

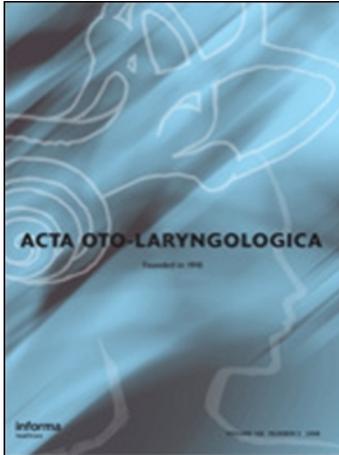
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Expression of uteroglobin in a murine model of allergic rhinitis

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Abstract

Conclusion. We observed for the first time the expression of Uteroglobin (UGB) in the nasal mucosa of mice. The results of our study suggest that UGB may play an important role in the regulation of inflammation in allergic rhinitis (AR) as well as in the lower airway allergic inflammations. **Objectives.** Uteroglobin is a protein secreted by epithelial lining of organs communicating with the external environment. Reports of its immunomodulatory effects in allergic disease have been made, but the true physiological role still remains to be elucidated. In this study we tried to observe the expression of UGB in the nasal mucosa of mice and determine its role in AR. **Materials and Methods.** Thirty BALB-c mice at 3 weeks of age (10 mice/group) were sensitized systemically by intraperitoneal ovalbumin injection and locally by ovalbumin inhalation. Control group were sensitized with PBS. Treatment group had intraperitoneal dexamethasone injection 1 hour before the initial sensitization while control and AR group were injected with PBS. Symptom scores, eosinophil counts, immunohistochemical staining as well as UGB mRNA expression in the nasal mucosa and lung tissue were analyzed. **Results.** The symptom scores and eosinophil counts between control and treatment group was significantly different from the AR group ($P < 0.01$). On immunohistochemical staining, UGB was localized in the epithelium and submucosal gland of the nasal mucosa as well as in the epithelium of respiratory bronchioles. UGB mRNA expression of the nasal mucosa and lung tissue was decreased in the AR group compared to the control group ($P = 0.022$). In the treatment group UGB expression was increased compared to the AR group ($P = 0.016$). The results of IHC and mRNA expression in the lung tissue correlated with the results in the nasal mucosa.

Keywords: Uteroglobin, CC10, allergic rhinitis, steroid

Introduction

Thirty years ago Joseph Daniel and Henning Beier discovered a small molecular weight protein in the uterus of pregnant rabbits that was thought to protect the blastocyst. The former named it blastokinin [1] while the latter named it uteroglobin (UGB) [2]. Fifteen years later, Singh found a protein secreted from the Clara cells of the bronchioles that could regulate inflammation, and called it Clara cell 10kDa protein (CC10) [3]. Further studies revealed that these were identical proteins and that it was also expressed in the kidney, digestive tract, prostate and blood [4–6], most of which are tissues that are in contact with the external environment. Furthermore, similar secreted proteins have been found in

rabbits, rat, mouse and humans suggesting that this protein is evolutionary conserved.

Though the same protein, UGB has been called by different names depending on their detection site and physiologic function. UGB is also known as blastokinin, CC10, clara cell secretory protein (CCSP), clara cell 16kDa protein (CC16), retinol binding protein, and polychlorinated biphenyl-binding protein. Recently, in a nomenclature committee a new generic name has been proposed to a number of similar secreted proteins in which UGB is the first, and is referred to as secretoglobin, family 1A, member 1 (SCGB1A1) [7].

Although discovered 30 years ago, the exact role of UGB has not been identified. Reports show that

UGB is a multifunctional protein. These include inhibition of phospholipase A2 and C [8–10], immune regulatory activity [11], anti-inflammatory activity [12,13], inhibition of tumor necrosis factor alpha [14,15] and inhibition of chemotaxis. Some have described UGB as the “natural immunosuppressor” [15].

The role of local mucosal immunity in the pathogenesis of allergic disease is important. There are reports that the level of UGB in the bronchoalveolar lavage (BAL) fluid is decreased in bronchial asthma [16], the representative allergic disease of the lower airway, suggesting that UGB may act as modulator of allergic inflammation in the lung. However, studies of UGB in the nose are lacking.

In this study we investigated the role of UGB in allergic rhinitis (AR). We first examined whether UGB is expressed in the murine nasal mucosa and then studied the effects of allergic inflammation and systemic steroids in the expression of UGB in a murine model of AR.

Materials and Methods

Experimental Animals

Five-week-old female specific pathogen free BALB-c mice each weighing 20–30 grams were used as experimental animals. Mice were divided into control, allergic rhinitis and treatment group with 10 mice each. The study reported herein followed the principles for laboratory animal research, as outlined in the Animal Welfare Act and Department of Health, Education and Welfare (National Institute of Health) guidelines for the experimental use of animals and experimental protocols were approved by our institution’s Animal Welfare Committee.

Sensitization, intervention, and allergen challenge

Allergen sensitization and challenge for the development of allergic rhinitis murine model is summarized in Figure 1. Briefly, on days 0, 7, 14, and 21, mice were immunized by intraperitoneal (i.p.) injection of 10 µg of ovalbumin (OVA) and 1 mg of aluminum hydroxide (alum) in 300 µL of phosphate-buffered saline (PBS). Ovalbumin (OVA, Grade V) and alum were purchased from Sigma Chemical Co. (St. Louis, MO).

One week after the last immunization (day 28), mice received a series of seven daily 1% OVA challenges via a nebulizer (PulmoAide, Somerset, PA). The aerosolized OVA (particle size 0.5 to 5.0 µm) was generated into a closed chamber of 8,800 cm³ (20 × 22 × 20 cm) made of acrylic box for 30 minutes. Control group received PBS inhalation instead of OVA. Treatment group received a

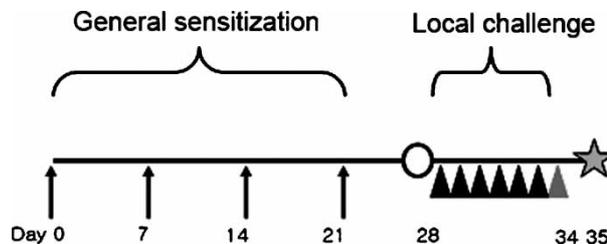


Figure 1. Allergic rhinitis and bronchial asthma model. BALB/c mice (ten per group) were sensitized with intraperitoneal injection of OVA and alum on four occasions spaced 1 week apart. One week after the last sensitization (day 28), mice received a series of six daily inhalation of OVA. The treatment group mice received dexamethasone one hour prior to initial OVA inhalation. Mice were killed for analyses 24 hours after OVA challenge. (arrow, OVA and alum i.p. injection; circle, dexamethasone or PBS i.p. injection, black triangle, OVA or PBS inhalation; grey triangle, OVA challenge; star, sacrifice).

single i.p. injection of 5 mg/kg of dexamethasone (DXM) purchased from Merck & Co. (West Point, PA) in PBS one day before the first OVA challenge. PBS was injected into the control and AR groups. Twenty-four hours after final OVA challenge (day 34), mice were sacrificed and nasal and lung tissues obtained for analysis.

Symptom score

On day 28, after the final OVA challenge, the frequency of sneezing and nasal rubbing events (four repetitive nasal rubbing movements equalled one event) were counted in each mouse for 15 minutes by two blinded observers, and the average count was recorded.

Nasal histology and eosinophil infiltration

After mice were sacrificed, the head was obtained and fixed in 10% formaldehyde solution. The head was decalcified with hydrochloric acid, embedded in paraffin, sectioned, and stained with haematoxylin and eosin (H & E). Two sections of the middle turbinate, 4 µm apart was made 5 mm posterior to the nasal vestibule. Eosinophils were counted in 5 different fields in the submucosal area on both sides of the nasal septum under light microscope (400 magnification; Laborlux K, Lekca, Wetzlar, Germany), using an eyepiece reticule. A single observer, not knowing the experimental conditions, counted all of the slides.

Immunohistochemical stain for UGB

Five mice per group were chosen randomly for immunohistochemical stain of the nasal and lung tissue. After deparaffinization, 3% H₂O₂ and 10% FBS were applied and rabbit polyclonal

anti-Uteroglobulin-antibody(CC10) (Santa Cruz Biotechnology, Santa Cruz, CA) was added after dilution at 1:200. The reaction took place for 10 hours at 4°C. After washing with PBS, biotinylated secondary anti rabbit-antibody (Dako, Ely, UK) and streptavidin HRP was added for 1hour and 20 minutes, respectively. Then it was reacted with 3, 3-diaminobenzidine tetrahydrochloride, and counterstained with hematoxylin.

Semi-quantitative RT-PCR of UGB

For examination of UGB mRNA expression, 5 mice were randomly selected and nasal mucosa from each of the 5 nose samples were obtained, immediately frozen at -70°C and used to synthesize cDNA. Trizol (Invitrogen, Carlsbad, CA) was used to extract the total RNA and AccuPower RT Premix tube (Bioneer, Seoul, Korea) was used for the reverse transcription reaction. A set of PCR primers was used to amplify UGB mRNA. The expected size of its PCR products was 216 bp. GAPDH was also amplified as an internal control for transcription and amplification and its expected size was 451 bp. The sequence of the sense and antisense primers for UGB and GAPDH were 5'TGA AGA GAC TGG TGG ATA CC3'/5'TTT ATT GCA AAG AGG AAG GA3', and 5'GTG GAT ATT GTT GCC ATC AAT GAC C3'/5'GCC CCA GCC TTC ATG GTG GT3' (Bioneer, Daejeon, Korea). The PCR cycle consisted of 94°C for 30 s, 58°C for 1 min and 72°C for 1 min 30 s. The amplified PCR products were analyzed on a 2% agarose gel containing 0.1 mg/ml ethidium bromide and scanned at 300 dots per inch. Semi-quantitative densitometric analysis was performed using NIH image analysis software (National Institutes of Health, Bethesda, MD). The expression of UGB over GAPDH (UGB/GAPDH) was used for semiquantitative analysis.

Statistical Analysis

Results in the different groups of mice were compared using the nonparametric Kruskal-Wallis test followed by post-hoc testing using Dunn's multiple comparison of means. Statistical analysis was performed using SPSS for Windows version 11.5 (SPSS Inc., Chicago, IL) and *P* values of <0.05 were considered statistically significant.

Results

Symptom score

Symptom score in the control, AR and treatment group was 8.2 ± 5.3 , 25.9 ± 4.5 , and 7.2 ± 5.1 respectively. The symptom score of the AR group was

significantly higher than those of the control and treatment group (*P* < .01). There was no difference between the control and treatment groups (Figure 2).

Eosinophil infiltration

The number of eosinophils in the control, AR and treatment group was 1.43 ± 1.12 , 9.50 ± 4.99 and 2.33 ± 1.51 respectively. The eosinophil infiltration in the AR group was significantly higher than those of the control and treatment group (*P* < .01). There was no difference between the control and treatment groups (Figure 3).

Immunohistochemical stain for UGB

Immunohistochemical staining of UGB in the mouse nasal mucosa showed that UGB was localized mainly in the cytoplasm of the nasal epithelium and in the acini of submucosal glands while the Goblet cells were spared. Reactions in the epithelium and submucosal glands were decreased in the AR group (Figure 4).

Immunohistochemical detection in the lung tissue revealed positive reactions that were mainly localized in the respiratory epithelium of the bronchioles. Reactions in the AR group showed a marked decrease (Figure 5).

Expression of UGB mRNA in the nasal mucosa

The expression of UGB was identified in all groups. To obtain semiquantitative results, an identical amount of cDNA was used for each PCR. UGB in

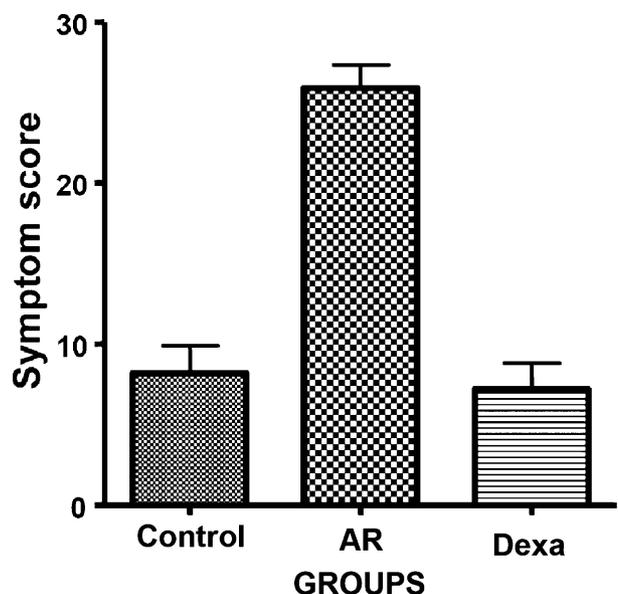


Figure 2. Symptom score during 15 minutes after OVA challenge. Symptom score in the allergic rhinitis group is significantly higher than those of the control and treatment group. **P* < .01.

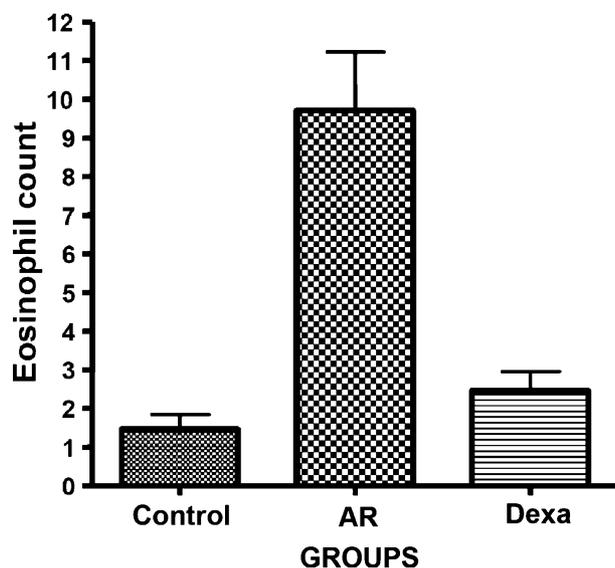


Figure 3. Number of eosinophils in the nasal mucosa of mice. The number of eosinophils in the allergic rhinitis group is significantly higher than those of the control and treatment group. * $P < .01$.

the AR group was significantly decreased ($P = .022$) compared to the control group. The expression was increased in the treatment group compared to the AR group ($P = .016$). There was no statistically significant difference between control and treatment groups (Figure 6).

Expression of UGB mRNA in the lung tissue

The expression of UGB in the AR group was significantly decreased ($P = .032$) compared to the control group. The expression was increased in the treatment group compared to the AR group ($P = .008$). There was no statistically significant difference between control and treatment groups (Figure 7).

Discussion

In this study we have demonstrated that UGB is expressed in the nasal mucosa of mice, and it is

localized in the nasal epithelium and submucosal glands. This will allow future studies of UGB and allergic rhinitis (AR) using the murine model. The expression of UGB is down-regulated in experimentally induced AR mice and systemic administration of dexamethasone prevented down-regulation of UGB. Finally the expression pattern of UGB in the lung matched the pattern of the nasal mucosa.

After reports of anti-inflammatory and immunomodulatory effects of UGB, much attention has been given on its role in airway allergy. Van Vyve first reported that UGB was decreased in BAL fluid of asthma patients [16], while Wang suggested that UGB might be involved directly or indirectly in the pathogenesis of bronchial asthma by showing elevated levels of neutrophil in BAL of UGB knock out (KO) mouse [17]. Furthermore, Hung showed that the increased allergic reaction in a UGB KO mice could be attenuated by administration of recombinant UGB, suggesting the possible therapeutic use of UGB in allergy.

However, studies regarding the role of UGB in allergic rhinitis are lacking. Lindahl first reported that the level of UGB was decreased in the nasal lavage fluid of four children with allergic rhinitis [18], and recently a decrease in the expression of UGB in a gene microarray study has been reported [19].

In the present study, we demonstrated for the first time the expression of UGB in the nasal mucosa of mice. We confirmed that UGB mRNA was expressed in all study group including the control group. However, the amount of expression was lower compared to the lung and therefore we used different amplification number between the nose and lung. Immunohistochemical staining using polyclonal UGB-antibody localized UGB in the epithelial cells and submucosal glands. It seems that UGB is secreted by ciliary epithelial cells and serous glands while the mucus producing Goblet cell and mucinous glands are spared. These findings are in accordance with the results of Benson who

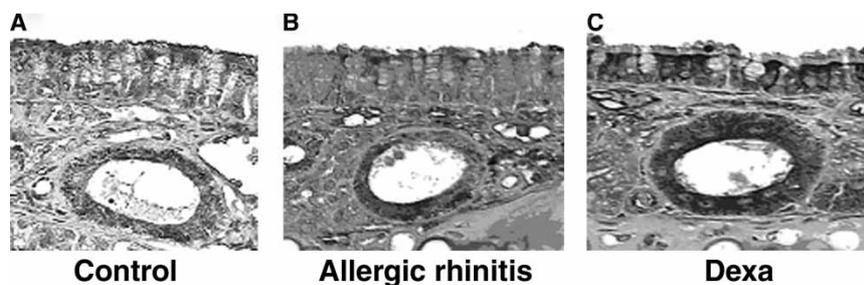


Figure 4. Immunohistochemical staining of uteroglobin(UGB) in the mouse nasal mucosa ($\times 400$). This study shows that UGB is localized mainly in the cytoplasm of the nasal epithelium and in the acini of submucosal glands (A). Reactions in the epithelium and submucosal glands are decreased in the allergic rhinitis group (B). Increased reactions are seen in the treatment group (C).

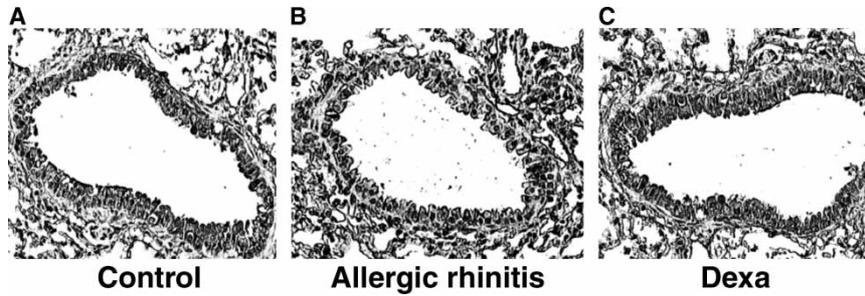


Figure 5. Immunohistochemical detection and localization of uteroglobin in the lung tissue ($\times 200$). Positive reactions are localized mainly in the respiratory epithelium of the bronchioles. Reactions in the positive control group shows marked decrease.

confirmed the expression of UGB in the human mucosa using immunofluorescent staining [20].

This is also the first study to analyze the change in expression of UGB in a murine model of allergic rhinitis. In mice sensitized and challenged with OVA the mRNA expression of UGB in the nasal mucosa was significantly down-regulated compared to the control mice. Regulation of UGB is not well known. Hung reported that UGB decreases the expression of inflammatory cytokines such as IL-4, IL-5, IL-13 and inhibits the proliferation of T cells while IFN- γ and IL-13 induced the expression of UGB, suggesting a link between these cytokines and UGB [21]. The results of the present and previous studies suggest that UGB is downregulated by the Th2 cytokines produced in the allergic inflammation. While Th2 cytokines were not measured in this

study, the increased eosinophil infiltration in the nasal mucosa can be an indirect evidence of local Th2 cytokines.

Steroids are commonly used to treat allergic rhinitis, and is known to inhibit T cells, as well as cytokines such as IL-2, IL-3, IL-4, IL-5, IL-13, IL-16, GM-CSF and TNF- α , thereby blocking the allergic response [22–24]. In the treatment group, where dexamethasone was administered systemically before local challenge, UGB expression was increased compared to the AR group. It was also increased compared to the control group, though not to a statistically significant level. There are conflicting reports on the effects of steroid on UGB. Some have reported no significant influence [25], while others have found that UGB is induced by steroids, suggesting that it could be another mechanism by

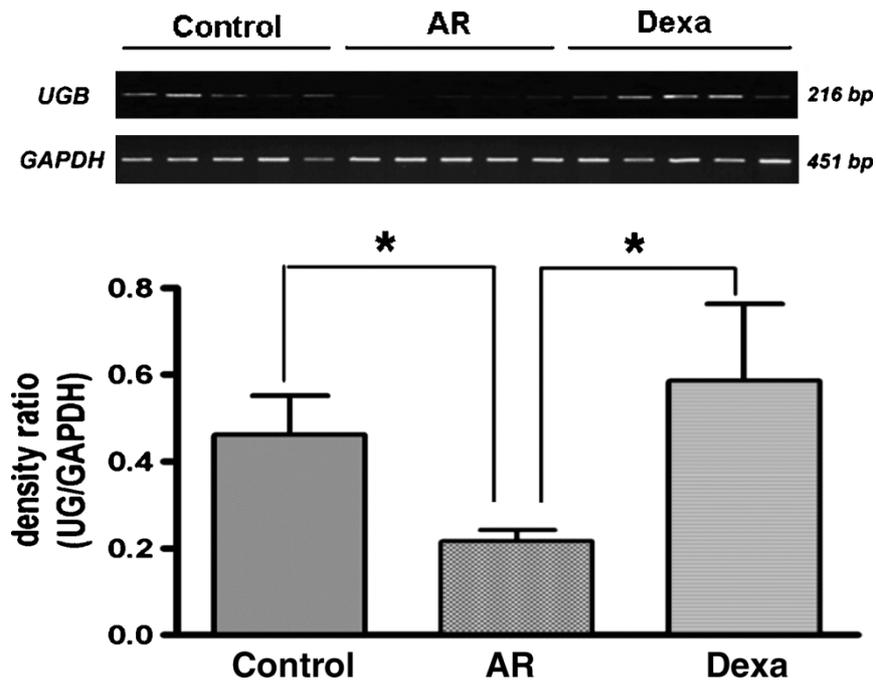


Figure 6. Expression of uteroglobin mRNA in the nasal mucosa of murine model of allergic rhinitis. Changes in UGB mRNA expression in control, allergic rhinitis and dexamethasone treated groups are shown. To obtain semiquantitative results for the assessment of UGB, an identical amount of cDNA is used for each PCR with primers amplifying the housekeeping gene GAPDH. The expression in the AR group is significantly decreased ($P = .022$) compared to the control group. The expression is increased in the treatment group compared to the AR group ($P = .016$). There is no statistically significant difference between control and treatment groups.

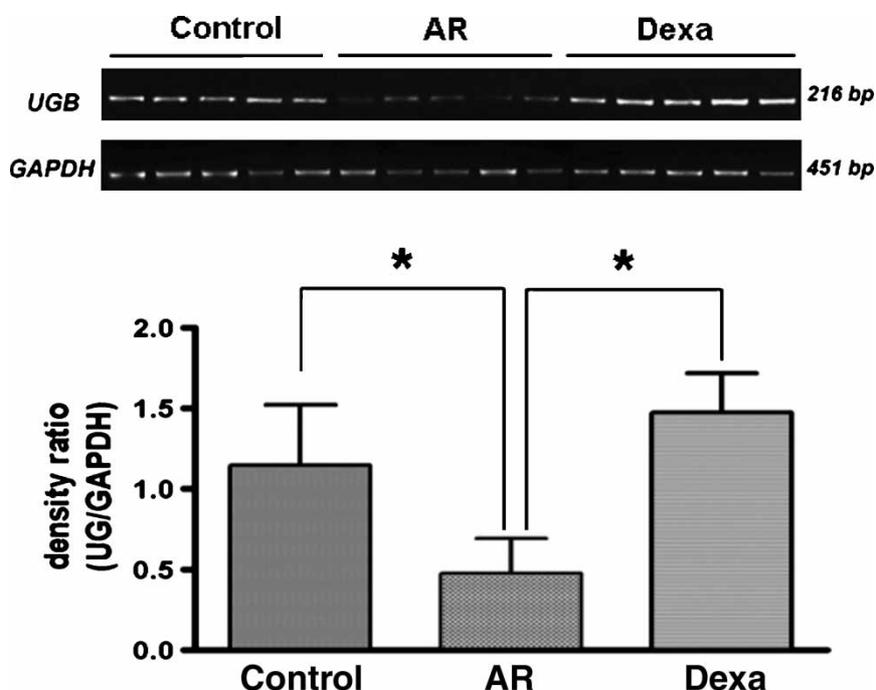


Figure 7. Expression of uteroglobin mRNA in the lung tissue. Changes in UGB mRNA expression in negative control, allergic rhinitis and dexamethasone treated groups are shown. The expression in the AR group is significantly decreased ($P=.032$) compared to the control group. The expression is increased in the treatment group compared to the AR group ($P=.008$). There is no statistically significant difference between control and treatment groups.

which steroids can inhibit airway inflammation [26,27]. In our study, the treatment group had significant reduction in symptom score and eosinophil infiltration. This can be interpreted as the inhibitory effect of dexamethasone on the inflammatory cytokines, or as an up-regulatory effect on UGB, or possibly both. The increased level of UGB compared to the control group, though not statistically significant, may support the latter possibility. Further studies to elucidate the relationship of steroids and UGB are warranted.

We also compared the expression of UGB in the lung and nose which has not been performed previously. Results show that they are in accordance. Allergic rhinitis and bronchial asthma are representative allergic diseases of the upper and lower airways, sharing common pathophysiologic features. Thus, once regarded as two separate disease entities they are thought to be a common disease with different clinical manifestations [28,29]. Our study adds evidence to the common airway theory. Furthermore, we expect UGB to play a role as a common regulator for both asthma and allergic rhinitis.

Conclusions

We observed for the first time the expression of UGB in the nasal mucosa of mice. The results of our study suggest that UGB may play an important role in the

regulation of inflammation in allergic rhinitis as well as in lower airway allergic inflammations. UGB can act as a common protective anti-inflammatory mediator in the allergic upper and lower airways.

There is no conflict of interest or financial support pertaining to this study.

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