Clinical and Experimental Immunology, 2023, **211**, 15–22
https://doi.org/10.1093/cei/uxac097
Advance access publication 11 November 2022
Research Article

Clinical & Experimental IMMUNOLOGY

OXFORD

Research Article

Signals from the TAFA4-PTEN-PU.1 axis alleviate nasal allergy by modulating the expression of FcεRI in mast cells

Caijie Zhou[1,‡], Meihua Li[1,‡], Yu Liu[2,‡], Xinxin Wang[3,4], Shuang Zhang[3,4], Li Guan[2], Jingyi Hong[3,4], Wei Zhou[3,4], Gaohui Wu[2], Weiliang Diao[1], Qinmiao Huang[*,2], Pingchang Yang[*,3,4]

[1]Longgang Traditional Chinese Medicine Hospital and Beijing University of Chinese Medicine Shenzhen Hospital, Shenzhen, China
[2]Department of Respirology & Allergy, Third Affiliated Hospital of Shenzhen University, Shenzhen, China
[3]Guangdong Provincial Regional Disease Key Laboratory, Shenzhen, China
[4]Institute of Allergy & Immunology of Shenzhen University, State Key Laboratory of Respiratory Diseases Allergy Division at Shenzhen University, Shenzhen, China

[‡]Equally contributed to this work.s
[*]Correspondence: Qinmiao Huang, Department of Respirology & Allergy, Third Affiliated Hospital of Shenzhen University, Shenzhen, China. Email: hqmwwb@163.com; Pingchang Yang. Room A7-509, 1066 Xueyuan Blvd, Shenzhen, China. Email: pcy2356@szu.edu.cn

Abstract

The high-affinity IgE receptor, FcεRI, plays a key role in the antigen-induced mast cell activation. Regulations for FcεRI are not yet well understood. TAFA4 is a molecule derived from neuron tissues, and has immune regulation functions. This study aims to clarify the role of TAFA4 in the regulation of FcεRI expression in mast cells. Nasal secretions were collected from patients with allergic rhinitis (AR) and healthy control (HC) subjects. TAFA4 levels of nasal secretions were evaluated by ELISA. A mouse model AR was developed using ovalbumin as the specific antigen. Negative correlation between TAFA4 and tryptase levels in nasal secretions was observed. TAFA4 could suppress the antigen-related mast cell activation. TAFA4 modulated the transcription of *Fcer1g* (FcεRI γ gene) in mast cells. Signals from the TAFA4-PTEN-PU.1 axis restricted FcεRI expression in mast cells. Administration of TAFA4 attenuated experimental AR. TAFA4 suppressed the expression of FcεRI in mast cells of airway tissues. TAFA4 can down regulate the expression of FcεRI in mast cells to suppress experimental AR. The data suggest that TAFA4 has translation potential to be developed as an anti-allergy therapy.

Keywords: nasal allergy, mast cell, IgE receptor, neuropeptide, immunity

Abbreviations: AR: allergic rhinitis; HC: healthy control; NSN: Nasal secretion; ELISA: Enzyme-linked immunosorbent assay; BMMCs: bone marrow derived mast cells; RE: relative expression; RT-qPCR: Real-time quantitative RT-PCR; NLF: nasal lavage fluid.

Introduction

Mast cells are the major effector cells in allergic diseases including allergic rhinitis (AR). Two types of IgE receptor, the higher affinity IgE receptor, FcεRI, and the lower affinity IgE receptor, CD23, have been described [1, 2]. FcεRI is the primary IgE receptor for allergic reactions. Mast cells and basophils express FcεRI. IgE binds FcεRI to form complexes to sensitize mast cells. Re-exposure to specific antigens cross links the complexes of IgE/FcεRI. This activates the mast cells to make the mast cells release allergic mediators, and therefore, to initiate allergic attacks [1, 2]. There is consensus that mast cells are canonical effector cells in allergic diseases. With regard to mast cells, numerous studies were conducted [1–3]. However, the key point about mast cell activation, the regulation of FcεRI expression in mast cells, is still poorly understood.

Neuropeptides have been found to contribute to the pathogenesis of allergic rhinitis [4]. Neuropeptides are produced by neurons. Some of neuropeptides and molecules, such as substance P, calcium ganglion related peptide, neuropeptide Y, and TAFA4, have powerful immune regulatory functions [5, 6]. It is reported that TAFA4 has immune regulatory functions, such as promoting the immune regulatory molecule, IL-10, in immune cells [7]. Whether TAFA4 can regulate the mast cell's activity has not been investigated. Therefore, based on the above information, we tested the effects of TAFA4 on the regulation of FcεRI expression in mast cells. We found that TAFA4 is effective at restricting the FcεRI expression in mast cells. Administration of TAFA4 could mitigate experimental AR.

Materials and Methods

Reagents

Antibodies (Abs) PTEN (A2B1), PU.1 (C-3), Pol II (B6-1), His (H-3), Flag (H-5), mast cell chymase (CC1, AF594), and FcεRI (H-5, AF488). TAFA4 was purchased from Santa Cruz Biotech (Santa Cruz, CA). ELISA kits of TAFA4 were

Received 22 June 2022; Revised 30 September 2022; Accepted for publication 24 October 2022

purchased from HSA Biotech (Shenzhen, China). ELISA kits of EPX, Mcpt1, IL-4, IL-5, IL-13, and OVA-specific IgE were purchased from CRK Pharma (Wuhan, China). Reagents and materials for immunoprecipitation (IP), chromatin IP (ChIP), RT-qPCR, and Western blotting were purchased from Invitrogen (Carlsbad, CA). Mouse TAFA4 protein was purchased from Huamei Biotech (Wuhan, China). Anti-OVA IgE Ab was purchased from Boyao Biotech (Shanghai, China).

Human subjects

The experimental procedures were examined and approved by our hospital's Human Ethics Committee (approval number: LGTM2021HE023). A written informed consent was obtained from each human subject. Patients with vivacious allergic rhinitis (AR) for more than a year were enrolled in the study at our allergy clinics from May 2021 to May 2022. The diagnosis of AR was made by our doctors in accordance with our routine procedures. All patients showed positive skin prick test (SPT; following published procedures [8]), positive specific serum IgE, typical AR clinical symptoms (including paroxysmal nasal itch, sneezing, profound nasal discharge, nasal congestion). To avoid being interfered by other inflammatory factors, patients suffering sinusitis were excluded. Additionally, subjects with any of the following conditions were also excluded: cancer, autoimmune diseases or serious organ diseases. Healthy control (HC) subjects were also recruited. All the HC subjects showed negative SPT and negative serum IgE results. The demographic data are presented in Table 1.

Table 1: demographic data of patients with allergic rhinitis

Items	AR	HC
Number	25	25
Age (years)	32.3 ± 4.5	31.6 ± 3.6
Male (%)	12 (48)	12 (48)
Female (%)	13 (52)	13 (52)
Serum total IgE (kU/L)	0.42 (0.05, 0.72)	0.21 (0.01, 0.33)
Serum sIgE (positive, %)	25 (100)	0 (0)
Co-suffer allergy		
Allergic asthma	0	0
Allergic dermatitis	0	0
Food allergy	0	0
Using corticosteroid history#	25	0
Blood neutrophil (10^9/L)	5.26 (4.43, 6.48)	5.81 (4.25, 6.19)
Blood eosinophil (10^9/L)	0.35 (0.16, 0.60)	0.12 (0.05, 0.15)
SPT results		
Bermuda grass	1 (4%)	0
Pine	2 (8%)	0
Poplar	2 (8%)	0
Rye	1 (4%)	0
Timothy grass	3 (12%)	0
Mugwort	2 (8%)	0
Mite mix	25 (100%)	0
Mold mix	5 (20%)	0
Animal dendar	3 (12%)	0

The data are presented as means ± SD or median (IQR). Specific IgE (sIgE) > 0.35 IU/ml was considered as positive. Patients were asked to stop using corticosteroid spray at least for 1 week before sample collection.

Assessment of antigen specific IgE (sIgE)

Blood samples (20 ml per person) were taken from each human subject through a ulnar venous puncture. The serum was isolated from the samples by placing the samples at 4°C overnight. Serum sIgE concentrations were determined by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). The positive criterion of sIgE was ≥ 35 IU/L.

Nasal secretion (NSN) collection

A piece of degreased cotton (approximately 2 grams) was gently inserted into the middle nasal meatus to adsorb the NSN. The cotton was taken off five minutes later. A 20 ml conical plastic tube (a piece of barrier was placed inside the tube to separate the cotton and NSN) was used to separate NSN. The tube was centrifuged at 5000 *g* for 5 min at 4°C. NSN was collected for further experiments.

Enzyme-linked immunosorbent assay

Cytokine and sIgE levels in the samples were measured by ELISA using specific reagent kits based on the recommended procedures.

Cell culture

Immune cells were cultured in PRMI1640 medium with supplements, including fetal calf serum (10%), penicillin (100 U/ml), streptomycin (0.1 mg/ml), and L-glutamine (2 nM) at 37°C in a 5% CO_2 atmosphere. For the BMMC degranulation assay (see below), the cells were cultured in medium containing no phenol red. Cell viability was assessed using the Trypan blue exclusion test prior to use in other experiments. The cell viability was greater than 99% throughout the experiments.

Generation of bone marrow derived mast cells

Bone marrow was removed from the femur bone of naive mice. After treatment with the red blood cell lysis buffer, the bone marrow cells were grown in a RPMI1640 medium supplemented by 2 ng/ml IL-3 and 5 ng/ml SCF. About 3 weeks culture, cells were harvested. $CD117^+$ $Fc\varepsilon RI^+$ bone marrow derived mast cells (BMMCs) were purified by FCM to be used in other experiments.

Assessment of BMMC degranulation

The BMMCs were sensitized by exposure to anti-OVA-IgE Ab (100 ng/ml) overnight. To induce BMMC degranulation, OVA (500 ng/ml) was added to the culture overnight with or without the presence of TAFA4 at gradient concentrations (10, 25, 50, and 100 pg/ml, respectively). Levels of β-hexosaminidase in culture supernatant were measured in accordance with published procedures [9], and considered to be an indicator of BMMC degranulation. Briefly, supernatants (50 µl) were mixed with 100 µl of substrate solution (2 mM PNAG in 0.1 M sodium citrate buffer, pH 4.5). The mixture was incubated for 1 h at 37°C. Glycine buffer (50 µl, 2 M, pH 10) was added to the sample to stop the reaction. The OD value of the samples was recorded in a microplate reader (Multi-mode microplate reader Synergy Neo2; BioTek Instruments; Germany) at 405 nm. The total β-hexosaminidase activity was determined using a whole cell lysate of the BMMCs lysed with 0.1% Triton X-100. The released β-hexosaminidase activity was expressed in percentage by using the following equation: released hexosaminidase

activity (%) = (sample OD – blank OD)/(total OD – blank OD) × 100.

Real-time quantitative RT-PCR (RT-qPCR)

RNA was extracted from the cells from the relevant experiments, converted to cDNA using a reverse transcription kit based on the recommended procedures. The cDNA samples were amplified in a qPCR device (CFX96, Bio-Rad) with a SYBR Green Master Mix kit in the presence of primers of *Fcer1g* (cagccgtgagaaagcagatg and gccaaccttcaaagcacaga) and *Pten* (ggcactgctgtttcacaaga and atcaccacacacaggcaatg). The results were computed using the 2-Ct method and presented in relative expression (RE).

Western blotting

Proteins were extracted from cells collected from relevant experiments, fractionated by SDS-PAGE, and transferred onto a PVDF membrane. The membrane was incubated with skim milk (5%) for 30 min at room temperature to inhibit the non-specific bond. Then, the membrane was incubated with primary Abs of interest (detailed in figures; diluted to 200 ng/ml), washed with Tris-buffered saline (TBST, containing 0.05% Tween 20) 3 times, incubated with secondary Abs (conjugated with HRP, diluted to 20 ng/ml). Immunoblots on the membrane were developed with the enhanced chemiluminescence, and photographed in an imaging device (UVP, Cambridge, UK).

Immunocomplex detection with IP

Proteins were extracted from cells collected from relevant experiments and precleared by incubation with protein G agarose beads for 2 h. The samples were centrifuged at 5000 *g* for 5 min. The supernatant was sampled, incubated with Abs of interest (detailed in the figures, the final Ab levels were 0.5 μg/ml) for 2 hours. The samples were centrifuged for 5 min at 5000 *g*. The beads were collected. The proteins on the beads were eluted and analyzed by western blot.

Evaluation of gene transcription activity using ChIP

Cells were fixed with 1% formalin for 15 min, incubated with a lysis buffer for 10 min, and sonicated to shear the DNA into small pieces. The samples were then processed with the IP procedures until the protein/DNA complex samples were obtained. DNA was recovered from the samples with a DNA extracting kit, and analyzed by qPCR in the presence of primers of the *Fcer1g* promoter (gctgtgtgtctctgtgtgtg and ggcagcaagacaccaagaaa). The results are presented as fold change of the input.

plasmids of *PTEN*, *SPI1*, and *FCER1G* promoter reporter

The plasmids of *PTEN*, *SPI1*, and *FCER1G* promoter luciferase reporter were supplied by the Sangon Biotech (Shanghai, China). Briefly, HEK293 cells were cultured in a 96-well microplate (3×10^4 cells per well). One day later, plasmids were added to the wells (100 ng/well) using transfection reagents (GeneJuice; Merk Millipore) based on recommended protocol. Cells were then used in further experiments.

Luciferase assay

HEK293T cells seeded on microplates (3×10^4 cells/well) were transiently transfected with *FCER1G* promoter luciferase reporter plasmid (100 ng), with or without the co-transfection with plasmids of *PTEN* and *SPI1* (100 ng each). On the next day, the luciferase activity in the total cell lysate was recorded.

Development of an AR mouse model

The animal experimental protocol was reviewed and approved by the Animal Ethics Committee of our hospital (approval number: LGTMME2021004). The animal research adhered to the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines). Male BALB/c mice (6-8-week-old) were purchased from Guangdong Experimental Animal Center (Foshan, China). Mice were maintained in a specific pathogen-free facility with free access to water and food. Mice were sensitized by subcutaneous injection of ovalbumin (OVA, 0.1 mg/mouse) mixed with 0.1 ml alum into the back skin on day 1 and day 7, respectively. Mice were boosted by treating with nasal instillation (20 μl/nostril, containing OVA 5 mg/ml with or without TAFA4 at 1 μg/ml) daily from day 9 to day 22. On day 23, mice were challenged by nasal instillation (20 μl/nostril, containing OVA 50 mg/ml). The AR response was assessed and recorded (see below).

Assessment of AR response

After the nasal challenge with OVA, nasal scratching times (the sign of nasal itch) and sneezing times were observed and recorded in a 30-min period. Mice were then anesthetized with ketamine injection (60 mg/kg). Blood samples were obtained using the eyeball pulling method. Mice were sacrificed through cervical dislocation. The trachea was exposed, and 1 ml of saline was injected into the trachea (toward the nose; the nostril was placed in a low position) to flush the nasal cavity. Saline solution was collected and used as nasal lavage fluid (NLF) in other experiments. Cytokines in NLF were quantified by ELISA.

Statistics

Student's *t* test or Mann Whitney test was performed to determine the difference between two groups. The ANOVA followed by the Dunnett test or the Bonferroni test was conducted for data in more than two groups. $P<0.05$ was set as a significant criterion.

Results

Negative association between TAFA4 and tryptase concentrations in nasal secretions

Nasal secretions were collected from patients with AR and HC subjects. We found that the AR group had lower TAFA4 levels compared to the HC group (Fig. 1A), whereas tryptase levels were higher in the AR group than in the HC group (Fig. 1B). A negative correlation was identified between TAFA4 levels and tryptase levels in nasal secretion samples (Fig. 1C). Given that tryptase is a representative mediator of mast cells, the results suggest that TAFA4 may help stabilize mast cells in the respiratory tract.

TAFA4 suppresses antigen-related mast cell activation

The relationship between TAFA4 and mast cell activation was further assessed. Bone marrow derived mast cells (BMMC) were prepared. BMMCs were sensitized by incubating with an ovalbumin (OVA)-specific IgE in culture overnight with or

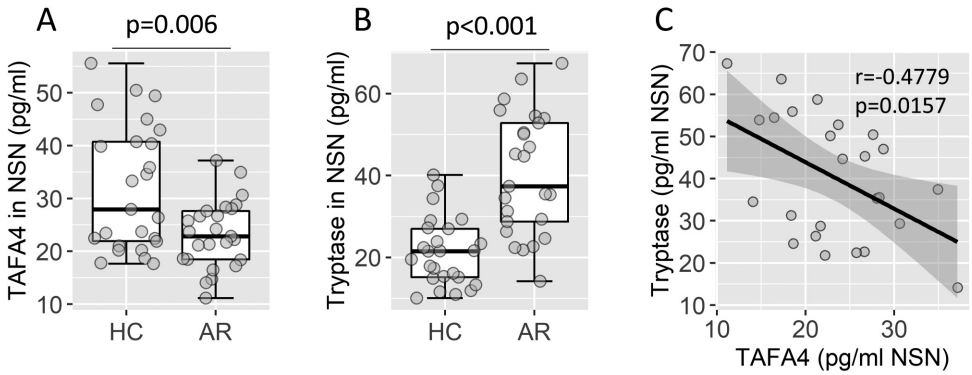

Figure 1: assessment of TAFA4 and tryptase in nasal secretions (NSN). NSN samples were collected from allergic rhinitis (AR) patients (n = 25) and healthy control (HC) subjects (n = 25), and analyzed by ELISA. A-B, median (IQR) of TAFA4 (A) or tryptase (B) levels in NSN. (C) Negative correlation between TAFA4 and tryptase levels in NSN.

without the presence of TAFA4 at gradient doses. Sensitized BMMCs released β-hexosaminidase (hexo, in short; used as an indicator of mast cell degranulation [9]) in response to OVA, but not to BSA. The results indicate that the sensitized BMMCs degranulate in response to specific antigens. The presence of TAFA4 was effective in suppressing degranulation of BMMCs in a dose-dependent manner (Fig. 2A). The evidence suggests that TAFA4 can inhibit mast cell activation. We also found that the expression of FcεRI in BMMCs was down regulated by TAFA4 in a dose-dependent manner (Fig. 2B). Results indicate that TAFA4 limits mast cell activation and suppresses FcεRI expression.

TAFA4 modulates *Fcer1g* (FcεRI γ-chain gene) transcription in mast cells

We then assessed the transcription activities of the *Fcer1g* gene in sensitized BMMCs following exposure to OVA or/and TAFA4 in culture. PU.1 is the transcription factor of *Fcer1g* [10, 11]. We found that the amounts of PU.1 and Pol II were significantly increased in the *Fcer1g* promoter in BMMCs after exposure to OVA. The increase of PU.1 and Pol II was countered when TAFA4 was present. Exposure to TAFA4 alone did not change the quantities of PU.1 and Pol II in promoter of *Fcer1g* (Fig. 3A-B). *Fcer1g* mRNA levels in BMMCs were increased following exposure to OVA, and were regulated in response to TAFA4 stimulation (Fig. 3C). The results demonstrate that TAFA4 can mitigate gene transcription of *Fcer1g* in mast cells.

The TAFA4-PTEN signals limit the expression of FcεRI in mast cells

The mechanism by which TAFA4 regulates the expression FcεRI was subsequently examined. It is recognized that PTEN plays a crucial role in maintaining immune homeostasis in cells [12]. Sensitized BMMCs were exposed to OVA (the specific antigen) in culture overnight. This caused lower PTEN levels in BMMCs. Exposure to TAFA4 increased the expression of PTEN to the baseline in BMMCs. Exposure of BMMCs to TAFA4 alone did not further increase the PTEN levels (Fig. 4A). Using an immunoprecipitation assay, we found a complex containing PTEN and PU.1 in BMMCs (Fig. 4B). The findings suggest that the PTEN is the link between the sensitization and upward regulation of the FcεRI expression. Furthermore, PTEN also serves as a link between TAFA4 and the downward regulation of FcεRI expression. To corroborate the results, a luciferase-linked *FCER1G* promoter reporter, a His-*PTEN* expressing plasmid, and a Flag-*SPI1* expressing plasmid were transfected into HEK293 cells. The results showed that the His-*PTEN*/Flag-*SPI1* complex was formed in HEK293 cells (Fig. 4C). The concomitant transfer of both *PTEN*-plasmids and *SPI1*-plasmids, but not either one alone, markedly activated the *FCER1G* promoter reporter (Fig. 4D). The results show that TAFA4 is capable of suppressing transcription of the *FCER1G* gene.

Administration of TAFA4 attenuates experimental AR

The effects of TAFA4 on modulation of the AR response were then tested. An AR mouse model was developed with ovalbumin (OVA) as a specific antigen. The mice exhibited AR-like response, including nasal itch, sneezing (Fig. 5A,B), an increase in the levels of allergic mediators (Mcpt1 and EPX) (Fig. 5C,D) and Th2 cytokines (IL-4, IL-5, IL-13) (Fig. 5E-G) in nasal secretions. OVA-specific IgE was detected in the serum (Fig. 5H). Administration of TAFA4 during the sensitization period, efficiently suppressed the AR response (Fig. 5). On the other hand, mice receiving the TAFA4 treatment did not show appreciable side effects.

TAFA4 suppresses the expression of FcεRI in mast cells of the airway tissues

At the time of sacrifice, respiratory tract tissues were excised in AR mice treated with or without TAFA4. Airway mononuclear cells (AMC) were isolated from the tissues with the enzyme-digestion approach, and analyzed by flow cytometry (FCM). We found that the number of mast cells in the airway tissues was increased in AR mice. Treatment of AR mice with TAFA4 did not appear to alter mast cell counts (Fig. 6A,B). However, the staining of FcεRI (reported by MFI, the median fluorescence intensity) in mast cells was significantly reduced in mast cells by TAFA4 (Fig. 6C,D). The results further verify that TAFA4 can down regulate the expression of FcεRI in mast cells.

Discussion

FcεRI in mast cells plays a crucial role in the IgE-mediated allergic reactions. Thus, to regulate the expression of FcεRI in

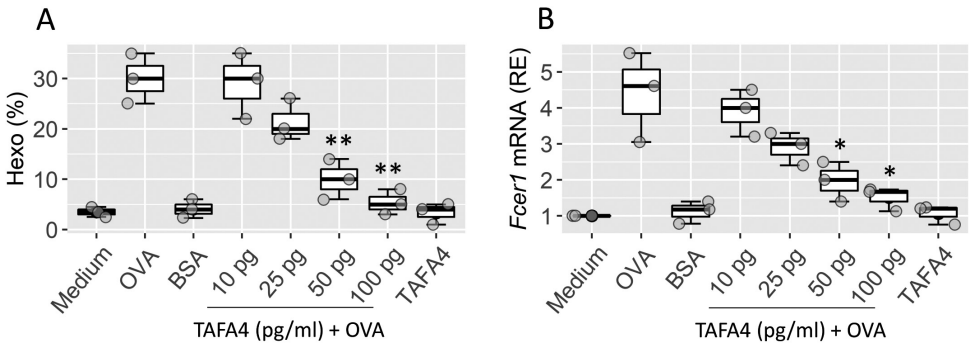

Figure 2: TAFA4 downregulates mast cell activation. BMMCs were sensitized to OVA-specific IgE, and treated with the procedures (denoted on the *X*-axis of boxplots) overnight. A, median (IQR) of hexo levels (compared against the hexo value induced by 0.1% Triton X-100) in supernatant from 3 experiments. B, median (IQR) of *Fcer1g* mRNA levels in BMMCs from 3 experiments. Hexo: β-hexosaminidase. OVA: Ovalbumin (0.5 μg/ml; the specific antigen). BSA: Bovine serum albumin (5 μg/ml; used as an irrelevant antigen).

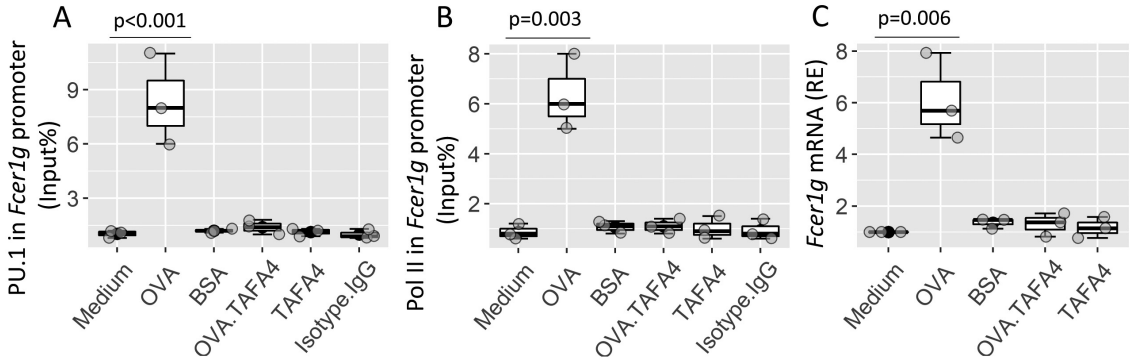

Figure 3: TAFA4 suppresses *Fcer1g* gene transcription. BMMCs were treated with the procedures denoted on the X axis of boxplots. ChIP results show the median (IQR) of PU.1 (A) and Pol II (B) in the *Fcer1g* promoter of BMMCs. (C), the median (IQR) of *Fcer1g* mRNA in BMMCs. BMMC: Bone marrow derived mast cells. ChIP: Chromatin immunoprecipitation. Pol II: RNA polymerase II.

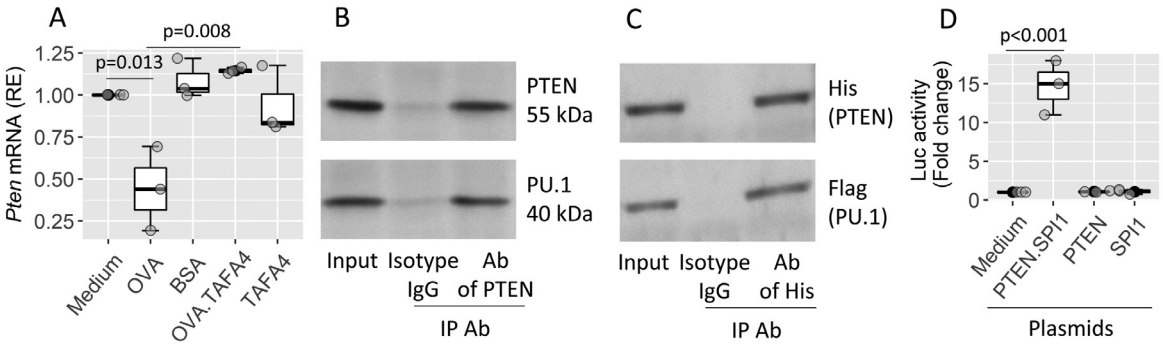

Figure 4: Assessment of the role of PTEN in regulating *Fcer1g* gene activity. BMMCs were treated with the procedures denoted on the *X*-axis of panel A. Boxplots show median (IQR) of *Pten* mRNA in BMMCs. B, immunoblots of IP show a complex of PTEN and PU.1 in sensitized BMMCs after treating with OVA.TAFA4. HEK293 cells were transfected with PTEN-plasmids or/and PU.1-plasmids, and a luciferase *FCER1G* promoter reporter. C, A complex of recombinant PTEN and PU.1 in HEK293 cells. D, Luciferase activity measurement.

mast cells is expected to alleviate allergic diseases. This study has identified a new agent, TAFA4, which can regulate the expression of FcεRI in mast cells. TAFA4 increases the activities of PTEN. The latter form a complex with PU.1, the transcription factor of FcεRI. Such physical contact between PTEN and PU.1 precluded movement of PU.1 toward the FcεRI promoter. This event restricts the transcription of the FcεRI gene. In this way, expression of FcεRI in mast cells is suppressed. The evidence suggests that TAFA4 may be a potential drug for treating allergic diseases.

The data demonstrate that TAFA4 can be released into the nasal cavity. Patients with AR have lower levels of TAFA4. TAFA4 is released from GINIP neurons. Published data indicate that TAFA4 relieves mechanical hypersensitivity [13],

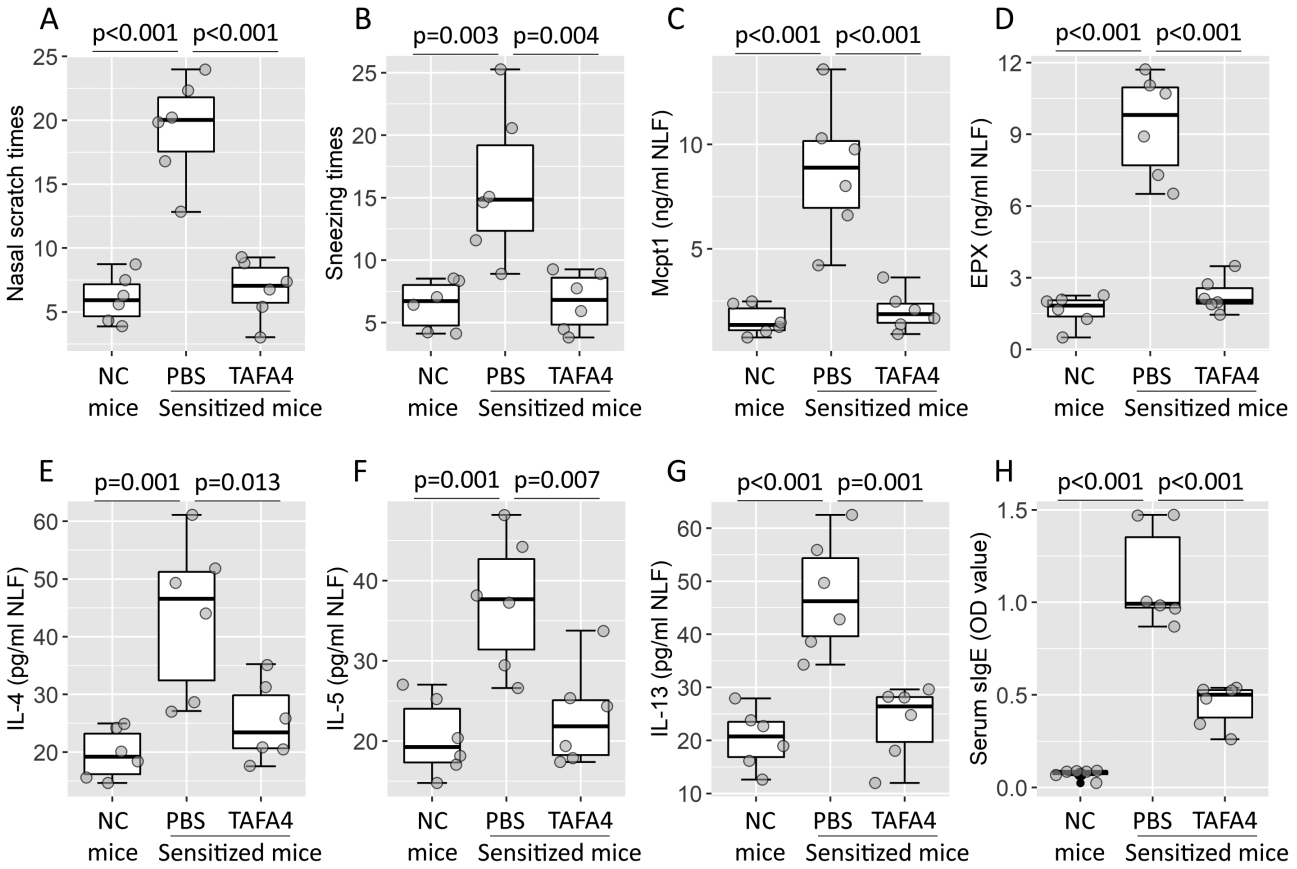

Figure 5: TAFA4 suppresses AR response. An AR mouse model was developed using OVA as a specific antigen. TAFA4 was employed in the period of immune boosting. A,B, AR clinical symptoms (Nasal itch and sneezing). C,D, allergic mediators (Mcpt1 and EPX) in NLF. E,G, Th2 cytokines (IL-4, IL-5, and IL-13) in NLF. H, serum sIgE levels. OVA: ovalbumin. NLF: nasal lavage fluid. PBS: using as a negative control agent. Each group consists of 6 mice.

involves in the pathogenesis of HPV infection [14], and promotes wound recovery [7]. Our data have added new insights to this field of study. The data demonstrate that TAFA4 can regulate FcεRI expression in mast cells. The data show that TAFA4 does not ablate the expression of FcεRI, the role of TAFA4 in this event can be defined maintaining the homeostasis of mast cells.

With the aim of alleviating allergic reactions, attempts were made to block IgE with appropriate antibodies. Clinical application of anti-IgE antibodies, such as omalizumab and ligelizumab, has shown positive results for the relief of allergic diseases [15]. Our evidence demonstrates an innovative approach to achieving this goal. The rationale behind this approach is based on the effects of TAFA4 on the regulation of FcεRI expression in mast cells. Through the administration of TAFA4, we did find that the expression of FcεRI in mast cells was regulated and the experimental AR was effectively attenuated.

The data show that the expression of PTEN can be up regulated by TAFA4. PTEN is an important molecule in the maintenance of homoeostasis in the cell. PTEN mutation has been observed in many types of cancers [16]. PTEN can relieve autoimmune diseases by controlling the STAT3-NF-κB signaling pathway [17]. It is proposed that PTEN can be used as a therapeutic tool for asthma treatment [18]. In this context, our data are consistent with previous studies. We found that PTEN could restrict the expression of FcεRI in mast cells. As aforementioned, PTEN did not ablate the expression of FcεRI in mast cells. The data verify the proposal that the TAFA4-induced PTEN on regulating the FcεRI expression in mast cells is to maintain the expression of FcεRI at a proper level.

The treatment of allergic conditions is currently unsatisfactory. Most treatment tools concentrate on symptom control. Of which administration of glucocorticoid agents or antihistamine agents is commonly employed in allergy clinics [19]. Most steroids and histamine blockades can suppress allergic reactions. However, these agents only have temporary therapeutic effects [20]. Specific antigen immunotherapy is an etiology targeting therapy. Its therapeutic effectiveness, however, remains in need of improvement [21]. Current data provide a potential new therapy for allergic illnesses. TAFA4 can suppress allergic reactions through down regulating the expression of FcεRI in mast cells. As mast cells are the canonical effector cells in allergic reactions, TAFA4 therapy has the translation potential to be developed for an anti-allergic drug.

In summary, the present data show that TAFA4 can suppress mast cell activity in allergic reactions. The mechanism behind this phenomenon is that TAFA4 activates the PTEN-PU.1 pathway to suppress the expression of FcεRI in mast cells. Administration of TAFA4 can efficiently alleviate experimental AR.

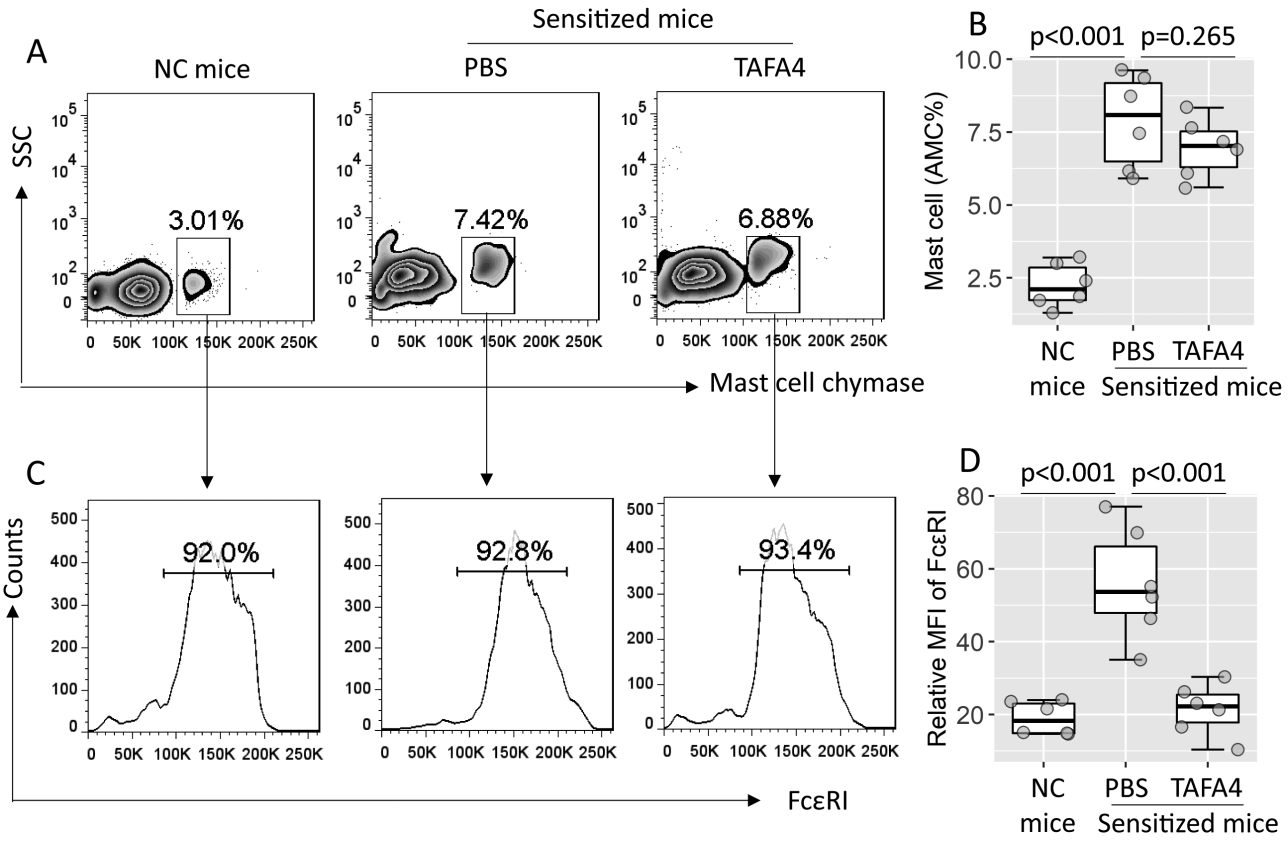

Figure 6: TAFA4 downregulates FcεRI in mast cells. An AR mouse model was developed using OVA as a specific antigen. TAFA4 was employed in the period of immune boosting. AMCs were prepared and analyzed by FCM. A, Gated FCM plots show mast cell counts. B, Median (IQR) of mast cell counts. C, Gated histograms show FcεRI$^+$ mast cell counts. D, Median (IQR) of relative MFI of FcεRI in mast cells of panel C. AR: allergic rhinitis. OVA: ovalbumin. AMC: airway mononuclear cells. FCM: flow cytometry. MFI: median fluorescence intensity.

Funding

This study was supported by grants of the National Nature and Science Foundation of China (32090052 and 32000655), Guangdong Provincial Key Laboratory of Regional Immunity and Diseases (2019B030301009), the Joint Youth Fund for Basic and Applied Basic Research of Guangdong Province (grant no. 2019A1515111095), the International Cooperative Research Project of Shenzhen Collaborative Innovation Program (grant no. GJHZ20190821173801659) and Shenzhen science, technology and innovation committee (KQTD20170331145453160)

Conflict of Interests

None declared.

Author Contribution

C.Z., M.L., YL., X.W., S.Z., L.G., J.H., W.Z., G.W., and W.D. performed experiments, analyzed data and reviewed the manuscript. Q.H. and P.Y. organized the study and supervised experiments. P.Y. designed the project and prepared the manuscript.

Ethical Guidelines

The experimental procedures were examined and approved by our hospital's Human Ethics Committee (approval number: LGTM2021HE023). A written informed consent was obtained from each human subject.

Data Availability

All the data are included in this paper.

Animal Ethic Guidelines

The animal experimental protocol was reviewed and approved by the Animal Ethics Committee of our hospital (approval number: LGTMME2021004). The animal research adhered to the ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines).

References

1. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med* 2012, 18, 693–704. doi:10.1038/nm.2755.
2. Elieh Ali Komi D, Wöhrl S, Bielory L. Mast cell biology at molecular level: a comprehensive review. *Clin Rev Allergy Immunol* 2020, 58, 342–65. doi:10.1007/s12016-019-08769-2.
3. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997, 77, 1033–79. doi:10.1152/physrev.1997.77.4.1033.
4. Li F, Jiang H, Shen X, Yang W, Guo C, Wang Z, et al. Sneezing reflex is mediated by a peptidergic pathway from nose to brainstem. *Cell* 2021, 184, 3762–3773.e10. doi:10.1016/j.cell.2021.05.017.
5. Perner C, Flayer CH, Zhu X, Aderhold PA, Dewan ZNA, Voisin T, et al. Substance P release by sensory neurons triggers dendritic cell migration and initiates the type-2 immune response to allergens. *Immunity* 2020, 53, 1063–1077.e7. doi:10.1016/j.immuni.2020.10.001.

6. Zhang Y, Zhang H, Jiang B, Tong X, Yan S, Lu J. Current views on neuropeptides in atopic dermatitis. *Exp Dermatol* 2021, 30, 1588–97. doi:10.1111/exd.14382.
7. Hoeffel G, Debroas G, Roger A, Rossignol R, Gouilly J, Laprie C, et al. Sensory neuron-derived TAFA4 promotes macrophage tissue repair functions. *Nature* 2021, 594, 94–9. doi:10.1038/s41586-021-03563-7.
8. Tantilipikorn P, Siriboonkoom P, Sookrung N, Thianboonsong A, Suwanwech T, Pinkaew B, et al. Prevalence of local allergic rhinitis to Dermatophagoides pteronyssinus in chronic rhinitis with negative skin prick test. *Asian Pac J Allergy Immunol* 2021, 39, 111–6. doi:10.12932/AP-170918-0408.
9. Okahashi N, Nakata M, Hirose Y, Morisaki H, Kataoka H, Kuwata H, et al. Streptococcal H2O2 inhibits IgE-triggered degranulation of RBL-2H3 mast cell/basophil cell line by inducing cell death. *PLoS One* 2020, 15, e0231101. doi:10.1371/journal.pone.0231101.
10. Herrmann N, Nümm TJ, Iwamoto K, Leib N, Koch S, Majlesain Y, et al. Vitamin D(3)-induced promotor dissociation of PU.1 and YY1 results in FcεRI reduction on dendritic cells in atopic dermatitis. *J Immunol* 2021, 206, 531–9. doi:10.4049/jimmunol.2000667.
11. Herrmann N, Koch S, Leib N, Bedorf J, Wilms H, Schnautz S, et al. TLR2 down-regulates FcεRI and its transcription factor PU.1 in human Langerhans cells. *Allergy* 2013, 68, 621–8. doi:10.1111/all.12145.
12. Mukherjee R, Vanaja KG, Boyer JA, Gadal S, Solomon H, Chandarlapaty S, et al. Regulation of PTEN translation by PI3K signaling maintains pathway homeostasis. *Mol Cell* 2021, 81, 708–723.e5. doi:10.1016/j.molcel.2021.01.033.
13. Yoo S, Santos C, Reynders A, Marics I, Malapert P, Gaillard S, et al. TAFA4 relieves injury-induced mechanical hypersensitivity through LDL receptors and modulation of spinal A-type K(+) current. *Cell Rep* 2021, 37, 109884. doi:10.1016/j.celrep.2021.109884.
14. Vink FJ, Dick S, Heideman DAM, De Strooper LMA, Steenbergen RDM, Lissenberg-Witte BI, et al. Classification of high-grade cervical intraepithelial neoplasia by p16(ink4a), Ki-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management. *Int J Cancer* 2021, 149, 707–16.
15. Gasser P, Tarchevskaya SS, Guntern P, Brigger D, Ruppli R, Zbären N, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun* 2020, 11, 165. doi:10.1038/s41467-019-13815-w.
16. Álvarez-Garcia V, Tawil Y, Wise HM, Leslie NR. Mechanisms of PTEN loss in cancer: It's all about diversity. *Semin Cancer Biol* 2019, 59, 66–79. doi:10.1016/j.semcancer.2019.02.001.
17. Lee SH, Park JS, Byun JK, Jhun J, Jung K, Seo HB, et al. PTEN ameliorates autoimmune arthritis through down-regulating STAT3 activation with reciprocal balance of Th17 and Tregs. *Sci Rep* 2016, 6, 34617. doi:10.1038/srep34617.
18. Kim SR, Lee YC. PTEN as a unique promising therapeutic target for occupational asthma. *Immunopharmacol Immunotoxicol* 2008, 30, 793–814. doi:10.1080/08923970802285164.
19. Simon D. Recent advances in clinical allergy and immunology 2019. *Int Arch Allergy Immunol* 2019, 180, 291–305. doi:10.1159/000504364.
20. Greuter T, Hirano I, Dellon ES. Emerging therapies for eosinophilic esophagitis. *J Allergy Clin Immunol* 2020, 145, 38–45. doi:10.1016/j.jaci.2019.10.027.
21. Yang L, Zhu R. Immunotherapy of house dust mite allergy. *Hum Vaccin Immunother* 2017, 13, 2390–6. doi:10.1080/21645515.2017.1364823.