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Inhibitory Effect of Propofol on Type II Inflammation in Mice with Allergic Rhinitis

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ABSTRACT

Propofol, a quick-acting systemic anesthetic agent widely used in general anesthesia, can alleviate airway T-helper 2 (T_H2) inflammation. Group 2 innate lymphoid cells (ILC2s) are a newly discovered group of lymphoid cells and play key roles in allergic rhinitis (AR). We aimed to investigate the regulation of ILC2s treated with propofol and its possible mechanisms in a mouse model.

An ovalbumin (OVA)-sensitized and challenged mouse model was established. Nasal lavage fluid (NLF) and tissue samples were collected for the detection of inflammatory cells, type II cytokines, and ILC2s using Giemsa staining, enzyme-linked immunosorbent assay, and flow cytometry. $CD4^+$ T cells and ILC2s were cocultured and detected by flow cytometry to confirm the proportion of T_H2 cells.

Compared with OVA-sensitized and challenged mice, propofol-treated model mice presented decreased type II cytokine levels and total numbers of cells, eosinophils, neutrophils, and macrophages in NLF. Mice treated with propofol presented decreased nasal ILC2 frequency. Moreover, the nasal expression of GATA binding protein 3 (GATA3) and retinoid-related orphan receptor α (*RORa*), as well as the levels of IL-5 and IL-13, were significantly inhibited after propofol treatment. Compared with those cultured alone, cocultures of ILC2s and $CD4^+$ T cells resulted in significantly more T_H2 cells. When propofol was added, the percentage of T_H2 cells significantly decreased. This effect was alleviated when anti-major histocompatibility complex class II (anti-MHC II) protein was added.

Our study provides preliminary evidence that propofol can play an inhibitive role in AR by regulating innate and adaptive immunity.

Keywords: Allergic rhinitis; Group 2 innate lymphoid cells; Propofol

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INTRODUCTION

Allergic rhinitis (AR) is a widespread health issue that is becoming more common and has significant medical and socioeconomic impacts. The investigation of biologics as potential treatments has thus far been limited to anti-immunoglobulin E (IgE) therapies and anti-inflammatory agents targeting type 2 responses.[1]

Propofol, a quick-acting systemic anesthetic agent widely used in general anesthesia, is characterized by quick recovery after anesthesia, mild gastrointestinal reaction, and fewer cumulative effects after continuous administration.[2] Accumulating evidence suggested that propofol has certain advantages in asthma patients due to its bronchodilatory effects.[3,4] Moreover, propofol has anti-inflammatory effects on various cells and animal models by inhibiting the production of inflammatory cytokines, such as interleukin (IL)-1b, IL-6, and tumor necrosis factor.[5-8] Propofol alleviates airway inflammation in asthmatic mice by inhibiting T-helper 2 (T_H2) differentiation.[9] Moreover, the inhibition of 5-hydroxytryptamine (5-HT) action by propofol is also involved in ovalbumin (OVA)-induced contraction.[10] These results suggest that propofol may be a potential treatment target for AR. Although the role of propofol in T_H2 response was studied, the effects of propofol on group 2 innate lymphoid cells (ILC2s) in AR are poorly understood.

ILC2s, a new population of lymphoid cells, play important roles in the development of immune response.[11] ILC2 cells are particularly effective at generating IL-5, which facilitates eosinophil activation and maintenance, as well as IL-13, a cytokine that modulates various facets of airway inflammation, mucus transformation, smooth muscle adaptation, and airway hyperreactivity.[12] Previous studies have shown that the frequency of ILC2s is elevated in allergic inflammation. For example, ILC2 numbers are greater in AR patients than in controls.[13] Our study explored the effect of propofol on ILC2s in a mouse model for the first time to provide a broader theoretical basis for the anti-inflammatory mechanism of propofol in AR and to provide laboratory evidence for its potential application in the treatment of AR in the future.

MATERIALS AND METHODS

Mouse Models

Sixty female 8-week-old BALB/c mice were purchased from the animal experimental center of Guangdong province. The mice were fed in a pathogen-free room with humidity of 50%–70% and temperature of 22–24℃. Our experiment received approval from the Ethics Committee of Guangzhou Women and Children's Medical Center (Approval No. 261A01).

Establishment of Allergic Mice Model

Sensitization was achieved through the intraperitoneal injection of 100 μL of phosphate-buffer saline (PBS) (FengHao, China) or OVA (50 μg, Sigma Aldrich, USA) and 1.6 mg aluminum hydroxide [$Al(OH)_3$, Sigma Aldrich, USA] dissolved in PBS on days 0, 7, and 14 as previously described.[14] Then, the challenge was performed by providing 20 μL of PBS or OVA (200 μg) intranasally on days 21, 22, and 23. Propofol (100 mg/kg, Sigma Aldrich, USA) was provided intraperitoneally half an hour before the challenge. The mice were grouped as follows (10 per group): (1) PBS (sensitization and challenge by PBS); (2) OVA (sensitization and challenge by OVA); (3) OVA + propofol (propofol treatment before challenge). The concentration of propofol was determined based on previous literature and our preliminary experiments.[15,16]

Nasal Lavage Fluid Collection and Cell Counting

As described previously, cold PBS (1 mL) was inserted into the nasal cavity and collected in a tube.[17] The collected samples were centrifuged and stored at −80°C for further analysis. Type II cytokines in the supernatant were tested using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA). The cell pellet was stained with Giemsa stain and counted to determine the number of eosinophils, lymphocytes, and total cells.

Determination of Nasal ILC2 Levels

The nasal tissues were obtained and minced for further use. After digestion with collagenase IV (1 mg/mL) (Fenghao, China) in Roswell Park Memorial Institute (RPMI) 1640 medium (Fenghao, China) for 2 hours, the cells were filtered through a strainer (70 μm and 40 μm) and collected for centrifugation. The percentage of the viable cells was determined using trypan blue staining (>96%). The mouse ILC2s were determined to be lineage negative CD3ε, CD11c, CD11b, Fc-epsilon receptor (FcεR)1α, γδ T cell receptor, B220, erythroid lineage (Ter-119), myeloid

differentiation antigen (Gr-1)), $CD45^+$, $CD127^+$, and suppression of tumorigenicity 2 $(ST2)^+$ cells and were sorted via a FACS ARIA flow cytometer (BD Biosciences, USA) with purity >92%. IL-5^+ ILC2 and IL-13^+ ILC2 cells were confirmed using Cytofix (BD Biosciences, USA).

Coculture of T_H2 and ILC2

$CD4^+$ T cells were purified from the blood of the mouse models using magnetic beads. The percentage of viable cells was determined using trypan blue staining (>96%). The $CD4^+$ lymphocytes (2×10^5 cells per well) were incubated in a comprehensive culture medium (RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin, all from Fenghao, China). T_H2 cells were prepared using an anti-murine CD3 antibody (concentration: 10 μg/mL) and an anti-murine CD28 antibody (concentration: 1 μg/ml), alongside murine IL-2 (10 ng/mL) and murine IL-4 (10 ng/ml) at 37°C, within a humidified environment containing 5% CO_2. All the antibodies were purchased from eBioscience (USA). The sorted ILC2s from mice were cultured with T_H2 cells (1:1) treated with propofol (30 μg/mL, eBioscience, USA), anti-major histocompatibility complex class II (anti-MHC II) monoclonal antibodies (mAb) (20 mg/mL, eBioscience, USA), propofol + anti-MHC II mAb for 96 hours as previously described. The T_H2 cell ($CD3^+CD4^+IL\text{-}4^+$) proportion and type II cytokine levels in the supernatant were subsequently determined using flow cytometry and ELISA. The concentration of drugs was also determined based on previous literature and our preliminary experiments.[9,18]

ELISA

The protein expression of cytokines was measured using ELISA kits (R&D systems, USA). The detection limits were as follows: IL-5, 3.9 pg/mL, IL-13, 125 pg/mL.

Quantitative Real-time Polymerase Chain Reaction

RNA was extracted from nasal tissue with TRIzol LS (USA). The RNA (1 μg) was reverse-transcribed to synthesize complementary DNA (cDNA). The relative expression of GATA binding protein 3 (*GATA3*) and RAR-related orphan receptor alpha (*RORA*) was detected via the ABI PRISM 7300 Detection System (USA). The relative expression of target genes was calculated via the ΔΔCt method and corrected for the beta-actin (*ACTB*) gene. The primers used in the test were as follows: *GATA3* sense, 5′-GCGGGCTCTATCACAAAATGA-3′; antisense, 5′-GCTCTCCTGGCTGCAGACAGC-3′; *RORA* sense, 5′-AAGGAGCCAGAAGGGATGAAC-3′; antisense, 5′-GGAACA ACAGACGCCAGTAAG-3′; *ACTB* sense, 5′-CGAAACTACCT TCAACTCCATC-3′; antisense, 5′-AGTGATCTCCTTCTGCAT CCT-3′.

Statistical Analysis

The data were analyzed using SPSS 18.0 with a *p* value of <0.05 indicating statistical significance. Comparison between groups was performed via the Mann-Whitney U test or Kruskal-Wallis H test.

RESULTS

Propofol Decreased Nasal Allergic Inflammation in Mouse Models

Allergic symptoms, OVA-specific IgE levels, and total numbers of cells, eosinophils, and lymphocytes in nasal lavage fluid (NLF) increased significantly after OVA sensitization and challenge (Figure 1). The propofol-treated mouse model presented with alleviated symptoms and decreased OVA-specific IgE levels, total number of cells, eosinophils, and lymphocytes in the NLF (Figure 1).

ILC2 Regulation by Propofol

In the OVA and propofol-treated group, the frequencies of nasal ILC2s, IL-5^+ ILC2s, and IL-13^+ ILC2s were lower than those in the OVA group (Figure 2). Moreover, the nasal expression of *GATA3* and *RORα* as well as the levels of IL-4, IL-5, and IL-13 were significantly inhibited after propofol treatment (Figure 2). The addition of anti-MHC II reversed the anti-inflammatory effect of propofol (Figure 2).

Propofol Inhibited Interaction Between ILC2s and T_H2 Cells Through MHC II

Compared with those cultured alone, cocultures of ILCs and $CD4^+$ T cells resulted in significantly more T_H2 cells (Figure 3). When propofol was added, the percentage of T_H2 cells significantly decreased (Figure 3). However, this effect was alleviated when anti-MHC II was added (Figure 3).

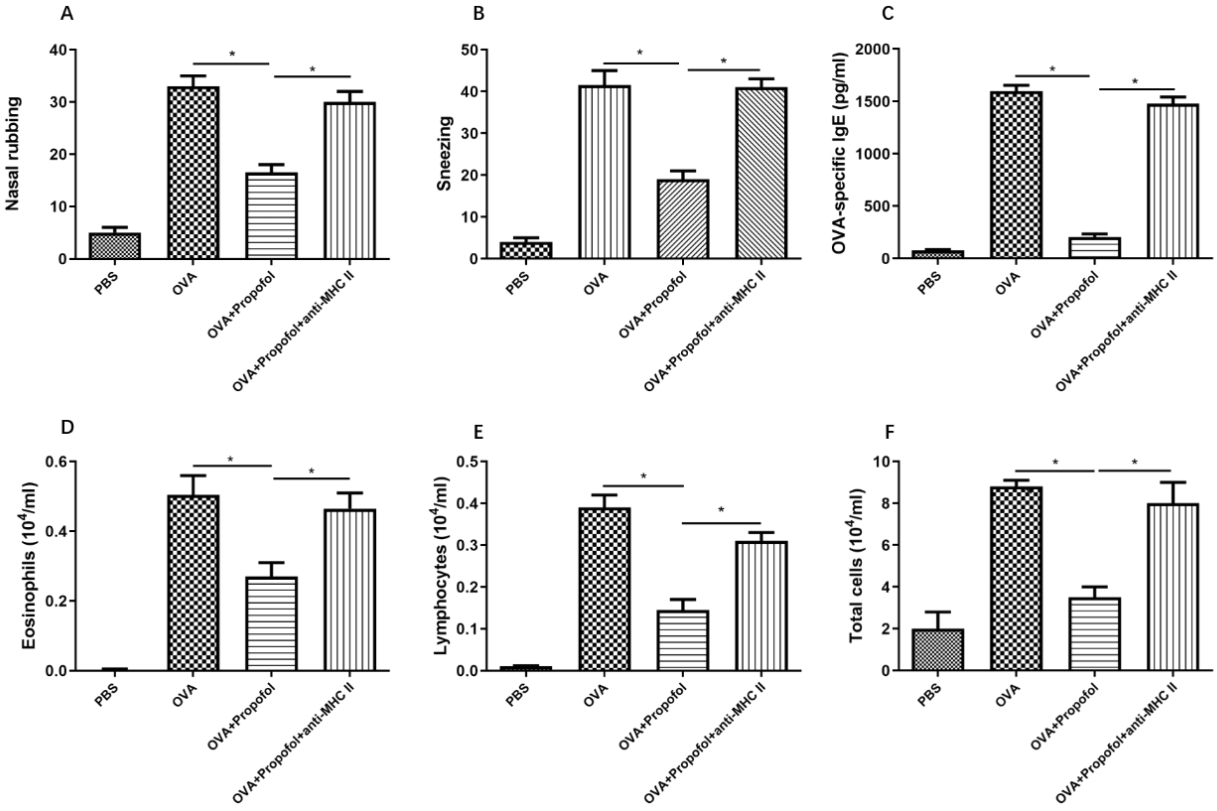

Figure 1. Establishment and intervention of an allergic rhinitis mouse model with propofol. A and B. Typical nasal symptoms after propofol treatments. C. Ovalbumin (OVA)-specific immunoglobulin E (IgE) levels were measured via enzyme-linked immunosorbent assay (ELISA). D–F. Total numbers of cells, eosinophils, and lymphocytes in the nasal lavage fluid of allergic mice after propofol treatment. * p<0.05; PBS: phosphate-buffered saline; MHC II: major histocompatibility complex class II.

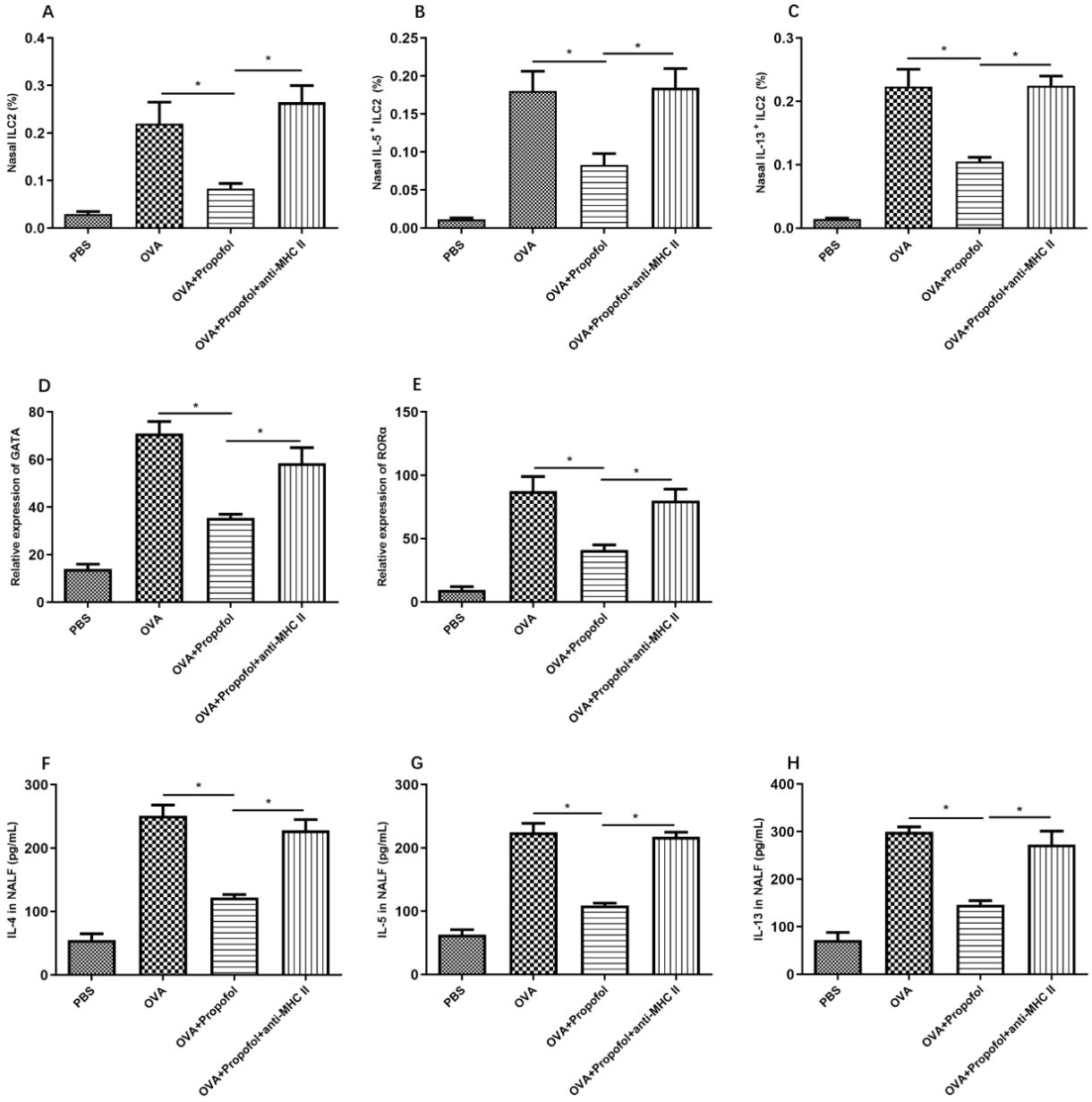

Figure 2. Propofol inhibits group 2 innate lymphoid cell (ILC2) responses in a mouse model. A–C: The proportions of nasal ILC2s, interleukin (IL)-5$^+$ ILC2s, and IL-13$^+$ ILC2s after propofol treatment were determined via flow cytometry. D and E: Relative mRNA expression of GATA binding protein 3 (GATA3) and RAR-related orphan receptor alpha (RORα) in nasal mucosa tissue. F–H: IL-4, IL-5, and IL-13 protein expression in nasal lavage fluid. * p<0.05; PBS: phosphate-buffered saline; OVA: ovalbumin; MHC II: major histocompatibility complex class II; NLF: nasal lavage fluid.

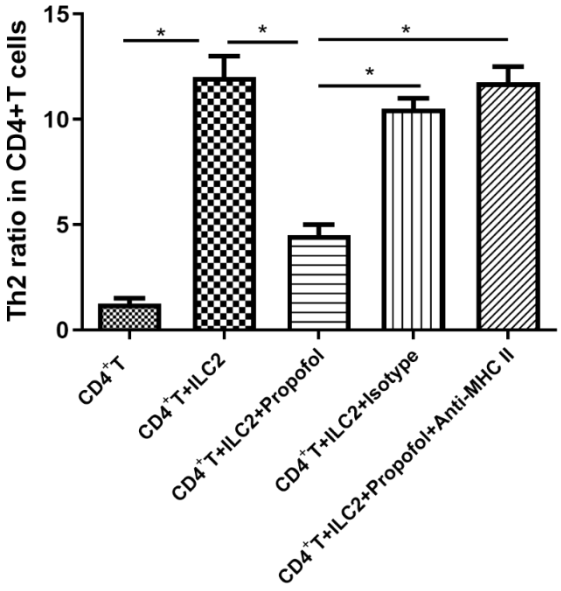

Figure 3. Propofol inhibited the interaction between group 2 innate lymphoid cells (ILC2s) and T-helper 2 (T_H2) cells through anti-major histocompatibility complex class II (anti-MHC II). Compared with those cultured alone, cocultures of ILC2s and $CD4^+$ T cells resulted in significantly more T_H2 cells. When propofol was added, the percentage of T_H2 cells significantly decreased. However, this effect was alleviated when anti-MHC II was added. * p<0.05.

DISCUSSION

In our study, we first demonstrated that propofol inhibited the proliferation and function of ILC2s as well as the interaction between ILC2s and T_H2 cells, providing a new target for AR treatment.

AR is a common upper airway disease worldwide and is traditionally characterized by T_H2 inflammation. ILC2s, a newly identified group of lymphoid cells, produce IL-5 and IL-13 after stimulation by epithelium-derived IL-25, IL-33, and thymic stromal lymphopoietin.[19] An increasing number of studies have shown that ILC2s play a central role in the development of allergic inflammation.[12]

Propofol is a common intravenous anesthetic with bronchodilatory effects.[20,21] Moreover, its anti-inflammatory effects have been confirmed in ischemia-reperfusion injury, lung injury, neuroinflammation, and various inflammatory cells, such as neutrophils, monocytes, macrophages, and T cells.[8] Our results suggested that propofol inhibited OVA-induced allergic inflammation in a mouse model, as manifested by decreased infiltration of inflammatory cells and type II cytokines in NLF. Similarly, Jing et al suggested that propofol inhibited T_H2-type inflammation in a mouse model by promoting apoptosis during T_H2 differentiation through activation of the GABA receptor.[8] A study by He et al suggested that propofol anesthesia upregulated T_H1 cytokine production and downregulated T_H2 cytokines production in a rat model of spinal cord injury.[22]

Our present study provided new evidence that propofol inhibits not only T_H2 inflammation but also ILC2 inflammation. In propofol-treated mice, nasal ILC2 proliferation and type II cytokine production were significantly decreased. Our data revealed that the expression of RORα and GATA3, key determinants of ILC2 differentiation and function, was also significantly inhibited by propofol.

A previous study confirmed that various cells, such as dendritic cells, B cells, and eosinophils can act as antigen-presenting cells by upregulation of MHC class II molecules.[23] Similarly, ILC2s also express MHC-II to act as antigen-presenting cells (APC).[24,25] Previous studies have shown that MHC-II expressed by APCs is engaged in T_H2 cell differentiation.[26] Consistently, our results also confirmed that propofol inhibited T_H2 cell differentiation by downregulation of MHC class II expression.

In conclusion, our study provides preliminary evidence for the first time that propofol inhibits the proliferation and function of ILC2s by decreasing the expression of transcription factors *GATA3* and *RORα* as well as type II cytokines (IL-5 and IL-13). We also found that propofol suppressed the interaction between ILC2s and T_H2 cells mediated by MHC II. Our results suggest propofol can play an inhibitory role in AR by regulating both innate and adaptive immunity.

STATEMENT OF ETHICS

Our experiment received approval from the Ethics Committee of Guangzhou Women and Children's Medical Center (Approval No. 261A01).

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The data supporting our findings are available upon reasonable request from the corresponding authors via email.

AI ASSISTANCE DISCLOSURE

Not applicable.

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