

Taurine Promotes the Production of CD4+CD25+FOXP3+ Treg Cells through Regulating IL-35/STAT1 Pathway in a Mouse Allergic Rhinitis Model

Jing Zhou

Dahua hospital

Yi Lu

Fudan University

Wei Wu

Fudan University

Yunhai Feng (✉ fengyunhai@sina.com)

Dahua hospital

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Abstract

Background: Allergic rhinitis (AR) is one of the most widespread immune conditions worldwide. However, common treatments often present with significant side effects or are cost-prohibitive for much of the population. A plethora of treatments have been used for the treatment of AR including antihistamines, steroids, and immune modulators. Among the treatments which have shown potential for efficacy in treating AR with a minimum of side effects but remains understudied is the conditionally essential amino acid taurine. Taurine has been previously shown to reduce AR symptoms. Here, we examine the role of taurine in modulating T regulatory cells, modulating the cytokine response in AR, and restoring healthy nasal mucosa.

Methods: Blood samples from 20 healthy donors and 20 AR patients were compared for CD4⁺CD25⁺FoxP3⁺ T regulatory (Treg) cell population percentage, cytokine release, and STAT1 signaling with and without taurine treatment or IL-35 neutralization. An OVA-induced AR mouse model was administered vehicle, taurine, or taurine plus an IL-35 neutralizing antibody and assayed for sneezing frequency, inflammatory cytokine response, nasal mucosa goblet cell density, and T regulatory cell percentage. CD4⁺ cells were further examined for cytokine release, STAT1 phosphorylation, and response to an anti-IL-35 antibody with and without a STAT1 inhibitor.

Results: Comparison of blood from normal donors and AR patients showed a reduction in CD4⁺CD25⁺FoxP3⁺ Treg cells in AR patients and a strong correlation between Treg percentage and IL-35 release. A similar pattern of Treg suppression was found in untreated AR mice when compared to normal control mice wherein there was a reduction in Treg percentage and a corresponding decrease in IL-35 release. AR mice also demonstrated increased sneezing frequency, an infiltration of goblet cell in nasal mucosa, and a reduction in IL-35 release from CD4⁺ cells. Conversely, IL-4, IL-5, and IL-13 secretion from CD4⁺ cells were increased in AR model mice, as was STAT1 phosphorylation. When AR mice were treated with taurine, sneezing frequency and nasal mucosa goblet cell content were reduced while Treg abundance was increased to that of normal mice. Accordingly, IL-35 release was restored, while IL-4, IL-5, and IL-13 secretion from CD4⁺ cells were suppressed. Likewise, STAT1 phosphorylation was inhibited with taurine treatment. Taurine-treated mice also given an IL-35 neutralizing antibody exhibited AR pathology including frequent sneezing and high nasal goblet cell content while retaining a restoration of Tregs. Furthermore, murine AR model CD4⁺ cells exposed to recombinant IL-35 responded with a reduction in inflammatory cytokine release and a decrease in STAT1 phosphorylation, mimicking the effect of taurine treatment.

Conclusions: Taurine induces release of IL-35 in AR; IL-35 promotes the production of CD4⁺CD25⁺FoxP3⁺ Treg cells via a STAT1-dependent pathway. The restoration of Treg populations by taurine normalizes the inflammatory response, reduces AR symptomology, and reduces histopathologic signs of AR.

Background

Allergic rhinitis (AR), commonly known as hay fever, is a very common condition with approximately 10–40% people affected globally ¹ and is the most widespread chronic disease in children ². AR, which is manifested by nasal congestion and excessive mucus production, results from a misdirection of the immune system towards non-pathogenic antigens. Frequently, this is airborne pollen with other common allergens being dust or animal dander. While AR is typically thought of a nuisance disorder it can become life-threatening to those with underlying respiratory issues, particularly asthma ³.

At the cellular level AR involves a complex system of immune cell interaction including mast cells ⁴, T cells ⁵, macrophages ⁶, and B cells ⁷ with an accompanying change in the cytokine and antibody milieu of nasal tissue ^{8,9}. The broad range of cellular and molecular actors which cause AR has made the development of treatments difficult as they will need to target multiple pathways which increase the likelihood of side effects.

As AR is a disease of an overactive immune system, treatments are primarily focused on blunting the immune response to reduce symptoms. Common medications include antihistamines ¹⁰, corticosteroids ¹¹, anticholinergics ¹², mast cell stabilizers ¹³, leukotriene inhibitors ¹⁴, and anti-IgE immunotherapies ¹⁵. Antihistamines are one of the first therapeutics for the treatment of AR ¹⁶ and remain a mainstay of therapy in either oral or topical formulation ¹; however this class of medication is plagued by common side effects such as excessive drowsiness. While corticosteroids are among the most effective treatments for AR, particularly when used in combination with other medications such as antihistamines, they present with rare but potentially serious side effects such as an increase in intraocular pressure ¹⁷. Therapeutics which more directly target immune function, such as mast cell and leukotriene inhibitors, typically are more effective with fewer side effects when compared to earlier treatments but can be costly ¹⁸. Regardless, there are no treatments which are universally effective and there remains a clear need for an efficacious AR treatment with a highly tolerable side effect profile.

One potential avenue for elucidating a mechanism for a novel AR therapy is to examine the effect of taurine on AR. Taurine, a conditionally essential natural amino acid, has been shown to suppress AR symptoms ¹⁹ and is a naturally occurring nutrient with an established safety record ^{20,21}. The mechanism by which taurine regulates immune function remains unclear, in part due to the array of related functions found to be regulated by taurine. Published data indicate a role for taurine for reducing an inflammatory response in AR via modulating the MAPK (ERK, p38, JNK) signaling pathway ²² which have known roles in AR ^{23–25}.

A central hallmark of autoimmune disease such as AR is dysregulation of the immune system resulting in an inappropriate response to benign stimuli. In healthy individuals the immune system is regulated in part by a subset of T cells, T regulatory cells (Treg), which function to inhibit the inflammatory response to prevent a response against the body's own tissues or harmless foreign bodies. Tregs are primarily identified by the surface markers CD4 and CD25 as well as the transcription factor Forkhead Box P3 (FoxP3) ^{26–29}. It has been reported that the relative abundance of CD4⁺CD25⁺Foxp3⁺ Tregs was

decreased significantly in a Guinea pig model of allergic rhinitis and that increasing the percentage of CD4⁺CD25⁺Foxp3⁺ Tregs restrains allergic reactions through an increase in IL-10 production³⁰. In fact, diminution of Treg populations increases the allergic response³¹ and AR patients have been shown to have reduced Treg numbers³²⁻³⁴. The importance of Tregs in the allergic response is clear, however the mechanism by which they regulate AR may depend on several functions, the most probable of which is cytokine release.

Among immunoregulatory cytokines, interleukin-35 (IL-35) has been demonstrated to play a critical, multifaceted role. IL-35 has been shown to convert resting B and T cells into IL-10 and IL-35 secreting B regulatory (Breg) and T regulatory (Treg) cells which serve to blunt the autoimmune response³⁵. Importantly, IL-35 promotes the maturation of naïve T cells via STAT1 and STAT4 signaling to form regulatory iT35 cells which, in turn, secrete additional IL-35³⁶.

While taurine has demonstrated effectiveness in AR, and CD4⁺CD25⁺FoxP3⁺ Tregs and IL-35 are known to play a central role in immune response regulation, the association between these components remains to be elucidated. In this study we use both blood from human AR patients as well as employ a validated murine AR model to examine the changes in cytokine release and alterations in Treg populations in response to taurine treatment. We examine baseline hematological differences between normal and AR samples as well as AR symptoms, histopathologic and cellular reactions to taurine treatment in a murine model. Included in this investigation is exploration into the role of IL-35 in relieving AR following taurine treatment.

Methods

Human blood samples

A total of 40 human blood samples were used in the present study to evaluate the relationship between IL-35 and CD4⁺CD25⁺FOXP3⁺ Tregs. Blood was obtained from healthy control subjects and from allergic rhinitis patients, n = 20 for each group. All patients signed written informed consent. This study was approved by the independent ethics committee of Dahua Hospital, Shanghai, China and strictly obeyed the Declaration of Helsinki.

Mouse model

Eight-week-old C57BL/6 mice (n = 48, n = 12/treatment group), free of murine-specific pathogens, were obtained from the animal department at Shengjing Hospital, China Medical University (Shenyang, China). The mice were housed in a controlled environment with a 12/12-h light/dark cycle with free access to food and water. They were maintained on an ovalbumin (OVA)-free diet. The experimental procedures

were approved by the ethical committee of Dahua Hospital, Shanghai, China. All mice were handled according to Institutional Animal Care and Use Committee (IACUC) guidelines and experiments were conducted following the institute's guidelines for animal experiments.

The allergic rhinitis model mice were sensitized by intraperitoneal (i.p.) injection with 1 mg/mL OVA (Sigma-Aldrich, St. Louis, MO, USA) and 20 mg/mL aluminum hydroxide (Sigma-Aldrich) in normal saline at a dose of 100 μ L/mouse. The control group mice were sensitized and challenged with saline. Taurine (3% w/v) and IL-35 antibody (10 ng/ml) were used to treat mice through tail intravenous injection, respectively. All mice were sacrificed via cervical dislocation at day 42 after injection, and nasal mucosa tissues were removed from the xenograft mice and fixed in 4% formalin for further analysis.

Human or mouse CD4⁺ T cell isolation

Human or mouse blood samples were diluted by PBS solution (1:1) and peripheral blood mononuclear cells were obtained by centrifugation on a lymphocyte separation medium. The concentration of lymphocytes was then adjusted to 1×10^6 /mL. Human or mouse CD4⁺ T cells were isolated from PBMC using CD4⁺ T cell isolation kits respectively (130-096-533 (human) and 130-104-454 (mouse)), Miltenyi Biotec, Germany). Manufacturer instructions were followed for all procedures.

Cell culture

Cells were grown in DMEM (Trueline, Kaukauna, WI, USA) supplemented with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) 2 mM L-glutamine (Solarbio, Beijing, P.R. China), and 1% penicillin/streptomycin (Solarbio, Beijing, P.R. China) and were maintained under a 5% CO₂ atmosphere, at 37 °C. Cells were treated with taurine (20 mmol/L) and IL-35 recombinant protein (10 ng/mL) as indicated. The STAT1 inhibitor fludarabine phosphate (Fludara, 50 μ mol/L, A8317, APEXBIO, USA) was dissolved in DMSO (D2650, Sigma, USA).

ELISA

IL-4, IL-5, IL-13, and IL-35 quantitative ELISA kits were obtained from Bioscience (Shjgogo, Shanghai, China) and used to determine the concentration of IL-4, IL-5, IL-13, and IL-35 released from mouse CD4⁺ T cells. All procedures were performed according to the protocol of the manufacturer. Briefly, biotin-labeled antibodies against IL-4, IL-5, IL-13, or IL-35 were incubated with cell supernatant in a 96-well ELISA plate at 37 °C for 2 h. The plate was washed 5 times with wash buffer and incubated with HPR-labeled avidin and re-washed. Detection was provided by colorimetric reaction (≤ 5 min) which was read on a microplate reader (Pulangxin, China) to determine the OD450 value. All samples were analyzed in triplicate.

Western blot

Whole protein lysates were extracted from indicated cells by RIPA lysis buffer (JRDUN, Shanghai, China) with EDTA-free Protease inhibitor Cocktail (Roche, Mannheim, Germany). Protein concentration was quantified by an Enhanced BCA protein assay kit (Thermo Fisher, Waltham, MA, USA). Equal mass of total protein (25 µg) were fractionated on 10% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Temecula, CA, USA) overnight. After being blocked with 5% nonfat dry milk for 1 h at room temperature, the membranes were probed at 4 °C overnight with primary antibodies followed by secondary antibody anti-mouse IgG (1:1000; Beyotime, Shanghai, China) for 1 h at 37 °C. Enhanced chemiluminescence system (Tanon, Shanghai, China) was used to detect protein abundance. The primary antibodies used are as follows: IL-35 (1:500, 701101, Invitrogen, Carlsbad, CA, USA), Foxp3 (1:500, ab75763, Abcam, Cambridge, UK), STAT1 (1:1000, ab31369, Abcam, Cambridge, UK), p-STAT1 (1:1000, ab4742, Abcam, Cambridge, UK) and GAPDH (1:1000, CST, Danvers, MA USA).

Flow cytometry (FCM) assay

Human or mouse mononuclear cells were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS; Gibco, New York, USA), penicillin (100 ul/ml; Solarbio, Beijing, China) and streptomycin (100 g/ml; Solarbio, Beijing, China). The Anti- CD4 FITC (11-0049-42 (human) and 11-0043-82 (mouse), Ebioscience, San Diego, CA, USA), Anti- CD25 APC (17-0259-42 (human) 17-0251-82 (mouse), Ebioscience, San Diego, CA, USA) and Anti- Foxp3 PE (12-4776-42 (human) and 12-5773-82 (mouse), Ebioscience, San Diego, CA, USA) were separately added (5 µL) to the diluted lymphocytes according to the manufacturer's instructions (Ebioscience, San Diego, CA, USA). A FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA, USA) was used to establish FCM and data analysis was performed using CellQuest software (Becton Dickinson, Bedford, MA, USA). Cells treated with a non-specific secondary antibody only were defined as blank control. The scatterplots are arranged as follows: the upper left quadrant contains CD4⁺CD25⁻FOXP3⁺ cells, the lower left quadrant contains CD4⁺CD25⁻FOXP3⁻ cells, while the upper and lower right contain CD4⁺CD25⁺FOXP3⁺ and CD4⁺CD25⁺FOXP3⁻ cells, respectively.

Histopathology assay

All sample tissues were fixed in 10% formalin for 48 hr and subsequently embedded in paraffin blocks and cut into slices using a microtome (Leike, Wuhan, China). Slides were deparaffinized and rehydrated in a xylene bath followed by ethanol mixed with increasing concentrations of water. Then, slices were exposed to diaminobenzidine (DAB) substrate for 1 minute followed by hematoxylin and eosin (H & E) staining. Three random fields on each slide were observed.

Statistical analysis

GraphPad Prism software Version 7.0 (La Jolla, CA, USA) was used for statistical analyses. Data were displayed as mean \pm SD for a minimum of three replicates. Statistical significance was determined by one-way ANOVA for multiple comparisons. A P-value < 0.05 indicates statistical significance.

Results

IL-35 secretion correlates positively with the percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells in the blood of patients with allergic rhinitis (AR).

PBMCs were isolated from whole blood of patients with AR or from healthy controls and magnetically sorted for CD4 expression followed FoxP3 and CD25 quantification by flow cytometry to identify Tregs. CD25⁺/FoxP3⁺ cells were significantly less abundant in AR patients when compared to normal controls (2.5% vs 7%); interestingly the reduction in double-positive cells can be accounted for by the loss of FoxP3 expression alone (3.9% vs 12% in AR and healthy samples, respectively, Fig. 1A). This is particularly noteworthy due to the well-defined role of FoxP3 in enabling proper Treg function. Moreover, IL-35 secretion from cultured PBMCs isolated from AR patients was significantly reduced compared to that from that secreted by PBMCs isolated from healthy controls, as measured by ELISA (Fig. 1B, left). We found a strong positive correlation ($r = 0.7941$, $p < 0.001$) between the percentage CD4⁺CD25⁺FoxP3⁺ cells and IL-35 secretion (Fig. 1B, right). Based on this correlation, the role of IL-35 was examined further in a murine model.

Antibody targeting IL-35 partly disrupted the function of taurine in a mouse AR model.

Taurine administration has been demonstrated to reduce AR-induced physiological response^{19,22}, increase the percentage of Tregs³⁷, and reduce phosphorylation of STAT1³⁸, however the role of taurine in influencing IL-35 in AR has not been described. To investigate the interplay between taurine and the immunoregulatory cascade it is known to impact, we administered taurine with and without co-administration of an IL-35 neutralizing antibody to an OVA-induced allergic rhinitis mouse model. Mice sensitized with only saline were used as a non-AR control and saline-treated AR mice served as a vehicle-treated control. First, sternutation was quantified for a 30-minute observation period as marker for general AR symptomology. Saline-sensitized negative control mice exhibited minimal sneezing. As expected, saline (vehicle) treated AR mice demonstrated frequent sneezing, a significant increase over control mice ($p < 0.001$). AR mice treated with taurine DOSE, however experienced a robust repression ($p < 0.001$) confirming an effective reduction in symptoms. To establish a potential role of IL-35 in this AR model an IL-35 neutralizing antibody was administered to AR mice also treated with taurine; IL-35 neutralization restored AR symptoms to nearly that of vehicle treated AR mice ($p < 0.05$) (Fig. 2A) strongly implicating IL-35 as mediator of sneezing in AR mice treated with taurine.

To delve in the mechanistic impact of cytokine release in a taurine-treated AR model, the concentrations of four key immunoregulatory cytokines were measured: IL-4, IL-5, IL-13, and IL-35. IL-4 and IL-13 are known to be increased in model AR³⁹ and IL-5 induces eosinophil infiltration during allergic response⁴⁰. As expected, the serum concentration of all three of the type 2 cytokines (IL-4, IL-5, and IL-13) were increased in the AR model compared to the saline control mouse group indicating an immune response (Fig. 2B, $p < 0.001$). In alignment with a reduction in AR symptoms, the concentrations of these cytokines decreased following taurine treatment (Fig. 2B, $p < 0.05$). Notably, the concentration of immunoregulatory IL-35 decreased significantly in the AR model and was restored to a nearly normal concentration with taurine treatment. Importantly, the AR symptomology directly correlates to serum IL-35 levels throughout this experiment and the concentration of IL-35 in the serum itself is amenable to taurine treatment, thus confirming the potential of taurine as an AR therapeutic and indicating the mechanism of action is via IL-35.

To verify the role of IL-35 in attenuating AR following taurine treatment, an anti-IL-35 antibody was employed to sequester the cytokine in mice also given taurine. Following anti-35 antibody treatment IL-4, IL-5, and IL-13 concentrations were partially restored ($p < 0.05$), indicating the anti-inflammatory effects of taurine were lost and suggesting taurine suppresses AR via an IL-35-dependent mechanism (Fig. 2B). Moreover, the resulting serum concentration of IL-35 in taurine plus antibody treated AR mice was lower compared to the taurine alone condition (Fig. 2B). This reduction is expected to be the result of a feedback loop resulting from the alterations in the cytokine cascade as well as the resulting change Treg populations (discussed below), although we cannot conclusively rule out an interaction between the neutralizing antibody and the ELISA assay itself.

Taurine alleviates AR damage to nasal mucosa

Comparison of H&E stains of nasal mucosa from vehicle treated AR mice show extensive infiltration of mucus-secreting goblet cells indicating both a successful creation of an AR model and providing a tissue-level explanation for the increase in sneezing noted above. In contrast, taurine-treated AR mice were found to have mucosa histology which was highly similar to that of non-AR (healthy) control mice with minimal goblet cell presence. Administration of an anti-IL-35 antibody in combination with taurine treatment in AR mice resulted in goblet cell-rich mucosa with morphology nearly identical to that of the saline-treated AR mice (Fig. 2C). The restoration of the goblet cell infiltration following antibody treatment parallels and explains the increase in sneezing frequency in these mice and confirms the therapeutic effects are dependent on IL-35. In addition to phenotypic and histologic response to taurine treatment we sought to examine the effects of taurine and anti-IL-35 antibody treatment on the Treg population.

In agreement with the patient samples analyzed, the percentage of CD4⁺CD25⁺FoxP3⁺ Tregs within the CD4⁺ population decreased from approximately 6% in non-AR mice to 2% in AR model mice.

Administration of taurine rescued the Treg population in AR mice to nearly 6% of CD4⁺ cells. Also, in alignment of our previous results, addition of an anti-IL-35 antibody plus taurine treatment inhibited the

increase in Treg numbers induced by taurine, resulting in approximately 4% CD4⁺CD25⁺FoxP3⁺ indicating taurine restores Treg populations through an induction of IL-35 (Fig. 2D).

To determine the tissue-level alterations in protein expression which may account for the impact of taurine we measured protein expression in the nasal tissues of AR and control mice. As expected, based on flow cytometry and ELISA data, both IL-35 and FoxP³ abundance were reduced in the AR model compared to control mice while STAT1 phosphorylation was increased. Taurine treatment rescued IL-35 and FoxP³ levels and reduced STAT1 phosphorylation to nearly that of non-AR tissue (Fig. 2E). To delve into the role of IL-35 on protein abundance we examined FoxP³ and pSTAT1 in nasal mucosa tissue from mice treated with both taurine and an anti-IL-35 antibody. FoxP³ levels were reduced compared to taurine-treated AR mice while STAT1 phosphorylation was increased indicating a partial reinstatement of the AR model state (Fig. 2E). The loss of IL-35 following antibody treatment is suspected to be the result of a feedback loop in which IL-35 production is dependent upon IL-35 stimulation. As STAT1 phosphorylation correlates inversely with IL-35 levels and positively with AR symptoms we expect that STAT1 plays a role in the regulation of AR by IL-35.

There was a robust decrease in FoxP3 abundance in AR nasal mucosa compared to non-AR mice, indicating a loss of CD4⁺CD25⁺FoxP3⁺ Tregs in this tissue. Importantly, AR mice treated with taurine had a complete restoration for FoxP³ levels, a clear indication of normalization of immune response ($p < 0.001$, Fig. 2E). Anti-IL-35 antibody diminished FoxP³ restoration following taurine treatment, resulting in protein abundance equidistant from the AR model and normal control mice ($p < 0.001$, Fig. 2E).

IL-35 targeting antibody disrupted taurine response in AR CD4 + T cells

We sought to delineate the interplay of taurine and IL-35 on CD4⁺ AR cells. Murine AR CD4⁺ cells were exposed to taurine with or without an anti-IL-35 antibody; cytokine release was measured by ELISA. Secretion of the pro-inflammatory cytokines IL-4, IL-5, and IL-13 were all reduced with taurine treatment suggesting a reduction in AR pathology. In contrast, anti-inflammatory IL-35 secretion was increased. Each of these effects were reversed when an IL-35 neutralizing antibody was administered with the taurine treatment, a clear indication that the anti-inflammatory impact of taurine is mediated via IL-35 (Fig. 3A). Accordingly, STAT1 phosphorylation was strongly reduced in CD4⁺ cells following taurine treatment and was fully restored when cells were cultured in the presence of an IL-35 neutralizing antibody (Fig. 3B). The correlation of STAT1 phosphorylation with IL-35 availability suggests that STAT1 is the downstream target of IL-35 in AR.

Recombinant IL-35 mimics taurine in AR CD4 T cells.

To elucidate the role of IL-35 in regulating the autoimmune cytokine cascade, recombinant IL-35 or taurine was administered to CD4⁺ cells isolated from AR mouse model blood. Measurement of the type 2 cytokines IL-4, IL-5, and IL-13 showed all were reduced significantly with either taurine or recombinant IL-35 treatment ($p < 0.001$, Fig. 4A). Recombinant IL-35 also induced further IL-35 secretion (Fig. 4A). Moreover, both taurine and recombinant IL-35 reduced STAT1 phosphorylation in accordance with a known IL-35 signaling pathway (Fig. 4B)⁴¹. The fact that taurine induced IL-35 release and that IL-35 presence, either secreted from CD4⁺ cells or administered exogenously, shows a dose-dependent trend in reducing STAT1 phosphorylation strongly suggests that taurine-induced IL-35 secretion controls STAT1 phosphorylation in AR.

STAT1 inhibition suppressed the IL-35 antibody function in normal CD4 T cells.

To further investigate the role of STAT1 in immunoregulation we employed a STAT1 inhibitor, fludarabine phosphate, in conjugation with anti-IL-35 antibody treatment of normal mouse CD4⁺ cells. As expected, exposure to anti-IL-35 antibody alone produced an increase in secretion of IL-4, IL-5, and IL-13 ($p < 0.001$, Fig. 5A). Treatment with the STAT1 inhibitor along an anti-IL-35 antibody diminished the response to the antibody and resulted in a reduction in cytokine release ($p < 0.001$, Fig. 5A). Accordingly, western blotting showed an increase in STAT1 phosphorylation (activation) in cells subjected to antibody treatment which was reversible with STAT1 inhibitor treatment (Fig. 5B). Interestingly, while STAT1 inhibitor treatment reduced STAT1 phosphorylation to below that of untreated cells, type 2 cytokine release was only partly blunted by the presence of the inhibitor, suggesting a parallel pathway may also be at play.

Discussion

In this study we described the interplay between taurine and IL-35 in moderating AR symptoms. While taurine has been known to relieve AR symptoms^{19,22}, we were able to describe this mechanism at multiple physiological levels. AR mice treated with taurine experience reduced sneezing frequency owing to a reduction in goblet cell infiltration into the nasal mucosa and taurine induced an expansion of IL-35 secreting CD4⁺CD25⁺FoxP3⁺ Tregs. IL-35 reduced STAT1 phosphorylation and decreases inflammatory cytokine release from CD4⁺ cells.

Taurine is a known immunoregulatory agent which has been shown to be impactful in several disorders⁴²⁻⁴⁴ in addition to AR^{19,22,45} however the bulk of this work has been broad and lacked an examination of detailed cellular mechanisms. The research presented here is, to the best of our knowledge, the first to focus on a detailed mechanistic analysis of taurine in AR. We believe that it is through this detailed approach will be promote the development of novel treatments based on the pathway used by taurine.

Secreted by CD4⁺FoxP3⁺ Treg cells, IL-35 plays a central role in AR. IL-35 has a well-defined inhibitory function on AR symptomology ⁴⁶, indeed serum IL-35 levels are inversely correlated with the Total Nasal Symptom Score (TNSS) in children with AR ⁴⁷, and recombinant IL-35 has been used successfully as an exploratory therapeutic in an OVA mouse AR model ⁴⁸. Our results align well with these data in that serum and tissue IL-35 levels are inversely correlated to sneezing, goblet cell infiltration of nasal mucosa, and inflammatory cytokine release resulting from AR. Mechanistically, we found that IL-35 had an inhibitory effect on STAT1 phosphorylation in AR cells, in agreement with previous research showing a role for STAT1 in murine AR, in particular for the regulation of cytokine secretion ⁴⁹ and with other research showing a decrease pSTAT1 abundance following coadministration of IL-35 and TNF- α + IL-2 β ⁴¹.

Our results can be used to form a framework for the development of AR therapeutics; knowing that the critical downstream mediators of taurine-mediated AR suppression are CD4⁺CD25⁺FoxP⁺ Treg cells, IL-35, and STAT1 can facilitate the development of agents such as drug-like taurine analogs or other compounds which target this pathway. Other such modalities can include immunotherapies which target IL-35 or its receptor in AR. With the increasing use of recombinant antibodies as therapeutics, both in the autoimmune and oncology spaces, we indeed envision a role for IL-35 in the arsenal of AR medications.

As the human immune system is nearly infinitely complex, there are factors which may play a role in the taurine/IL-35/Treg pathway which may remain important but are outside of the scope of this work. These include cAMP, which is enriched in Tregs and functions through direct cell:cell interactions to mediate immune regulation ⁵⁰, CTLA-4, a well-described immune checkpoint protein ⁵¹, and the immuno-oncology target PD-1 ⁵². Moreover, we found that anti-IL-35 treatment only partially reversed the effects of taurine, suggesting an additional immunoregulatory pathway may be involved in AR suppression. We verified the importance of FoxP3⁺ Treg cells in AR here, future research will be required to determine if there is any consequential difference between FoxP3^{hi} vs FoxP3^{Lo} Treg cells ⁵³.

Abbreviations

AR☐allergic rhinitis☐

Treg: T regulatory;

FoxP3: Forkhead Box P3;

Breg: B regulatory ;

OVA☐ ovalbumin☐

FCM☐flow cytometry

Declarations

Ethics approval and consent to participate

This study was approved by the independent ethics committee of Dahua Hospital, Shanghai, China and strictly obeyed the Declaration of Helsinki. The experimental procedures were approved by the ethical committee of the Dahua Hospital, Shanghai, China. The handling of mice and all animal experiments were performed according to the Institutional Animal Care and Use Committee (IACUC) guidelines and the institute's guidelines.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests

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Not available

Author Contributions

Yunhai Feng designed this project and revised the manuscript; Jing Zhou performed the experiments and wrote the draft; Yi Lu analyzed the data and edited diagrams. Wei Wu helped to technical assistance.

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References

1. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, Zuberbier T, Baena-Cagnani CE, Canonica GW, Van Weel C, Agache I, Ait-Khaled N, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA2LEN and AllerGen). *Allergy Eur. J. Allergy Clin. Immunol.*2008;63:8–160.
2. Pediatric Otolaryngology - Charles D. Bluestone - Google Books [Internet]. [cited 2020 Jun 27];Available from: <https://books.google.com/books?id=y5rpEdeKtJEC&pg=PA1065#v=onepage&q&f=false>
3. Wheatley LM, Togias A. Allergic rhinitis. *N Engl J Med [Internet]*2015 [cited 2020 Jun 27];372:456–63. Available from: <http://www.nejm.org/doi/10.1056/NEJMcp1412282>
4. Pawankar R, Yamagishi S, Yagi T. Revisiting the Roles of Mast Cells in Allergic Rhinitis and its Relation to Local IgE Synthesis. *Am J Rhinol [Internet]*2000 [cited 2020 Jun 21];14:309–17. Available from: <https://pubmed.ncbi.nlm.nih.gov/11068656/>
5. Kaminuma O, Nishimura T, Saeki M, Mori A, Hiroi T. T cell-mediated nasal hyperresponsiveness in allergic rhinitis. *Biol Pharm Bull [Internet]*2020 [cited 2020 Jun 21];43:36–40. Available from: <https://pubmed.ncbi.nlm.nih.gov/31902929/>
6. Hirano M, Ogita-Nakanishi H, Miyachi W, Hannya N, Yamamoto-Kimoto Y, Sakurai K, Miyoshi-Higashino M, Tashiro-Yamaji J, Kato R, Ijiri Y, Tanaka K, Kanazawa A, et al. Essential role of macrophages in the initiation of allergic rhinitis in mice sensitized intranasally once with cedar pollen: Regulation of class switching of immunoglobulin in B cells by controlling interleukin-4 production in T cells of submandibular lymph nodes. *Microbiol Immunol [Internet]*2012 [cited 2020 Jun 21];56:392–405. Available from: <https://pubmed.ncbi.nlm.nih.gov/22404751/>
7. Luo J, Guo H, Liu Z, Peng T, Hu X, Han M, Yang X, Zhou X, Li H. Analysis of peripheral B cell subsets in patients with allergic rhinitis. *Allergy, Asthma Immunol Res [Internet]*2018 [cited 2020 Jun 21];10:236–43. Available from: </pmc/articles/PMC5911442/?report=abstract>
8. Eifan AO, Durham SR. Pathogenesis of rhinitis. *Clin Exp Allergy [Internet]*2016 [cited 2020 May 31];46:1139–51. Available from: <http://doi.wiley.com/10.1111/cea.12780>
9. König K, Klemens C, Eder K, San Nicolás M, Becker S, Kramer MF, Gröger M. Cytokine profiles in nasal fluid of patients with seasonal or persistent allergic rhinitis. *Allergy Asthma Clin Immunol* 2015;11:26.
10. Kawauchi H, Yanai K, Wang DY, Itahashi K, Okubo K. Antihistamines for allergic rhinitis treatment from the viewpoint of nonsedative properties [Internet]. *Int. J. Mol. Sci.*2019 [cited 2020 Jun 27];20. Available from: </pmc/articles/PMC6337346/?report=abstract>
11. Sur DKC, Plesa ML. Treatment of Allergic Rhinitis. *Am Fam Physician* 2015;92:985–92.
12. Georgitis JW. The anticholinergic treatment of allergic perennial rhinitis. *J Allergy Clin Immunol* 1992;90:1071–6.
13. Can I, Samim E. Mast Cell Stabilizers in the Treatment of Allergic Rhinitis. *Antiinflamm Antiallergy Agents Med Chem [Internet]*2008 [cited 2020 Jun 27];7:9–15. Available from:

<http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1871-5230&volume=7&issue=1&spage=9>

14. Parnes SM. The role of leukotriene inhibitors in allergic rhinitis and paranasal sinusitis. [Internet]. *Curr. Allergy Asthma Rep.*2002 [cited 2020 Jun 27];2:239–44. Available from: <https://pubmed.ncbi.nlm.nih.gov/11918866/>
15. Bayar Muluk N, Bafaqeeh SA, Cingi C. Anti-IgE treatment in allergic rhinitis. *Int. J. Pediatr. Otorhinolaryngol.*2019;127.
16. Frankland AW, Gorrill RH. Summer hay-fever and asthma treated with antihistaminic drugs. *Br Med J* 1953;1:761–4.
17. Bensch GW. Safety of intranasal corticosteroids. *Ann Allergy, Asthma Immunol [Internet]* 2016 [cited 2020 Jun 21];117:601–5. Available from: <https://pubmed.ncbi.nlm.nih.gov/27979016/>
18. Scow DT, Luttermoser G, Dickerson K. Leukotriene Inhibitors in the Treatment of Allergy and Asthma. *Am Fam Physician* 2007;75:65–70.
19. Zhou J, Lu Y, Li F, Wu W, Xie D, Feng Y. In vitro and in vivo Antiallergic Effects of Taurine on Allergic Rhinitis. *Int Arch Allergy Immunol* 2020;181:1–13.
20. Shao A, Hathcock JN. Risk assessment for the amino acids taurine, l-glutamine and l-arginine. *Regul Toxicol Pharmacol [Internet]* 2008 [cited 2020 Nov 1];50:376–99. Available from: <https://pubmed.ncbi.nlm.nih.gov/18325648/>
21. Ding L, Yang Z, Liu G, Ran N, Yi M, Li H, Zhao H, Tang L, Cheng H, Zhao J, Zhang Y, Ji X, et al. Safety and efficacy of taurine as an add-on treatment for tics in youngsters. *Eur J Neurol [Internet]* 2020 [cited 2020 Nov 1];27:490–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/31618495/>
22. Nam SY, Kim HM, Jeong HJ. The potential protective role of taurine against experimental allergic inflammation. *Life Sci* 2017;184:18–24.
23. Li Q, Chen Y, Ma Y, Zhang Y, Sun C. [Role of P-JNK and P-c-Jun of JNK transduction pathway on the nasal mucosa remodeling in allergic rhinitis rats]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi [Internet]* 2015 [cited 2020 Jun 21];29:2057–9. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27101678>
24. Liu J, Liu L, Cui Y, Zhang J, Jiang H. P38 MAPK regulates Th2 cytokines release in PBMCs in allergic rhinitis rats. *J Huazhong Univ Sci Technol - Med Sci [Internet]* 2010 [cited 2020 Jun 21];30:222–5. Available from: <https://pubmed.ncbi.nlm.nih.gov/20407878/>
25. Yang J, Zhong W, Xue K, Wang Z. Epigenetic changes: An emerging potential pharmacological target in allergic rhinitis [Internet]. *Int. Immunopharmacol.*2019 [cited 2020 Jun 21];71:76–83. Available from: <https://pubmed.ncbi.nlm.nih.gov/30878818/>
26. Valencia X, Lipsky PE. CD4+CD25+FoxP3+ regulatory T cells in autoimmune diseases. *Nat. Clin. Pract. Rheumatol.*2007;3:619–26.
27. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *J Immunol [Internet]* 2017 [cited 2020 Jun 20];198:981–5. Available from: <https://pubmed.ncbi.nlm.nih.gov/12522256/>

28. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *J Immunol [Internet]* 2017 [cited 2020 Jun 20];198:986–92. Available from: <https://www.nature.com/articles/ni904>
29. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet [Internet]* 2001 [cited 2020 Jun 20];27:20–1. Available from: https://www.nature.com/articles/ng0101_20
30. Leech MD, Benson RA, deVries A, Fitch PM, Howie SEM. Resolution of Der p1-Induced Allergic Airway Inflammation Is Dependent on CD4 + CD25 + Foxp3 + Regulatory Cells . *J Immunol* 2007;179:7050–8.
31. Palmer C, Mulligan JK, Smith SE, Atkinson C. The Role of Regulatory T Cells in the Regulation of Upper Airway Inflammation. *Am J Rhinol Allergy [Internet]* 2017 [cited 2020 Nov 8];31:345–51. Available from: <http://journals.sagepub.com/doi/10.2500/ajra.2017.31.4472>
32. Han D, Wang C, Lou W, Gu Y, Wang Y, Zhang L. Allergen-specific IL-10-secreting type I T regulatory cells, but not CD4+CD25+Foxp3+ T cells, are decreased in peripheral blood of patients with persistent allergic rhinitis. *Clin Immunol [Internet]* 2010 [cited 2020 Nov 8];136:292–301. Available from: <https://pubmed.ncbi.nlm.nih.gov/20403730/>
33. Genc S, Eroglu H, Kucuksezer UC, Aktas-Cetin E, Gelincik A, Ustyol-Aycan E, Buyukozturk S, Deniz G. The decreased CD4 +CD25 + FoxP3 + T cells in nonstimulated allergic rhinitis patients sensitized to house dust mites. *J Asthma [Internet]* 2012 [cited 2020 Nov 8];49:569–74. Available from: <https://www.tandfonline.com/doi/abs/10.3109/02770903.2012.695418>
34. Huang X, Chen Y, Zhang F, Yang Q, Zhang G. Desequilíbrio imunológico periférico mediado por células Th17/Treg em pacientes com rinite alérgica. *Braz J Otorhinolaryngol* 2014;80:152–5.
35. Dambuza IM, He C, Choi JK, Yu CR, Wang R, Mattapallil MJ, Wingfield PT, Caspi RR, Egwuagu CE. IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease. *Nat Commun* 2017;8.
36. Collison LW, Delgoffe GM, Guy CS, Vignali KM, Chaturvedi V, Fairweather D, Satoskar AR, Garcia KC, Hunter CA, Drake CG, Murray PJ, Vignali DAA. The composition and signaling of the IL-35 receptor are unconventional. *Nat Immunol [Internet]* 2012 [cited 2020 Jun 20];13:290–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/22306691/>
37. Miao J, Zhang J, Zheng L, Yu X, Zhu W, Zou S. Taurine attenuates Streptococcus uberis-induced mastitis in rats by increasing T regulatory cells. *Amino Acids [Internet]* 2012 [cited 2020 Jun 21];42:2417–28. Available from: <https://pubmed.ncbi.nlm.nih.gov/21809074/>
38. Huang J-S, Chuang L-Y, Guh J-Y, Huang Y-J, Hsu M-S, Huang J-S, Chuang L-Y, Guh J-Y, Huang Y-J. Antioxidants attenuate high glucose-induced hypertrophic growth in renal tubular epithelial cells. *Am J Physiol Ren Physiol [Internet]* 2007 [cited 2020 Jun 21];293:1072–82. Available from: <http://www.ajprenal.org>

39. Zhao C, Yu S, Li J, Xu W, Ge R. Changes in IL-4 and IL-13 expression in allergic-rhinitis treated with hydrogen-rich saline in guinea-pig model. *Allergol Immunopathol (Madr) [Internet]* 2017 [cited 2020 Jun 21];45:350–5. Available from: <https://pubmed.ncbi.nlm.nih.gov/28215576/>
40. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease [Internet]. *Respir. Res.* 2001 [cited 2020 Jun 21];2:71–9. Available from: </pmc/articles/PMC59571/?report=abstract>
41. Kanai K, Park A-M, Yoshida H, Tsunoda I, Yoshie O. IL-35 Suppresses Lipopolysaccharide-Induced Airway Eosinophilia in EBI3-Deficient Mice. *J Immunol* 2017;198:119–27.
42. Lu Y-F, Zhang Q-Y, Wang L-H, Liu X-Y, Zhang S-X. The protective effects of taurine on experimental autoimmune myocarditis. *Eur Rev Med Pharmacol Sci [Internet]* 2017 [cited 2020 Jun 20];21:1868–75. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28485791>
43. Lutz NW, Fernandez C, Pellissier JF, Cozzzone PJ, Béraud E. Cerebral Biochemical Pathways in Experimental Autoimmune Encephalomyelitis and Adjuvant Arthritis: A Comparative Metabolomic Study. *PLoS One [Internet]* 2013 [cited 2020 Jun 20];8. Available from: <https://pubmed.ncbi.nlm.nih.gov/23457507/>
44. Gambino CM, Di Bona D, Aiello A, Carru C, Duro G, Guggino G, Ferrante A, Zinellu A, Caruso C, Candore G, Accardi G. HLA-C1 Ligands are Associated With Increased Susceptibility to Systemic Lupus Erythematosus. *Hum Immunol [Internet]* 2018 [cited 2020 Jun 20];79:172–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/29395276/>
45. Lee H-S, Goh E-K, Wang S-G, Chon K-M, Kim H-K, Roh H-J. Detection of Amino Acids in Human Nasal Mucosa Using Microdialysis Technique: Increased Glutamate in Allergic Rhinitis. 2005. 213–219p
46. Xue JM, Ma F, An YF, Suo LM, Geng XR, Song YN, Mo LH, Luo XQ, Zhang XW, liu DB, Zhao CQ, Yang PC. Probiotic extracts ameliorate nasal allergy by inducing interleukin-35–producing dendritic cells in mice. *Int Forum Allergy Rhinol [Internet]* 2019 [cited 2020 Jun 20];9:1289–96. Available from: <https://pubmed.ncbi.nlm.nih.gov/31623025/>
47. Ruan G, Wen X, Yuan Z. Correlation between miR-223 and IL-35 and their regulatory effect in children with allergic rhinitis. *Clin Immunol [Internet]* 2020 [cited 2020 Jun 20];214. Available from: <https://pubmed.ncbi.nlm.nih.gov/32169441/>
48. Suzuki M, Yokota M, Nakamura Y, Ozaki S, Murakami S. Intranasal administration of IL-35 inhibits allergic responses and symptoms in mice with allergic rhinitis. *Allergol Int [Internet]* 2017 [cited 2020 Jun 20];66:351–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/27707583/>
49. Hattori H, Rosas LE, Okano M, Durbin JE, Nishizaki K, Satoskar AR. STAT1 is involved in the pathogenesis of murine allergic rhinitis. *Am J Rhinol [Internet]* 2007 [cited 2020 Jun 28];21:241–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/17424888/>
50. Bopp T, Becker C, Klein M, Klein-Heßling S, Palmetshofer A, Serfling E, Heib V, Becker M, Kubach J, Schmitt S, Stoll S, Schild H, et al. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. *J Exp Med [Internet]* 2007 [cited 2020 Jun 20];204:1303–10. Available from: </pmc/articles/PMC2118605/?report=abstract>

51. Sojka DK, Hughson A, Fowell DJ. CTLA-4 is required by CD4+CD25+ treg to control CD4+ T-cell lymphopenia-induced proliferation. *Eur J Immunol [Internet]* 2009 [cited 2020 Jun 20];39:1544–51. Available from: <https://pubmed.ncbi.nlm.nih.gov/19462377/>
52. Roskopf S, Jahn-Schmid B, Schmetterer KG, Zlabinger GJ, Steinberger P. PD-1 has a unique capacity to inhibit allergen-specific human CD4+ T cell responses. *Sci Rep [Internet]* 2018 [cited 2020 Jun 28];8:1–11. Available from: <https://www.nature.com/articles/s41598-018-31757-z>
53. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, et al. Functional Delineation and Differentiation Dynamics of Human CD4+ T Cells Expressing the FoxP3 Transcription Factor. *Immunity [Internet]* 2009 [cited 2020 Jun 20];30:899–911. Available from: <https://pubmed.ncbi.nlm.nih.gov/19464196/>

Figures

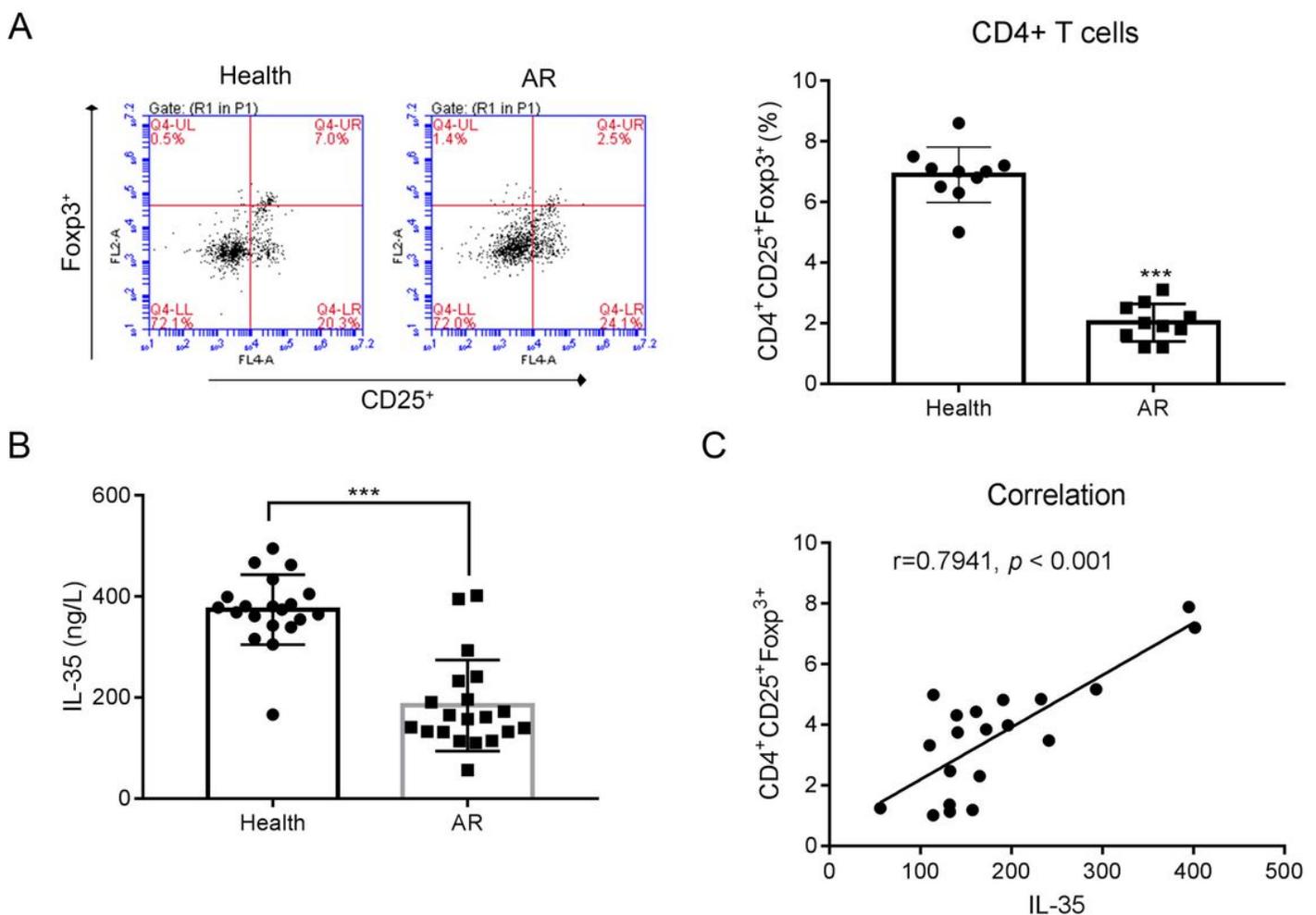


Figure 1

IL-35 secretion correlates positively with the percentage of CD4+CD25+Foxp3+ Treg cells in the blood of patients with allergic rhinitis (AR).

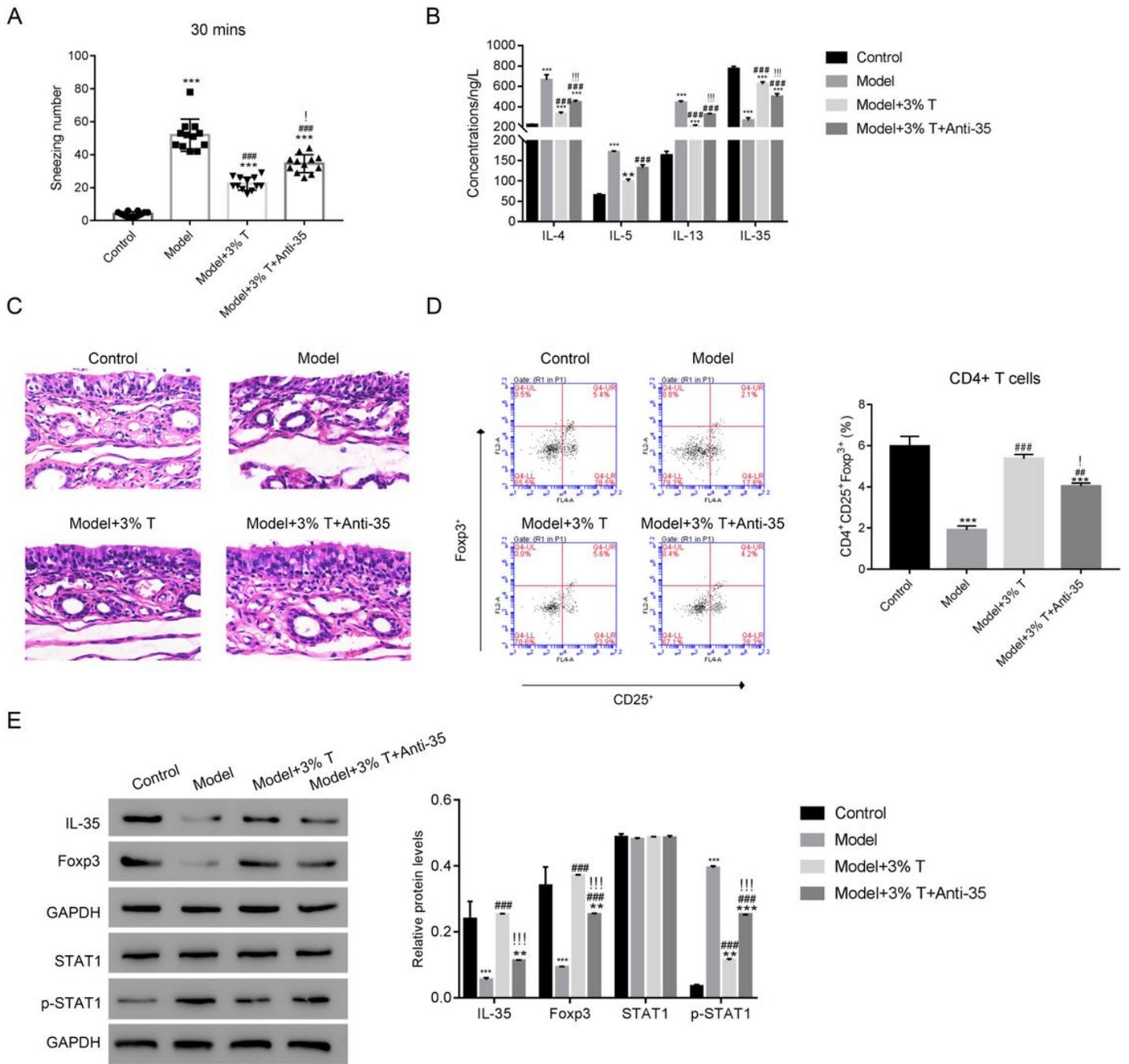


Figure 2

Taurine alleviates AR damage to nasal mucosa

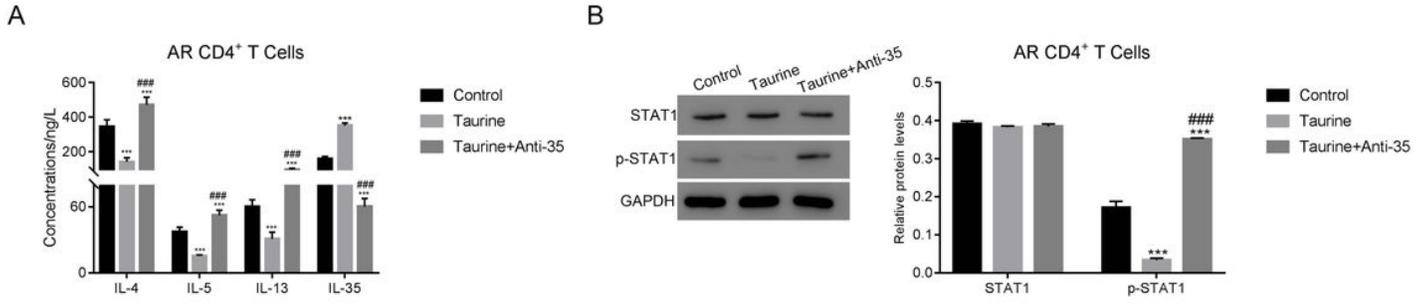


Figure 3

IL-35 targeting antibody disrupted taurine response in AR CD4+ T cells

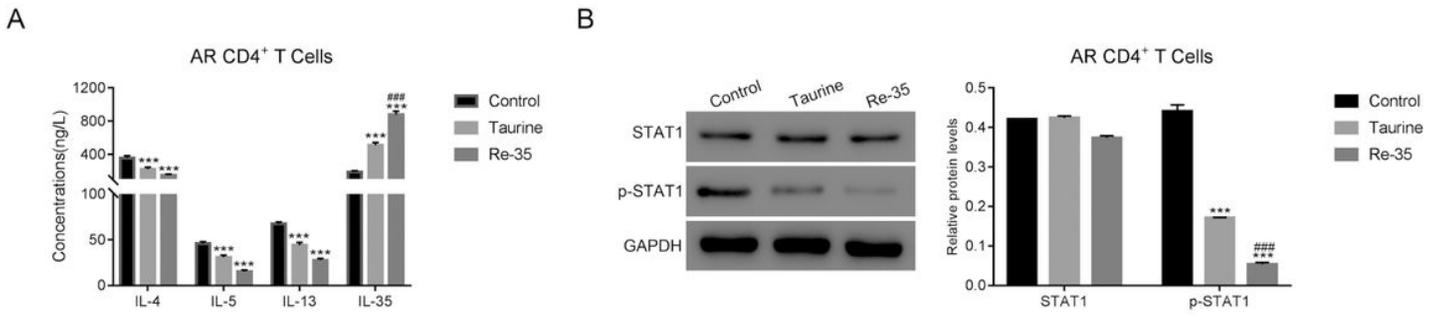


Figure 4

Recombinant IL-35 mimics taurine in AR CD4 T cells.

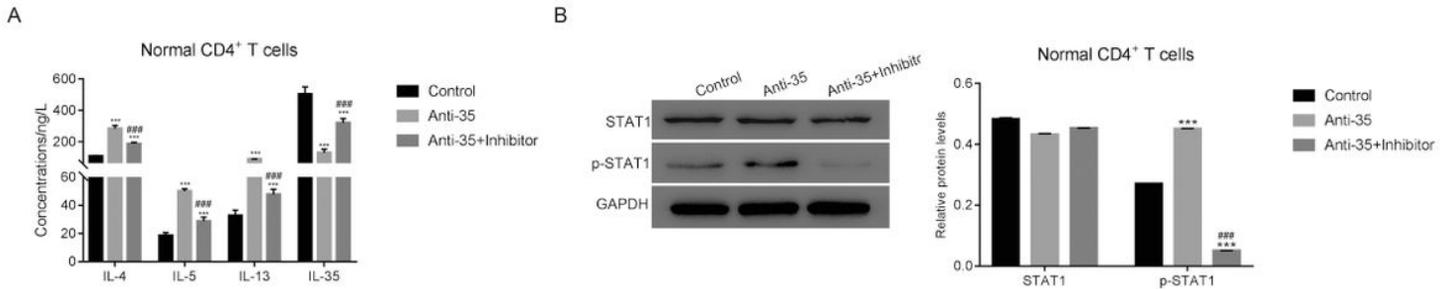


Figure 5

STAT1 inhibition suppressed the IL-35 antibody function in normal CD4 T cells.