

Astragalus Polysaccharide Relieves Inflammatory Responses in Guinea Pigs with Allergic Rhinitis via Ameliorating NF- κ B-Mediated Treg/Th17 Imbalance

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Abstract

Background: Allergic rhinitis (AR) is regarded as a prevalent and non-infectious inflammation in nasal mucosa, and astragalus polysaccharide (APS) could mitigate inflammation.

Objectives: Herein, this study probed the specific mechanism of APS in inflammatory responses in AR.

Methods: Firstly, AR guinea pig models were established through the stimulation and sensitization of ovalbumin (OVA) and received APS treatment. Changes in nasal symptoms were assessed through counting the sneezing and rubbing times of guinea pigs. The change patterns of OVA-specific immunoglobulin-E (OVA-sIgE), OVA-specific immunoglobulin-G1 (OVA-sIgG1), tumor necrosis factor (TNF)- α , and interleukin (IL)-6 in guinea pig serum were identified. Meanwhile, the levels of IL-17, transforming growth factor (TGF)- β , IL-10, and forkhead box protein P3 (Foxp3) in the guinea pig tissues or serum were examined, and CD25⁺Foxp3⁺Treg or CD4⁺IL17⁺Th17 cell proportion was detected. Afterwards, nuclear factor-kappa B (NF- κ B) expression in guinea pig nasal mucosa tissues were examined. Rescue experiments were designed to probe the role of NF- κ B overexpression in inflammatory responses and Treg/Th17 imbalance in AR guinea pigs.

Results: APS treatment reduced sneezing and rubbing times of AR guinea pigs and suppressed OVA-sIgE, OVA-sIgG1, TNF- α , and IL-6 levels in guinea pig serum, and meanwhile, increased CD25⁺Foxp3⁺Treg cell proportion while reduced CD4⁺IL17⁺Th17 cell proportion in AR guinea pig serum or tissues, in a dose-dependent manner. NF- κ B was highly-expressed in AR guinea pigs and down-regulated after APS treatment. NF- κ B overexpression facilitated inflammatory responses and Treg/Th17 imbalance in AR.

Conclusion: APS reduced Treg/Th17 imbalance via suppressing NF- κ B expression, thereby ameliorating inflammatory responses in AR.

Keywords

astragalus polysaccharide, allergic rhinitis, NF- κ B, Treg, Th17, transcription factor, ovalbumin, inflammatory response

Introduction

Allergic rhinitis (AR), typically classified into seasonal AR and perennial AR, is defined as a prevalent and non-infectious type of atopic disorders, with nasal obstruction, paroxysmal sneezing, rhinorrhea, and rhinocnesmus as its clinical manifestations.¹ Though AR is not life-threatening, it still brings about detrimental effects on human life, such as decreased concentration, energy loss, and even depression.² Pathologically, AR is an immunoglobulin E-mediated inflammatory disorder occurring in nasal mucosa, and the correspondent

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inflammatory mediators released from eosinophils, mast cells, lymphocytes, and epithelial cells are primary contributors to the induction of AR inflammatory responses.³ On a separate note, Treg/Th17 cell dynamic balance which is strictly regulated under normal conditions is crucial to immune homeostasis, and Treg/Th17 imbalance would trigger a series of abnormal immune responses, thereby inducing inflammation and autoimmune disorders.^{4,5} Moreover, Treg/Th17 imbalance is proposed as a one pathogenesis of AR and is closely implicated in AR progression.⁶ Hence, it is prudent and imperative to conduct an in-depth investigation on inflammatory responses and Treg/Th17 imbalance in AR.

Polysaccharide, a carbohydrate chain bound by glycosidic bonds, is classified as a type of macromolecular polymer consisting of over 10 monosaccharides.⁷ Polysaccharide from plants confers various roles in anti-oxidation, anti-inflammation and anti-aging.^{8,9} Astragalus polysaccharide (APS), as the primary bioactive ingredient of *Astragalus membranaceus*, possesses multiple physiological and pharmacological functions, and anti-inflammatory/oxidant effects on various diseases and cancers.¹⁰ Notably, APS could mitigate inflammation-related symptoms in nasal mucosa area of AR rats.¹¹ Nonetheless, the role of APS in AR-induced Treg/Th17 imbalance remains elusive.

In addition, previous study has elucidated that APS exerts its functions via modulating transcription factors (TFs) in diseases, such as osteoarthritis, ulcerative colitis, and Alzheimer's disease.^{12–14} Nuclear factor-kappa B (NF- κ B) is regarded as one of critical and pleiotropic TFs that could modulate genes and affect various biological processes, including immune responses, cellular growth and maturation.¹⁵ Besides, NF- κ B is previously proven to accelerate transcription of gene encoding pro-inflammatory cytokine and is correlated with the pathogenesis and progression of inflammation-related disorders, such as inflammatory bowel disease and AR.^{16,17} Furthermore, NF- κ B activated by pro-inflammatory cytokines modulates miR-34a, thus disturbing Treg/Th17 balance in autoimmune diseases.¹⁸ Therefore, NF- κ B was introduced as a focus in our study.

Given the abovementioned evidence, we raised a hypothesis that APS may alleviate inflammatory responses in AR through improving NF- κ B-mediated Treg/Th17 imbalance, and accordingly set out to explore the functions of APS on AR as a promising direction for AR treatment.

Materials and Methods

Experimental Animals

All animal experiments were conducted with the approval of the protocols ratified by the Animal Ethics Council of The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University, and in conformity with the *Guidelines for the Care and Use of Laboratory Animals*.¹⁹ Guinea pigs (aged 6–7 weeks and weighing 240 g $\pm$ 30 g;

Guangdong Institute for Drug Control, Guangzhou, China; SYXK (Guangdong) 2020-0241) were fed with a standard diet and raised under 12-h light/dark cycles. The guinea pigs received experimental treatments after 1 week of adaptive feeding.

Animal Models and Processing

Based on the previously reported method,²⁰ the AR guinea pig models were established using OVA to stimulate and sensitize guinea pigs. In brief, guinea pigs of the OVA group were sensitized through an intraperitoneal injection of OVA (25 μ g/guinea pig) 3 times a week for 2 consecutive weeks. After 2 weeks, OVA solution (100 μ g/guinea pig) was dropped into the bilateral nasal cavities of guinea pigs through micropipettes once a day for 1 consecutive week. The guinea pigs were orally administered¹¹ APS with different concentrations (25, 50, and 100 mg/kg/day)^{21,22} or intranasally infused with lentivirus-packaged oe-NF- κ B daily 30 min prior to the intranasal OVA treatment from the 22nd to 31st day. In order to ensure better transfection efficiency, the guinea pigs were intranasally infused with 4 μ L lysophosphatidylcholine (1% w/v, phosphate buffered saline (PBS); Sigma-Aldrich, St. Louis, MO, USA)²³ before the infusion of lentiviral vectors, and afterwards, guinea pigs in the lentivirus-treated group were intranasally infused with 20 μ L lentivirus-packaged oe-NF- κ B (1×10^8 TU/mL), guinea pigs in the negative control group were intranasally infused with the same dose of lentivirus-packaged oe-NC. Guinea pigs of sham group received normal saline (NS) treatment. Lentivirus-packaged oe-NF- κ B and its negative control oe-NC were supplied by GenePharma (Shanghai, China).

Assessment of Rhinitis Symptoms

After the final oral administration, all guinea pigs were put into separate cages and then observed for 30 min, and the sneezing and rubbing times of guinea pigs within 30 min were recorded.

Enzyme-Linked Immunosorbent Assay (ELISA)

The guinea pigs were intraperitoneally injected with 40 mg/kg pentobarbital sodium for anesthesia 24 h after the symptom assessment. Guinea pig blood was harvested through intraorbital venipuncture and were centrifuged at 1000 g at for 10 min to separate serum. Afterwards, the levels of OVA-specific immunoglobulin-E (OVA-sIgE), OVA-specific immunoglobulin-G1 (OVA-sIgG1), tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-17, transforming growth factor (TGF)- β , IL-10, and forkhead box protein P3 (Foxp3) in the serum were examined by ELISA based on the manufacturer's instructions (ThermoFisher Scientific, Waltham, MA, USA).

Table 1. qPCR Primers.

	Forward Primer (5'-3')	Reverse Primer (5'-3')
IL-17	ATCAGGACGCGCAAACATG	TGATCGCTGCTGCCTTCAC
TGF- β	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGGC
IL-10	TAAT AAGCTCCAAGACCAAG	TAGAATGGGAACTGAGGTATC
Foxp3	GCGCCCTCTTGGACAGC	TTCTTTCTCTGGGCACACTGG
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA

Quantitative Real-Time Polymerase Chain Reaction (RT-Qpcr)

The levels of IL-17, TGF- β , IL-10, and Foxp3 in guinea pig nasal mucosa tissues were examined via RT-qPCR. Briefly, the guinea pigs were anesthetized through an intraperitoneal injection of 40 mg/kg pentobarbital sodium 24 h after the symptom assessment, after which guinea pig nasal mucosa tissues were taken out. The total RNA content in guinea pig nasal mucosa tissues was separated using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and synthesized into the complementary DNA using the cDNA synthesis kits (Invitrogen) based on the manufacturer's instructions. RT-qPCR was conducted on StepOne Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The primers of PCR are shown in Table 1. The relative gene expression was analyzed using the $2^{-\Delta\Delta C_t}$ method, with GAPDH as the endogenous reference.

Western Blot Analysis

The total protein content in guinea pig nasal mucosa tissues was separated using protein extraction kits (Beyotime, Shanghai, China) and quantified using bicinchoninic protein analysis kits (BCA; Pierce, Rockford, IL, USA). Afterwards, the protein samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane, after which the membrane was blocked with Tris-buffered saline with Tween-20 buffer (TBST) supplemented with 20 mM Tris-HCl, 5% skim milk, 150 mM NaCl, and 0.05% Tween 20 (pH 7.5) at room temperature for 2 h, incubated overnight at 4°C with rabbit anti-NF- κ B (bs-23217R, 1:500, Bioss Biotech, Beijing, China) and rabbit anti- β -actin (bs-0061R, 1:500, Bioss Biotech), and then incubated with goat anti-rabbit IgG (ab6721, 1:2000, Abcam, Cambridge, UK) at room temperature for 2 h. The protein bands were developed with the help of enhanced chemiluminescence (ECL) Western blotting reagent (Millipore, Billerica, MA, USA) and quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA), with β -actin as the internal reference.

Flow Cytometry

The collected guinea pig blood was added with heparin for anticoagulation, and peripheral blood mononuclear cells (PBMCs) were isolated from the heparinized blood samples, after which isolated cells were rinsed with PBS for 3 times. Subsequently, the surface of PBMCs was stained with CD4-PE and CD25-PE, followed by cell staining using anti-Foxp3 or anti-IL-17 staining kits. Finally, CD4⁺IL-17⁺Th17 cells and CD25⁺Foxp3⁺Treg cells were examined using a flow cytometer.

Statistical Analysis

Data analysis and graphing were performed using the SPSS21.0 software (IBM SPSS Statistics, Chicago, IL, USA) and GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Measurement data were indicated as mean $\pm$ standard deviation and in compliance with the normal distribution and the homogeneity of variance. The *t*-test was employed to assess data between two groups; one-way ANOVA was adopted for comparisons among multiple groups; Tukey's multiple comparisons test was employed for the post-hoc test; the *p* value was obtained from the two-sided test, and a value of *p* < 0.05 was regarded statistically significant and *p* < 0.01 was regarded highly statistically significant.

Results

AR Guinea Pigs Presents with Treg/Th17 Imbalance and Inflammatory Responses

Initially, OVA was used to stimulate and sensitize guinea pigs to establish the AR guinea pig models. Afterwards, the sneezing and rubbing times of guinea pigs were counted, which showed that the sneezing and rubbing times of AR guinea pig were increased (*p* < 0.05, Figure 1(A) and (B)). Meanwhile, OVA-sIgE and OVA-sIgG1 levels in AR guinea pig serum were increased (*p* < 0.05, Figure 1(C) and (D)), and TNF- α and IL-6 levels in the serum were elevated (*p* < 0.05, Figure 1(E) and (F)). Preceding studies proposed that AR induced Treg/Th17 imbalance.^{6,24} As a result, levels of the Treg cell cytokines IL-10 and Foxp3 as well as

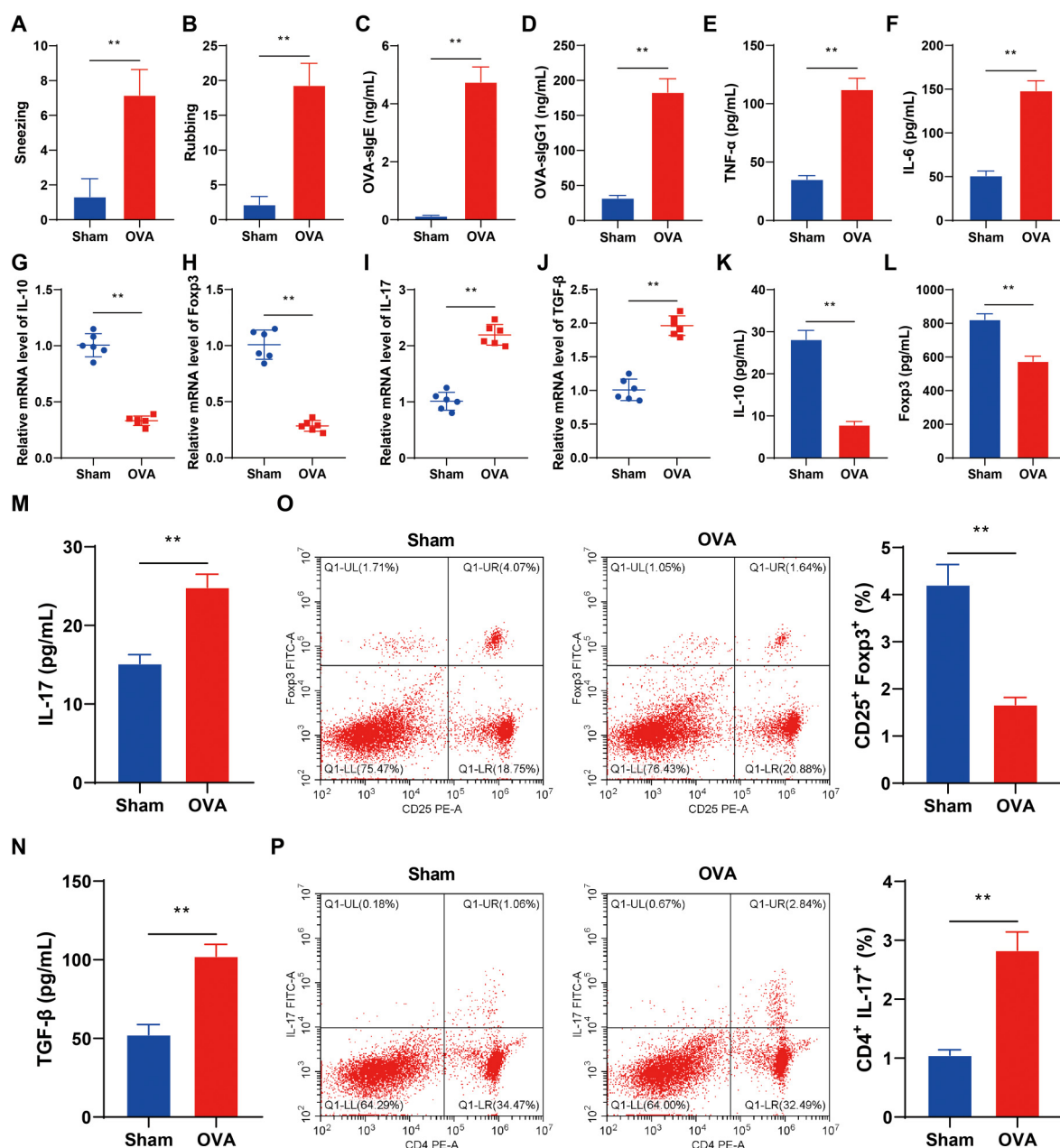


Figure 1. Ar guinea pigs presents with Treg/Th17 imbalance and inflammatory responses. The AR guinea pig models were established using OVA to stimulate and sensitize guinea pigs. (A–B) The sneezing and rubbing times of guinea pigs were counted; (C–F) ELISA was conducted to examine the concentrations of OVA-sIgE, OVA-sIgG1, TNF- α , and IL-6 in guinea pig serum; (G–J) RT-qPCR was performed to identify the mRNA levels of IL-10, Foxp3, IL-17, and TGF- β in guinea pig tissues; (K–N) ELISA was used to examine the concentrations of IL-10, Foxp3, IL-17, and TGF- β in guinea pig serum; (O–P) Flow cytometry was conducted to detect CD25⁺Foxp3⁺Treg or CD4⁺IL17⁺Th17 cell percentage in the guinea pig serum; N = 6, data in panels A–F and K–P were expressed as mean $\pm$ standard deviation; data in panels A–P were analyzed using the t-test; ** $p < 0.01$.

the Th17 cell cytokines IL-17 and TGF- β in guinea pig tissues or serum were examined. The testing results revealed that IL-10 and Foxp3 levels were declined whereas IL-17 and TGF- β levels were increased ($p < 0.05$, Figure 1(G) to (N)). The testing results of flow cytometry showed that

CD25⁺Foxp3⁺Treg cell percentage in AR guinea pig serum was decreased while CD4⁺IL17⁺Th17 cell percentage was increased ($p < 0.05$, Figure 1(O) and (P)). Together, the above findings indicated that AR guinea pigs developed Treg/Th17 imbalance and inflammatory responses.

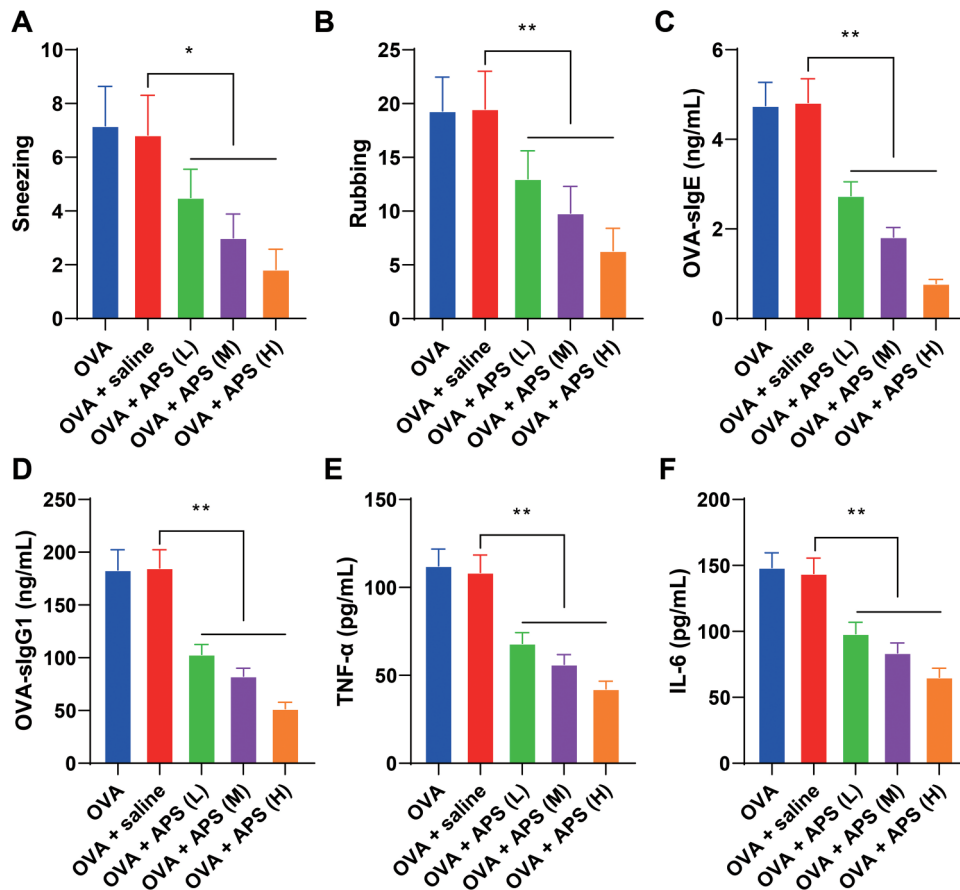


Figure 2. APS ameliorates inflammatory responses in AR guinea pigs. AR guinea pigs were treated with different concentrations of APS (Low (L): 25 mg/kg/day; Medium (M): 50 mg/kg/day; High (H): 100 mg/kg/day). A–B: The sneezing and rubbing times of guinea pigs were counted; C–F: ELISA was performed to identify the concentrations of OVA-sIgE, OVA-sIgG1, TNF- α , and IL-6 in guinea pig serum; N = 6, data in panels A–F were expressed as mean $\pm$ standard deviation; data in panels A–F were analyzed using one-way ANOVA, followed by Tukey's post-hoc test; * $p < 0.05$ and ** $p < 0.01$.

APS Ameliorates Inflammatory Responses in AR Guinea Pigs

Existing study mentioned that APS could alleviate AR symptoms,¹¹ but its specific mechanism remains to be studied. To further explore the role of APS in AR, AR guinea pigs were treated with different concentrations of APS. The testing results revealed that APS reduced the sneezing and rubbing times of guinea pigs in a dose-dependent manner ($p < 0.05$, Figure 2(A) and (B)). In addition, APS dose-dependently suppressed OVA-sIgE and OVA-sIgG1 levels ($p < 0.05$, Figure 2(C) and (D)), and TNF- α and IL-6 levels in AR guinea pig serum ($p < 0.05$, Figure 2(E) and (F)). Collectively, the above results suggested that APS could mitigate inflammatory responses in AR guinea pigs.

APS Reduces Treg/Th17 Imbalance in AR Guinea Pigs

Subsequently, we determined to probe the effects of APS on Treg/Th17 cell imbalance in AR guinea pigs. Firstly, the

detection results showed that, after APS treatment, IL-10 and Foxp3 levels in the guinea pig tissues and serum were up-regulated while IL-17 and TGF- β levels were down-regulated, and the above changes were dose-dependent ($p < 0.05$, Figure 3(A) to (H)). Flow cytometry showed that APS treatment increased CD25⁺Foxp3⁺Treg cell percentage while decreased CD4⁺IL17⁺Th17 cell percentage in a dose-dependent manner ($p < 0.05$, Figure 3(I) and (J)). Altogether, the above results indicated that APS could reduce Treg/Th17 imbalance in AR guinea pigs. And APS concentration (H) with better effect on alleviating AR was adopted for subsequent experiments.

NF- κ B Overexpression Reverses the Alleviative Effect of APS on Inflammatory Responses in AR Guinea Pigs

Previous studies documented that NF- κ B was prominently expressed in AR,¹⁷ and NF- κ B could mediate Treg/Th17 imbalance in various autoimmune diseases.¹⁸ Accordingly, NF- κ B expression in guinea pig nasal mucosa tissues were

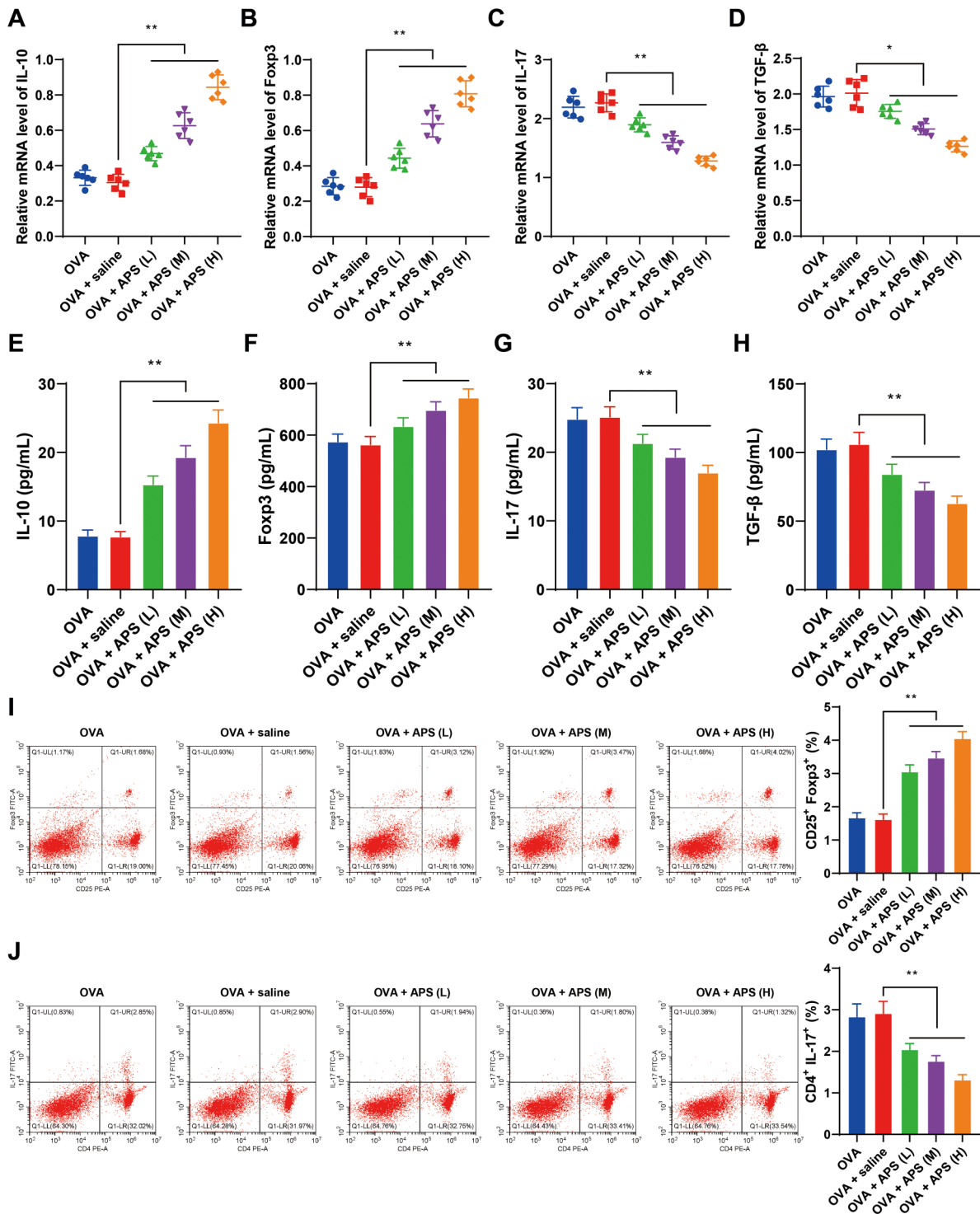


Figure 3. APS Reduces Treg/Th17 imbalance in AR guinea pigs. A–D: RT-qPCR was performed to examine the mRNA levels of IL-10, Foxp3, IL-17, and TGF- β in the guinea pig tissues; E–H: ELISA was conducted to examine the concentrations of IL-10, Foxp3, IL-17, and TGF- β in guinea pig serum; I–J: Flow cytometry was used to detect CD25⁺Foxp3⁺Treg or CD4⁺IL17⁺Th17 cell percentage in guinea pig serum; N=6, data in panels E–J were expressed as mean $\pm$ standard deviation; data in panels A–J were analyzed using one-way ANOVA, followed by Tukey's post-hoc test; * p < 0.05 and ** p < 0.01.

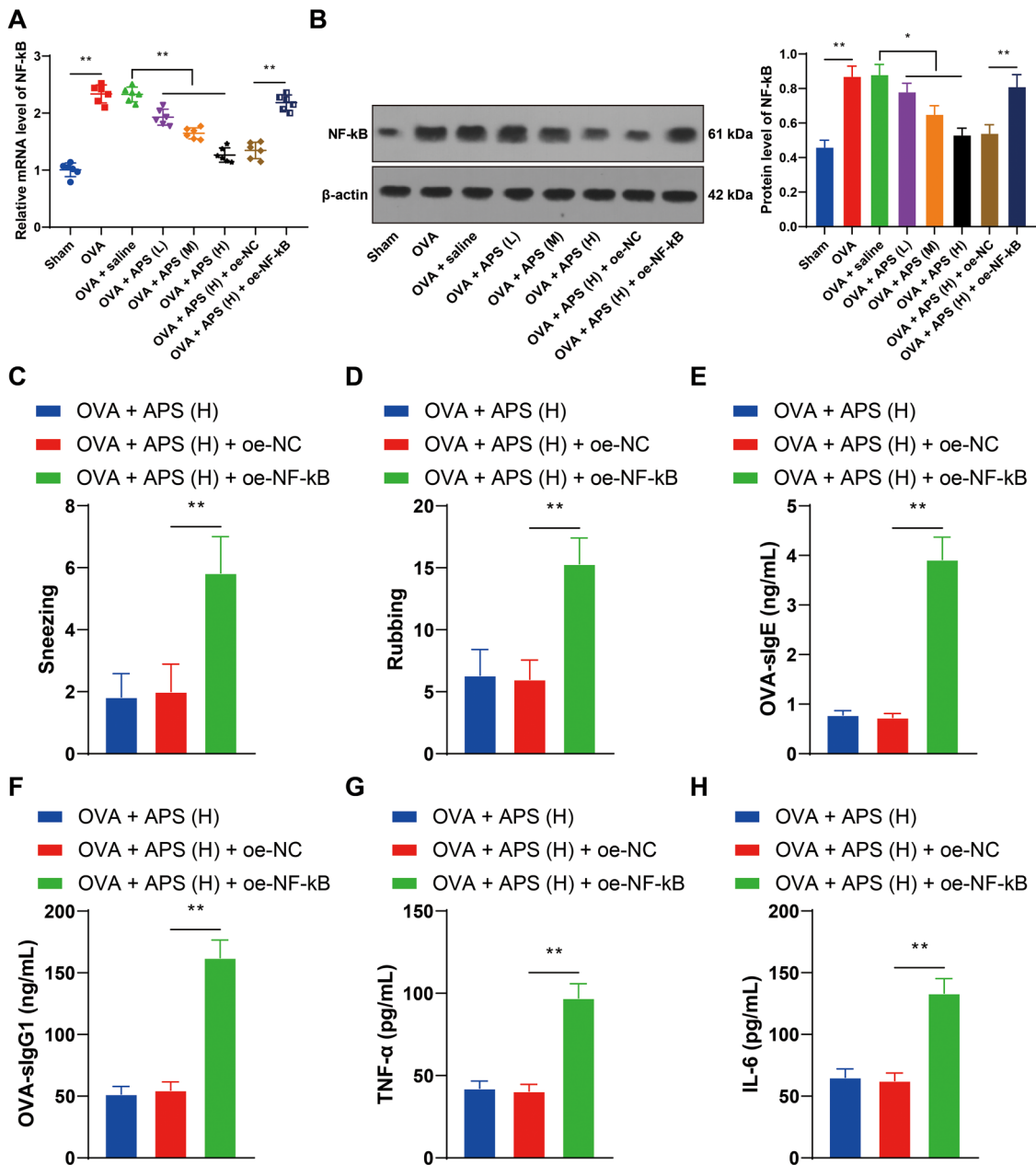


Figure 4. NF-κB overexpression reverses the alleviative effects of APS on inflammatory responses in AR guinea pigs. The guinea pigs were intranasally infused with lentivirus-packaged oe-NF-κB, with the infusion of oe-NC as the negative control. A–B: qRT-PCR and Western blot were used to examine NF-κB expression in guinea pig nasal mucosa tissues; C–D: The sneezing and rubbing times of guinea pigs were counted; E–H: ELISA was conducted to examine the concentrations of OVA-sIgE, OVA-sIgG1, TNF-α, and IL-6 in guinea pig serum; N=6, data in panels B–H were expressed as mean ± standard deviation; data in panels A–H were analyzed using one-way ANOVA, followed by Tukey's post-hoc test; * $p < 0.05$ and ** $p < 0.01$.

examined, which showed that NF-κB was up-regulated in guinea pig AR models and down-regulated after APS treatment ($p < 0.05$, Figure 4(A) and (B)). Subsequently, lentivirus-packaged oe-NF-κB was intranasally infused into the guinea pigs to overexpress NF-κB ($p < 0.05$, Figure 4(A) and (B)), and then the guinea pigs were subjected to a joint experiment with APS. Afterwards, NF-κB

overexpression increased the sneezing and rubbing times of guinea pigs ($p < 0.05$, Figure 4(C) and (D)), and elevated OVA-sIgE and OVA-sIgG1 levels ($p < 0.05$, Figure 4(E) and (F)) and TNF-α and IL-6 levels ($p < 0.05$, Figure 4(G) and (H)) in the serum. Overall, the above results showed that NF-κB overexpression counteracted the alleviative effect of APS on inflammatory responses in AR guinea pigs.

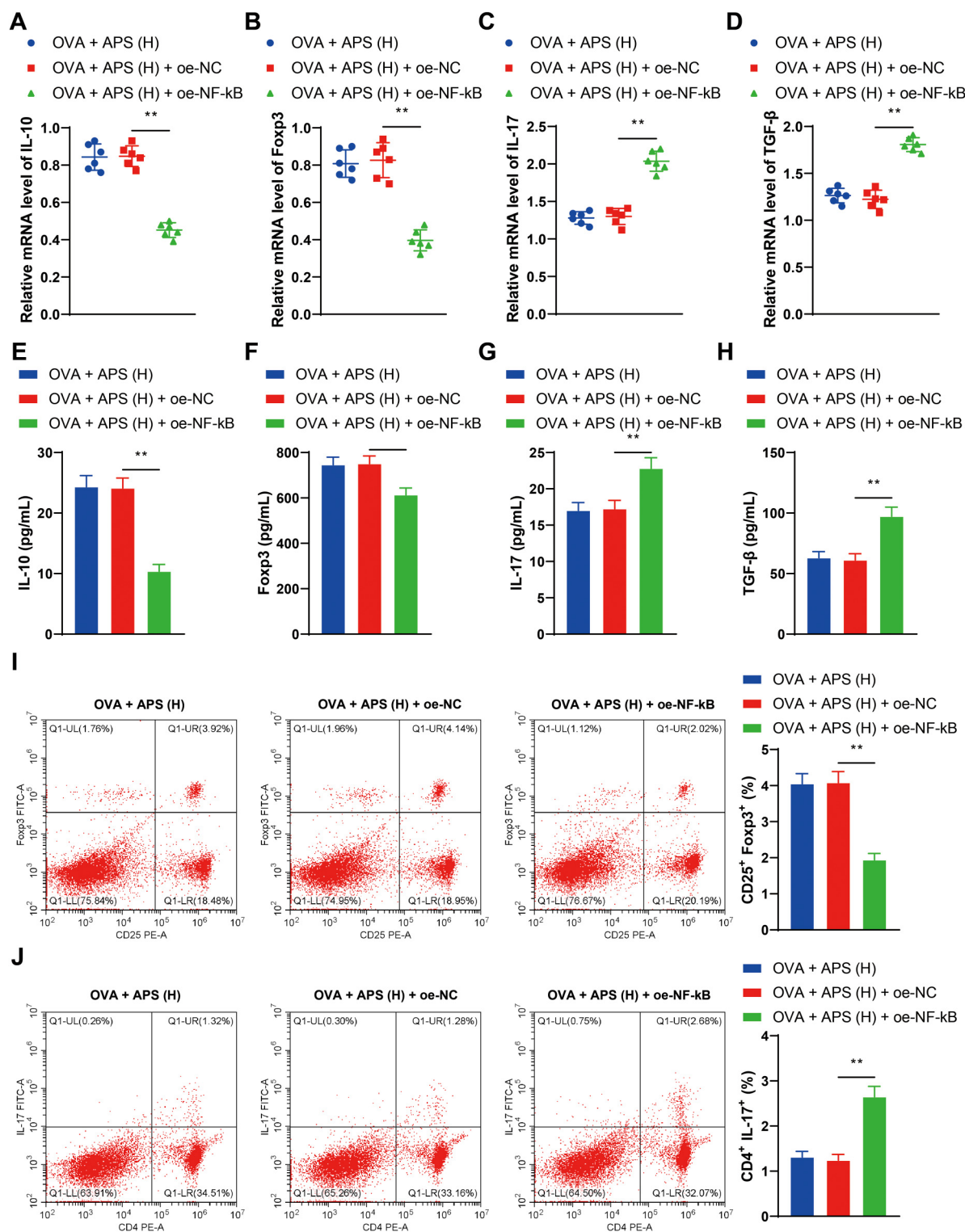


Figure 5. NF- κ B overexpression reverses the alleviative effects of APS on Treg/Th17 imbalance. A–D: RT-qPCR was used to identify the mRNA levels of IL-10, Foxp3, IL-17, and TGF- β in the guinea pig tissues; E–H: ELISA was performed to examine the concentrations of IL-10, Foxp3, IL-17, and TGF- β in guinea pig serum; I–J: Flow cytometry was conducted to detect CD25⁺Foxp3⁺Treg or CD4⁺IL17⁺Th17 cell percentage in guinea pig serum; N=6, data in panels E–J were expressed as mean $\pm$ standard deviation; data in panels A–J were analyzed using one-way ANOVA, followed by Tukey's post-hoc test; ** $p < 0.01$.

NF- κ B Overexpression Reverses the Alleviative Effect of APS on Treg/Th17 Imbalance

To further probe the effects NF- κ B exerted on AR guinea pigs, IL-10, Foxp3, IL-17 and TGF- β levels in guinea pig tissues and serum were identified, which showed that NF- κ B overexpression declined IL-10 and Foxp3 levels whereas increased IL-17 and TGF- β levels ($p < 0.05$, Figure 5(A) to (H)). Additionally, the results of flow cytometry revealed that NF- κ B overexpression reduced CD25⁺Foxp3⁺Treg cell percentage but increased CD4⁺IL17⁺Th17 cell percentage ($p < 0.05$, Figure 5(I) and (J)). To conclude, the above findings indicated that NF- κ B overexpression can reverse the improvement of APS on Treg/Th17 imbalance in AR guinea pigs.

Discussion

AR is considered as a prevailing chronic inflammation disorder worldwide, and the overall incidence of AR is increasing annually and varies by regions and by the gender and age of the population.²⁵ In addition, Treg/Th17 imbalance is closely associated with inflammation-related diseases.²⁶ Mounting studies proved that polysaccharide derived from plants not only possess anti-inflammatory activity, but also affects Treg/Th17 imbalance.^{27–29} Herein, our study highlighted that APS ameliorated AR inflammatory responses via reducing NF- κ B-mediated Treg/Th17 imbalance.

Th17 cells are known to produce pro-inflammatory cytokines while Treg cells possess the opposite function through secreting anti-inflammatory cytokines, and during inflammation, Th17 cells account for a large proportion in Treg/Th17 balance.³⁰ Of note, Treg cell reduction and Th17 cell amplification exist in AR patients and this imbalance is correlated with AR progression.⁶ On a separate note, a former review by Rahim et al highlighted that extracts derived from certain medicinal plants possess an anti-AR effect.³¹ APS derived from *Astragalus membranaceus* has various pharmacological properties and is regarded as a plant component with promising potential to improve or treat multiple diseases.³² Furthermore, APS is used for treating airway inflammation dominated by neutrophil and eosinophils, and could attenuate allergic symptoms of AR.^{11,33} However, the alleviating effects exerted by APS on AR remains to be clarified. OVA is widely adopted to induce the AR model, and OVA-sIgE up-regulation is observed in AR.³⁴ Consequently, we determined to establish AR guinea pig models through OVA stimulation and sensitization to probe the specific roles of APS in AR. Afterwards, OVA induction elevated the levels of OVA-sIgE, OVA-sIgG1, TNF- α , and IL-6, and meanwhile, declined CD25⁺Foxp3⁺Treg cell percentage while elevated CD4⁺IL17⁺Th17 cell percentage in AR guinea pig serum, which indicated that AR guinea pigs developed Treg/Th17 imbalance and inflammatory responses. Subsequently, APS with different concentrations was adopted to treat AR guinea

pigs, after which the sneezing and rubbing times of AR guinea pigs were reduced, and the levels of OVA-sIgE, OVA-sIgG1, and pro-inflammatory cytokines were declined in a dose-dependent manner. Relevant studies demonstrated that APS could effectively alleviate inflammatory responses induced by lipopolysaccharide in inflammatory osteolysis³⁵ and intestinal inflammatory damages in goslings,³⁶ further supporting that APS imparted an ameliorating role to inflammatory responses in AR guinea pigs.

Previous studies documented that polysaccharide from *Epimedium* could reducing Th17 cell proportion but increasing Treg cell proportion in aplastic anemia,²⁸ and polysaccharide from *Inonotus obliquus* could ameliorate Treg/Th17 imbalance through down-regulating Th17 cell proportion while upregulating Treg cell proportion in colitis.³⁷ Therefore, we speculated that APS may modulate Treg/Th17 balance in AR guinea pigs. Then, the subsequent results revealed that APS treatment upregulated IL-10 and Foxp3 levels whereas downregulated IL-17 and TGF- β levels, and increased CD25⁺Foxp3⁺Treg cell proportion and decreased CD4⁺IL17⁺Th17 cell proportion. The study reported by Zhao et al elucidated that APS treatment rescued the reduction of Treg cells and declined IL-17 level in Peyer's patches, thus alleviating colitis in rats.³⁸ However, conflicting studies elucidated the opposite trends that APS could reduce Treg proportion in polymicrobial sepsis and hepatocellular carcinoma,^{39,40} which may be due to difference types of diseases. In turn, our findings suggested that APS could increase Treg cell proportion and improve Treg/Th17 imbalance in AR guinea pigs, for the first time.

Increasing studies proved that polysaccharide could modulate pro-inflammatory cytokines via regulate NF- κ B-related pathways.^{9,41} Furthermore, NF- κ B was upregulated in AR,¹⁷ and participated in Treg/Th17 imbalance through modulating Foxp3 expression.¹⁸ Hence, we determined to explore the effects of NF- κ B on the alleviating role of APS in AR. Later, our experiments validated NF- κ B upregulation in nasal mucosal tissues of AR guinea pigs, and revealed that APS could down-regulate NF- κ B expression. On top of that, NF- κ B was overexpressed and underwent a joint experiment with APS in AR guinea pigs. NF- κ B overexpression increased the sneezing and rubbing times of AR guinea pigs, the levels of OVA-sIgE, OVA-sIgG1, and pro-inflammatory cytokines. In addition, its overexpression declined IL-10 and Foxp3 levels but increased IL-17 and TGF- β levels, and reduced CD25⁺Foxp3⁺Treg cell proportion while increased CD4⁺IL17⁺Th17 cell proportion. Preceding studies illustrated that APS exerted a protective role against colitis through suppressing NF- κ B activation,¹⁶ and mitigated virus myocarditis via repressing TLR4/NF- κ B p65 pathway.⁴² Besides, polysaccharide from *Inonotus obliquus* promoted Treg/Th17 balance via blocking TLR4/NF- κ B pathway in mice infected with *Toxoplasma gondii*.⁴³ Altogether, it is plausible to suggest that NF- κ B overexpression could neutralize the improvements of APS

on inflammatory responses and Treg/Th17 imbalance in AR guinea pigs.

Conclusion

In summary, our findings revealed that APS promoted Treg/Th17 balance via suppressing NF- κ B expression *in vivo*, thus reducing inflammatory responses in AR guinea pigs, which may provide a therapeutic approach for alleviating AR as well as inflammation-related diseases in the clinic. However, though APS could modulate multiple TFs, we only chose NF- κ B as a focus for experimentations. Additionally, we failed to explore the upstream or downstream genes of NF- κ B that may be implicated in the regulation of APS in AR guinea pigs. Going forward, we shall probe the regulatory effects exerted by APS on other genes in AR guinea pigs, and further explore the target genes of NF- κ B involved in the treatment of APS on AR guinea pigs.

Availability of Data and Materials

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

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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