoneal injection of yohimbine (Sigma) 1 mg/kg for 5 days; Group OVA + DEX + Yohimbine ($n = 10$), a single intraperitoneal dose of dexmedetomidine 25 µg/kg + yohimbine 1 mg/kg for 5 days (Fig. 2).

2.4. Allergic asthma induction

BALB/c mice were first sensitized by intraabdominal injection of 200 µl of sensitizing liquid (20 mg Al(OH)₃(Sigma) + 200 µg Ovalbumin (Sigma.5503)/ml) on days 5 and 15, followed by inhalation of nebulized excitation liquid (i.e. 200 mg ovalbumin, 25 µl, PRONEB compression nebulizer) (on day 25, day 35, day 45 and day 55. After a total of 55 days, the OVA-induced asthma model was successfully established. Ten

of these mice (group Control, $n = 10$) were randomly selected for invasive lung function testing to prove the success of the asthma model and served as a control group (i.e. non-treated group). The other BALB/c mice ($n = 40$) were treated with dexmedetomidine and then subjected to invasive lung function tests. This 40 asthma mice were further divided into 4 groups as described above (Fig. 2).

2.5. Measurement of airway hyperresponsiveness (AHR)

Bronchopulmonary resistance were measured 24 h after the last OVA challenge. The mice were subjected to tracheostomy under anaesthesia (1% Pentobarbital, intraperitoneal injection), and they were ventilated and airway resistance was measured by the FlexiVent system (SCIREQ, Canada). The animals were ventilated at a tidal volume of 8 ml/kg at a rate of 150/min with a positive end-expiratory pressure of 2 cm H₂O. Aerosolized Ach was nebulized through an inlet of the main chamber for 6 min in increasing concentrations (from 0 (Equivalent volume of saline), 6, 12, 24 or 48 mg/ml). Measurement of airway hypersensitivity is conducted by measuring parameters for airway resistance (cmH₂O.s/mL), which included total respiratory resistance (Rrs) and elastance (Ers), proximal airway resistance (Rn) and distal airway dampening (G) and elastance (H). These parameters were recorded and averaged for 6 min after each nebulization, using the Snapshot-150 perturbation manoeuvre method of the flexion system.

2.6. Collection of peripheral blood

Blood samples were collected promptly by trans-ocular blood sampling after AHR measurement. 0.5–1.0 ml of blood was collected, centrifuged at 3000 r/min for 10 min after leaving at room temperature for 1–2 h and the serum was collected with stored at -20°C for ELISA testing.

2.7. Bronchoalveolar lavage fluid (BALF)

After collecting blood specimens, a small incision was made into the trachea in a sterile fashion under anaesthesia and a sterile 22 G indwelling needle was inserted (fixed to avoid damaging the lung too deeply). After this, the left lung was immediately ligated and 0.5 ml of 0.9% NaCl solution at 4 °C was slowly injected into the right lung with a syringe and then slowly aspirated. This injection-aspiration was repeated three times with a total injection volume of 1.5 ml and a recovery volume of approximately 1.2 ml (80–90% recovery). The lysate was filtered through gauze and the resulting BALF was centrifuged at 2000 r/min for 10 min at 4 °C. After centrifugation, the cells were suspended in PBS and then stained with Giemsa stain with the total number of cells counting and cell sorting were performed under a Fluorescent microscope (Orindos BX53, Japan). The supernatant was separated and stored at –20 °C for ELISA.

2.8. Histological analysis and evaluation

The lungs were then embedded in paraffin wax for slicing. The slices were stained with hematoxylin and eosin (HE, solar. China) or periodic acid-Schiff (PAS, Sigma) or Immunohistochemistry (Rabbit anti-MUC5AC, anti-TLR4, anti-NF-κB polyclonal antibody Biyuntian Institute of Biotechnology). They were analyzed under a Fluorescent microscope (Orindos BX53, Japan). The extent of perivascular and peribronchial inflammation was estimated by a score calculated using utilizing quantification of inflammatory cells in lung sections stained with HE. HE staining for the inflammatory score; PAS staining for sputum production with cupped cell chemosis and immunohistochemical mean luminosity values were analyzed by Image J software.

2.9. ELISA

Serum and BALF IL-4 and IgE levels were measured in strict accordance with the ELISA kit instructions. (Mouse IL-4 Elisa Kit and Mouse IgE Elisa Kit, Dr D).

2.10. qPCR

1. RNA extraction. RNAs were extracted using the TIUZOL (TIUZOL kits, Invitrogen Company)) method. **2. Lung tissue RNA integrity testing.** After extraction of total RNA from the lungs, the extracted RNA was subjected to 1% agarose electrophoresis to check the integrity of the RNA. **3. Reverse transcription of RNA from lung tissue.** Reverse transcription of mRNA was performed using the Universal Reverse Transcription Kit (Sollerbauer & Co). **4. PCR amplification of DNA from lung tissue.** After reverse transcription of the lung tissue cDNA, SYBR® Remix Ex Taq™ I was selected for the PCR reaction. Target gene primers designed by Invitrogen Company. The following forward and reverse primers were used:

Tlr2 forward, 5'-GCAAACGCTGTTCTGCTCAG-3'
Tlr2 reverse, 5'-AGGCGTCTCCCTCTATTGTATT-3'
Tlr4 forward, 5'-ATGGCATGGCTTACACCACC-3'
Tlr4 reverse, 5'-GAGGCCAATTTTGCTCTCCACA-3'
Il-13 forward, 5'-CCTGGCTCTTGCTTGCCTT-3'
Il-13 reverse, 5'-GGTCTTGTTGATGTTGCTAC-3'
Il-4 forward, 5'-GGTCTCAACCCCAAGCTAGT-3'
Il-4 reverse, 5'-GCCGATGATCTCTCTCAAGTGAT-3'
Tslp1 forward, 5'-AGAAGCCCTCAATGACCAC-3'
Tslp1 reverse, 5'-AGGTTTGATTGACGCAGAT-3'
Muc5ac forward, 5'-GTGGTTTGACACTGACTTCCC-3'
Muc5ac reverse, 5'-CTCCTCTCGGTGACAGAGTCT-3'
Nfκb forward, 5'-AGGCTTCTGGGCCTTATGTG-3'
Nfκb reverse, 5'-TGCTTCTCTCGCCAGGAATAC-3'

2.11. q-PCR system. Determination of q-PCR results

At the end of the experiment, the Ct value of each qPCR measurement was recorded and averaged three times, using β -actin as the internal reference. The data were processed according to the $2^{-\Delta\Delta Ct}$ method. ΔCt = mean $\pm$ standard deviation of Ct values for (target gene - reference gene); $\Delta\Delta Ct$ = mean $\pm$ standard deviation of ΔCt for (sample to be tested - reference sample); mean $\pm$ standard deviation of initial template amount for relative samples = $(2^{-\Delta\Delta Ct})$. Relative expression of target gene (RQ value) = $2^{-\Delta\Delta Ct}$.

2.12. Statistical analysis

In this study, Image J software was used for image processing. The data were analyzed using GraphPad Prism 8.0 software. Data analysis by applying Shapiro-Wilk normality test, Levene's test, Homogeneity of covariances assumption/Box's M-test, Mauchly's Test for Sphericity, Two-way mixed ANOVA, Simple effect, Pairwise Comparisons of Estimated Marginal Means, Levene's test and Independent samples T-test. Significance markers: ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (see Fig. 2).

3. Results

3.1. Successful establishment of an OVA allergic asthma airway hyperresponsiveness mouse model

The OVA-induced allergic asthma BALB/c mouse model was constructed after 2 abdominal injections of sensitizing solution and 4 intranasal titrations of challenge solution (Fig. 3-a). We elicited airway responses by increasing doses of acetylcholine (Ach) and performed invasive lung function tests. Airway resistance parameters Rrs (total respiratory resistance), Ers (total elasticity), Rn (proximal airway resistance), G (distal airway resistance) and H (tissue elasticity) were measured and all parameters were significantly different in the OVA group compared to the control group at 6 mg, 12 mg, 24 mg and 48 mg Ach. At a dose of 0 mg Ach (Fig. 3-b, c, d, e, f). The parameter H was significantly different in the OVA group compared to the control group (Fig. 3-e). At this time it was concluded that there was airway hyper-responsiveness in the OVA allergic asthma mouse model and the model was successfully constructed.

3.2. Dexmedetomidine reduces total airway resistance and proximal airway resistance in OVA allergic asthmatic mice

DEX (Dexmedetomidine, 25 μ g/kg intraperitoneally for 5 days) was shown to be a better effective therapeutic dose based on previous studies [19]. After treatment airway resistance was measured as described in the previous section (Fig. 4 a). The Rn and Rrs responses to Ach were suppressed in the OVA + DEX compared to the OVA group, particularly at the higher doses of Ach (Fig. 4 b and c). G responses to Ach were blunted insignificantly in OVA + DEX in comparison to group OVA (Fig. 4 d). On the other hand, the response of Ers and H to increasing doses of Ach was unaffected by treatment (Fig. 4 e and f). These results suggest that dexmedetomidine has a preventive effect on distal airway resistance (parameter G), proximal airway resistance (parameter Rn) and total respiratory resistance (parameter Rrs) in asthmatic mice, particularly in terms of reducing G and Rn. However, it had a limited effect on the reduction of total airway resistance and no effect on tissue elasticity (parameter H) and total elasticity (parameter Ers).

3.3. Reduction of airway inflammatory response in OVA allergic asthma after dexmedetomidine intervention

Extracted BALF was subjected to Giemsa staining; as well as PAS staining and HE staining of lung tissue. Compared with the OVA group,

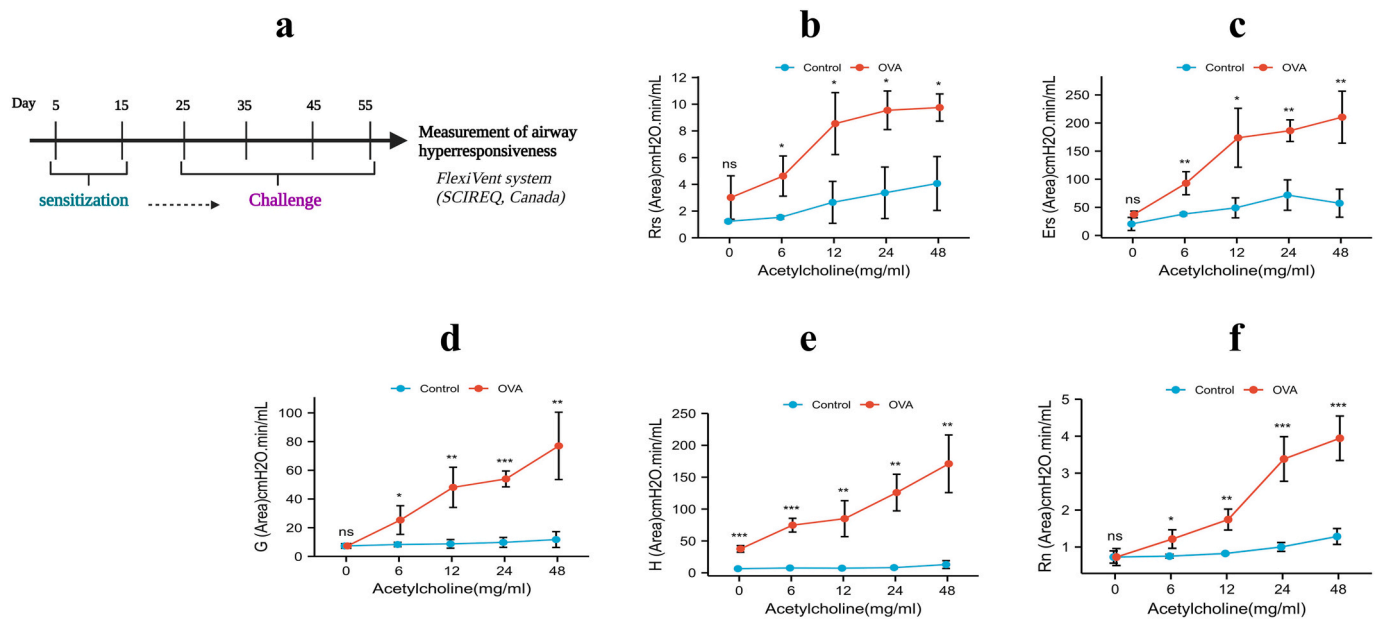


Fig. 3. Invasive airway resistance monitoring confirms successful airway hyperresponsiveness modelling. Fig. 3-a shows airway resistance tested by applying the FlexiVent system after 2 doses of sensitizing fluid and 4 doses of challenge fluid. Fig. 3b-f shows graphs of the changes in airway resistance parameters Rrs, Ers, G, H, and Rn with increasing dose stimulation with Ach, respectively, and all OVA groups were statistically different. ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, by Two-way mixed ANOVA, Simple effect and Pairwise Comparisons of Estimated Marginal Means.

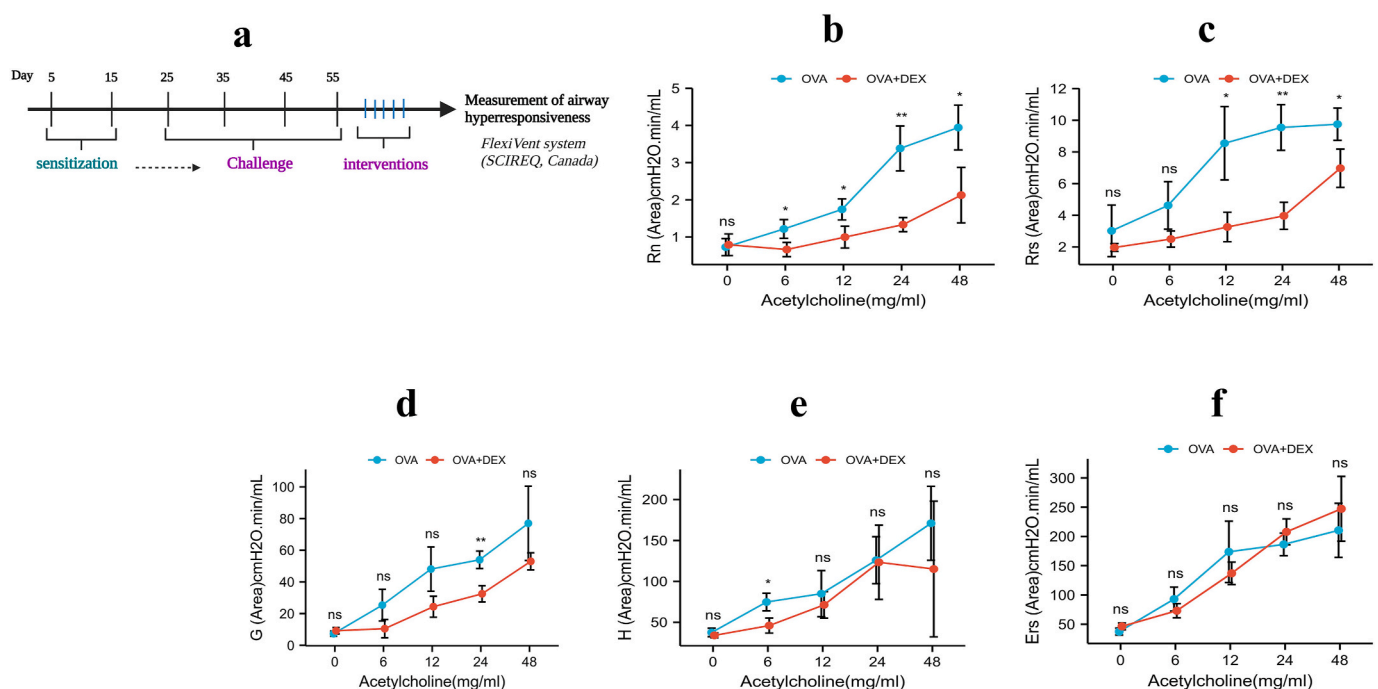


Fig. 4. Dexmedetomidine reduces airway hyperresponsiveness. Fig. 4-a shows the successful construction of the OVA asthma model after 5 consecutive days of dexmedetomidine intervention to monitor airway resistance; Fig. 4-b,c,d,e,f represent the resistance parameters Rn, Rrs, G, H, and Ers, respectively. Rn and Rrs were statistically different after the DEX (dexmedetomidine) intervention with increasing doses of Ach; G showed a trend towards significant improvement but was not statistically significant; H and Ers did not show significant improvement. ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, by Two-way mixed ANOVA, Simple effect and Pairwise Comparisons of Estimated Marginal Means.

Giemsa staining showed that inflammatory cells were significantly reduced in the OVA + DEX group, and there was a statistical difference ($P < 0.05$) (Fig. 5-a); PAS and HE staining showed that the OVA + DEX group had less structural destruction of cells and a reduced area of inflammatory infiltration, which was also statistically different (Fig. 5-a); with further ELISA detection in blood and BALF Both IgE and IL-4 were

significantly reduced in the blood and BALF of the OVA + DEX group compared to the OVA group, with a statistically significant difference ($P < 0.05$) (Fig. 4-b). However, the OVA + DEX + Yohimbine (dexmedetomidine antagonist) group showed no significant change compared to the OVA group; compared to the Control and OVA + DEX groups, both showed a significant and statistically significant increase (Fig. 5-a and

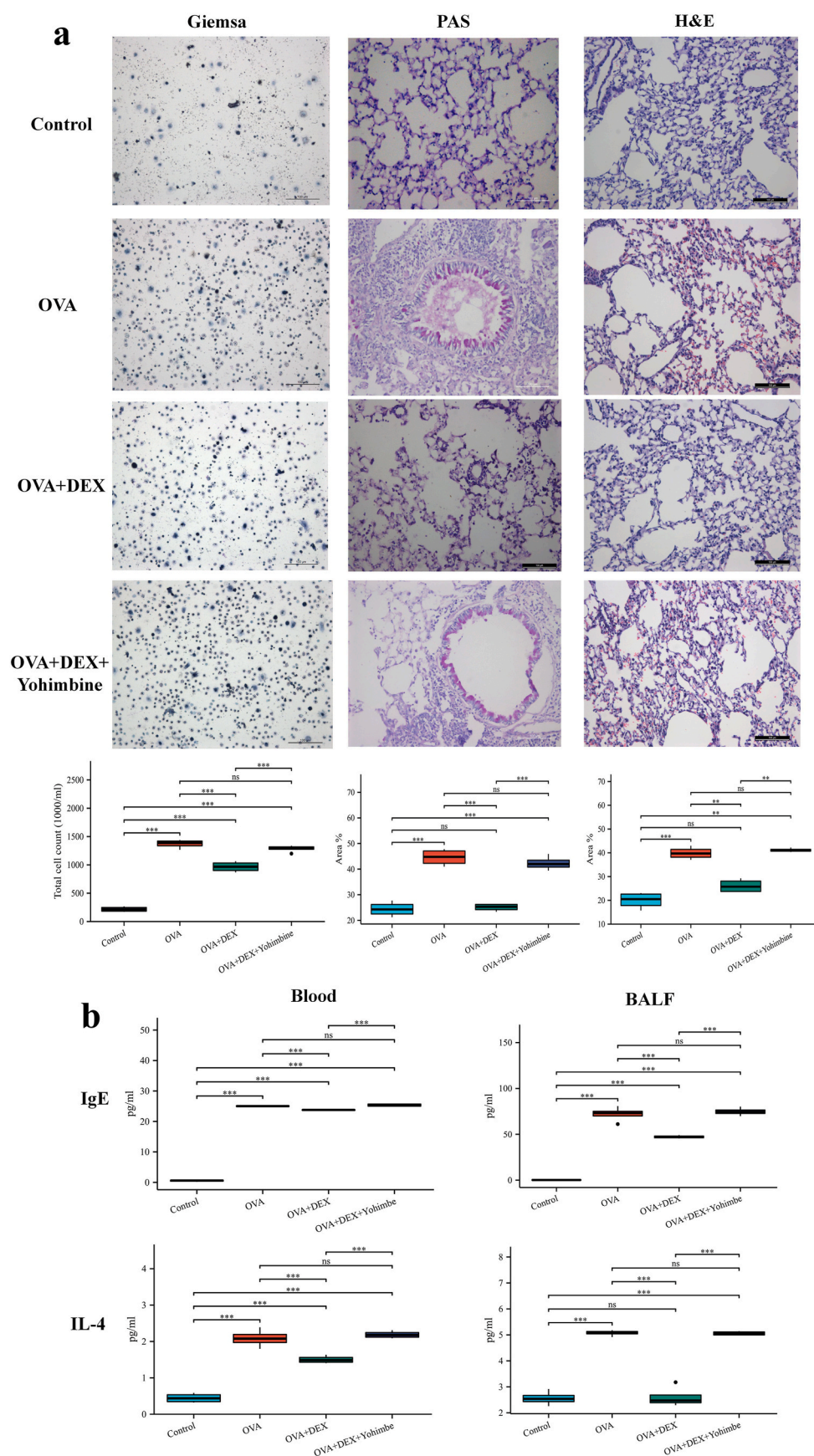


Fig. 5. Dexmedetomidine attenuates the airway inflammatory response in OVA allergic asthma. Fig. 5-a shows Giemsa staining, PAS staining and HE staining as well as a statistical plot of cell counts after Giemsa staining with percentage of inflammatory area for PAS staining and HE staining. Fig. 5-b shows ELISA analysis of IgE and IL-4 concentrations in blood and BALF. ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by One-way ANOVA test, Tukey HSD post-hoc test, Levene's test and Independent samples T-test.

b). Therefore, it is concluded that dexmedetomidine reduces the airway inflammatory response in OVA allergic asthma.

3.4. Dexmedetomidine attenuates the airway inflammatory response in OVA allergic asthma independent of the TLR2/NF- κ B pathway

To further investigate the mechanism of DEX (dexmedetomidine) to attenuate airway inflammation, lung tissue was taken for qPCR experiments. The results showed a statistically significant reduction in NF- κ B (Fig. 6-b and d) and its downstream IL-4 (Fig. 6-h and k) in the OVA + DEX group compared to the OVA group, but no statistically significant reduction was seen in the upstream TLR2 (Fig. 6-c and e) and downstream IL-13 (Fig. 6-f and i). Therefore, we consider that dexmedetomidine attenuates this airway inflammatory response probably independent of the TLR2/NF- κ B pathway.

3.5. Dexmedetomidine inhibits airway inflammation via the TLR4/NF- κ B pathway

To investigate the mechanism by which DEX attenuates airway inflammation and the reason for the reverse expression of TLR2 and NF- κ B, further qPCR assays were performed on TLR4, a channel receptor upstream of NF- κ B. The changes in TLR4 and NF- κ B were found to be in the same direction and statistically different in the OVA + DEX group compared to the OVA group (Fig. 6-g and j). In addition, immunohistochemical staining of lung tissues for TLR4 and NF- κ B was performed and the percentage of area occupied was counted and found to be statistically different as well (Fig. 6-l, m and n). The changes in TLR4 and NF- κ B direction were consistent from the gene level and protein level with both decreases after dexmedetomidine treatment.

4. Discussion

In this study, we showed that dexmedetomidine downregulated the expression of components of the TLR4/NF- κ B signalling pathway, which in turn affected the secretion of IL-4 and IgE.

The role of TLR4/NF- κ B signalling in airway inflammation has been well studied [11]. Allergens and air pollutants trigger epithelial cell NF- κ B production by activating TLRs or protease-activated receptors (PARs), leading to the activation of dendritic cells (DCs). Activated DCs migrate to priming nodes to promote Th2 development and activate the recruitment of innate immune cells to the airways, which subsequently produce many of the mediators that contribute to airway inflammation. Other studies have reported that neutrophils amplify recruitment and inflammatory responses in neutrophilic asthma by stimulating airway epithelial cells to activate the TLR4/NF- κ B pathway and secrete chemokines [17]. Inhibition of TLR4/NF- κ B signalling reduced lipopolysaccharide-induced injury and inflammation in bronchial epithelial cells has also been reported [18]. In our study, we found that TLR4/NF- κ B was significantly increased after OVA exposure, as were the inflammatory factor IL-4 and the antigen-presenting agent IgE. Our results are consistent with these studies and proved that TLR4/NF- κ B signalling pathway has an important role in the airway inflammatory response and AHR.

Dexmedetomidine (DEX) is a highly selective α -adrenergic agonist (α : α 1 selectivity of 1600:1). α -receptors are widely expressed in the central and peripheral nervous systems as well as on other cells and tissues such as endothelial cells and epithelial cells [9,20]. DEX has been reported to reduce ventilator-induced lung injury by activating the ERK1/2 pathway [7] and by modulating the immune phenotype of macrophages [21]. It has also been shown to improve endotoxin-induced acute lung injury in vivo by maintaining mitochondrial dynamic homeostasis through the HIF-1 α /HO-1 signalling pathway [8]. Administration of dexmedetomidine is protective in chronic obstructive pulmonary disease [22]. Based on reports and previous studies, we have explored the optimal dose of dexmedetomidine for

mitigating airway hyperreactivity employing through a dose-escalation approach [19–22]. Here, we showed that dexmedetomidine attenuated airway resistance and protected the lung against injury due to inflammation in an animal asthma model. These results establish the role of this drug in suppressing AHR protection during an asthma attacks.

Inflammatory responses and immune responses are key to the development and progression of AHR. The main specific cytokines associated with inflammation are IL-4, IL-5 and IL-13 secreted by Th2 cells. The main protein associated with the immune response is allergen-specific B cells synthesized immunoglobulin E (IgE) [9,14]. In the current study, IL-4 and IgE were found to be involved in lung inflammation and were inhibited by dexmedetomidine. However, dexmedetomidine did not affect IL-13. Both IL-4 and IL-13 are secreted by Th2 cells. However, IL-4 production is dependent on calcium-regulated neurophosphatase, whereas IL-13 production is only partially dependent on calcium-regulated neurophosphatase [16]. Calcium-regulated neurophosphatase is positively correlated with intracellular Ca^{2+} [23]. Since dexmedetomidine is an α 2 receptor agonist, we believe that dexmedetomidine reduces intracellular Ca^{2+} concentration by inhibiting Ca^{2+} inward flow via voltage-gated Ca^{2+} channels as a result of α 2 receptor agonism [9]. As a result, the activity of calcium-regulated neurophosphatase is reduced leading to lower IL-4 production. However, our data are limited since we have not examined the differential production and expression of IL-4 and IL-13. Nevertheless, our study opens the door for future studies on the effect of dexmedetomidine on intracellular Ca^{2+} mediated AHR.

TLRs are a subfamily of pattern recognition receptors that recognize invading pathogens and endogenous noxious stimuli to induce innate and adaptive immune responses. Among TLRs, TLR4 is the major lipopolysaccharide (LPS) receptor. When expressed on the cell membrane TLR4 activates NF- κ B. TLR4/NF- κ B can increase cytokines (e.g. tumour necrosis factor (TNF) α , interleukin (IL)-1 β /IL-6), chemokines, enzymes and reactive oxygen species [24]. Several studies have demonstrated that the inflammatory response can be suppressed by inhibiting the TLR4/NF- κ B signalling pathway [24,25]. Dexmedetomidine has been reported to attenuate the inflammatory response by inhibiting the TLR4/NF- κ B signalling pathway in both inflammatory [26] and ischemia-reperfusion lung injury [27]. In this study, we have confirmed the inhibitory effect of dexmedetomidine on the expression of TLR4/NF- κ B thus suppressing inflammation and on AHR thus protecting the lungs. It is important to note that the effect of dexmedetomidine was attenuated in the group OVA + DEX + Yohimbine, which further supports that α 2 receptors are involved in the inhibition of TLR4/NF- κ B signalling pathway generation. This mechanism is different from that of nebulized lidocaine which involves inhibition of airway smooth muscle cell proliferation and migration by downregulating TLR2 to improve allergic airway inflammation [28] and blocking the TLR2/NF- κ B/NFATc1 signalling pathway [29]. Furthermore, we did not detect the involvement of TLR2 in our study. Activation and correct assembly of TLR4 with upstream differentiation protein 2 (MD-2) require relatively stable concentrations of cations of substances such as Ca^{2+} and Mg^{2+} [24,30], while activation of TLR2 is involved with upstream of Pam3CSK4 [28,32]. There are no reports on the mechanism of action between alpha receptors and the TLRs/NF- κ B signalling pathway. However, IL-4 and TLR4 are inversely regulated with IL-13 and TLR2, and such an outcome could be due to Ca^{2+} changes, but there is a lack of data to prove this. This mechanism may be one of the future research directions.

In summary, dexmedetomidine can reduce airway hyperreactivity and airway inflammatory responses. The mechanism underlying this effect may be the regulation of the inflammatory response through the TLR4/NF- κ B signalling pathway mediated by and down-regulating the inflammatory factor IL-4 and the immune protein IgE, which is also an important cause of airway hyperreactivity. Furthermore, it reduces airway hyperresponsiveness.

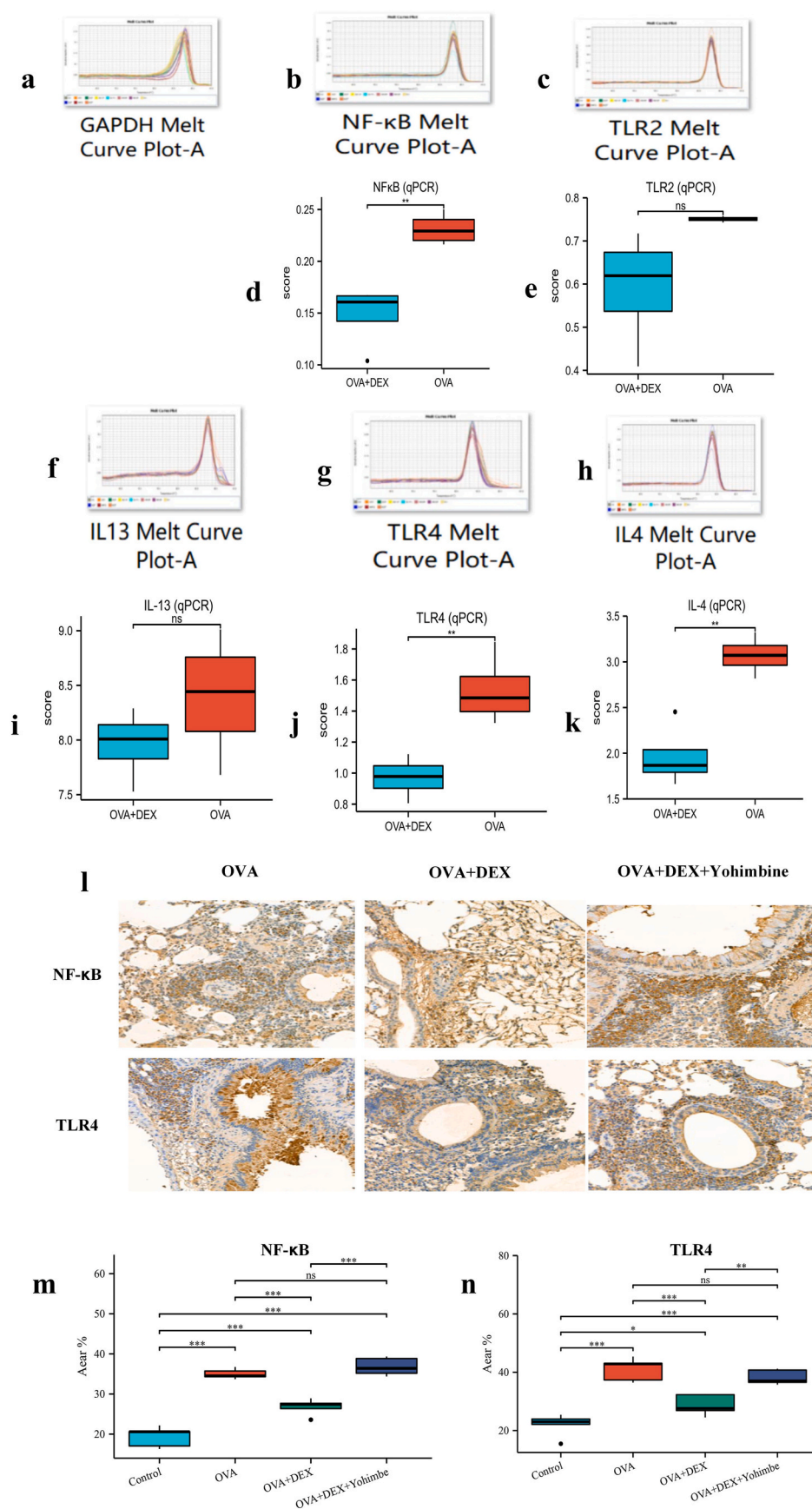


Fig. 6. Inhibition of airway inflammation by dexmedetomidine may be associated with the TLR4/NF-κB pathway but not the TLR2/NF-κB pathway. Fig. 6-a to k show qPCR amplification curves and Ct values calculated for genes GAPDH, NF-κB, TLR2, IL13, TLR4 and IL-4. Fig. 6-l, m and n are percentages of area immunohistochemically and stained for NF-κB and TLR4 proteins. ns, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by One-way ANOVA test, Tukey HSD post-hoc test, Levene's test and Independent samples T-test.

5. Conclusions

Dexmedetomidine reduced the production of IL-4 and IgE through downregulation of TLR4/NF κ B signalling pathway, thereby reducing the pulmonary inflammatory response as well as airway resistance with thus demonstrating a preventive effect on airway hyperresponsiveness in the OVA-induced asthma model.

Ethics approval and consent to participate

Experimental animals were handled under a protocol approved by the Institutional Animal Care and Use Committee of Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.

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Author's contributions

Dong Yang is responsible for conceiving, designing, supervising, writing, (and sometimes performing the experiments). Xiyu DU, Juan Zhi and Qirui Duan: performed the experiments and analyzed. Juan Zhi and Qirui Duan: Contributed reagents/materials/analysis tools. Qirui Duan: design and conducted experiments, wrote the manuscript.

Consent for publication

All authors agree to publish.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's statement

The data, statistical results and figures in the manuscript are the authors' actual experimental results. There is no conflict of interest between the authors and they are published uniformly.

Declaration of competing interest

The authors have declared that there are no conflicts of interest.

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Further reading

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