

Staphylococcal enterotoxin B compromises the immune tolerant status in the airway mucosa

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Summary

Background The breakdown of immune tolerance plays a critical role in allergic disorders; the mechanism of breaching immune tolerance remains largely unknown.

Objective The present study aimed to investigate the role of Staphylococcal enterotoxin B (SEB) in the interference of the immune tolerance in the nasal mucosa.

Methods The immune tolerant components, tolerogenic dendritic cells (ToIDC) and regulatory T cells (Treg), were assessed in the surgically removed nasal mucosa from patients with allergic rhinitis (AR) or non-AR chronic rhinitis. The contents of SEB and integrin α 5 β 1 (avb6) in the nasal epithelium were assessed using enzyme-linked immunoassay. The ability of avb6 on ToIDC induction and the effect of SEB on suppression of avb6 in nasal epithelial cells were observed in cell culture.

Results Compared with that in the non-AR nasal mucosa, the frequencies of ToIDCs/Tregs were lower, the contents of SEB were higher and the contents of avb6 were lower in the AR nasal mucosa. Avb6 had the ability to induce the development of ToIDCs *in vitro*; the latter had the ability to induce Treg development. The expression of avb6 was detected in nasal epithelial cells in culture that could be suppressed by SEB.

Conclusions and clinical relevance The components of immune tolerance machinery, ToIDCs and Tregs were suppressed in the AR nasal mucosa. The increases in SEB and decreases in avb6 in nasal epithelium are associated with the compromises of immune tolerance in the nasal mucosa. SEB has the ability to suppress the expression of avb6 in nasal epithelial cells.

Keywords allergic rhinitis, immune tolerance, integrin, nasal mucosa, Staphylococcal enterotoxin B

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Introduction

The prevalence of airway allergy is increased tremendously in recent decades [1]; its aetiology and pathogenesis still remain largely unknown although research in this area has been advanced rapidly in recent years. The nasal cavity is opened to the external environment directly; airborne antigens and microbes can easily access the epithelial layer of the mucosa. The epithelial cells and dendritic cells (DC) in the subepithelial region can respond to the stimuli of antigen or/and microbial

products resulting in physiological responses or pathological changes in the mucosa.

Staphylococcal enterotoxin B (SEB) is an enterotoxin produced by the bacterium *Staphylococcus aureus*. SEB may contaminate ingested food and induce gastrointestinal dysfunction [2, 3]. It is reported that SEB interferes with the function of the immune system in the airway mucosa, such as to be involved in the pathogenesis of airway allergy [4, 5]. The mechanism by which SEB facilitates immune dysfunction in the airways remains incompletely understood.

Immune tolerance indicates a condition that the immune system does not respond to antigenic or microbial stimuli that the immune system is supposed to respond [6]. The mechanism of immune tolerance

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includes clonal deletion, T cell energy and active suppression, among which the immune suppression is regarded as the most important part in immune tolerance [7]. The major components in immune suppression machinery are regulatory T cells (Treg) including type 1 Treg (Tr1), Th3 cells and CD4⁺ CD25⁺ Foxp3⁺ Tregs [7]. Tregs are distributed in every compartment in the body to fulfil the immune suppressive function over other effector T cells to maintain the immune homeostasis. Therefore, the condition of immune polarization in allergic disease like airway allergy with the Th2 polarization as the major immune pathological feature, implicates that the immune tolerance is broken in local tissues; in other words, it implicates the immune suppressor cells, such as Tregs dysfunction [8]. The mechanism that causes a low frequency of Tregs in the airway mucosa is not clear.

Integrin alphavbeta6 (avb6) is produced by epithelial cells in response to external stimuli, such as wound and inflammation [9]. Our recent study data also show that intestinal epithelial cells express detectable avb6 that has proteolytic activity and can convert the precursor of transforming growth factor (TGF) β into the active form of TGF β [9]. TGF β plays a critical role in the Treg development [10]. Tolerogenic DCs (ToIDC) express TGF β and aldehyde dehydrogenase (ALDH) that can induce CD4⁺ CD25⁺ T cells to Foxp3⁺ Tregs [11, 12]. There is an epithelial layer covering the surface of the nasal mucosa. The epithelial cell-derived avb6 may be involved in the Treg development in the nasal mucosa.

As SEB can facilitate the development of allergic responses in animal models [13], SEB plays a role in sustaining the allergic status in the body [14]. Tregs are the main component of immune tolerance machinery, and we hypothesize that SEB may interfere with Treg development in the airway mucosa that results in Th2 polarization in the airway mucosa. In this study, we collected a batch of nasal mucosal specimens from patients with allergic rhinitis (AR). We found that the frequencies of ToIDC and Treg were markedly less in the AR nasal mucosa than that in the non-AR nasal mucosa. High levels of SEB were detected in nasal mucosa specimens of patients with AR. SEB could inhibit CD4⁺ CD25⁺ T cells to develop to Tregs.

Materials and methods

Reagents

Western blotting reagents were purchased from Invitrogen (Shanghai, China). Recombinant avb6, enzyme-linked immunosorbent assay (ELISA) kit of immunoglobulin (Ig)E, was purchased from GenWay Biotech (San Diego, CA, USA). Recombinant IL-2, ELISA kits of IL-4 (sensitivity = 10 pg/mL) and IFN γ (sensitiv-

ity = 8 pg/mL) were purchased from R&D Systems (Shanghai, China). SEB ELISA kit was purchased from Tetracore, Inc. (Rockville, MD, USA; sensitivity = 5 pg/mL). The ALDEFLUOR[®] kit was purchased from Stem-Cell Tech (Vancouver, BC, Canada). Integrin beta6 (C-19; isotype: goat IgG), TGF- β (3C-11, isotype: mouse IgG1), CD103 (H-260; isotype: rabbit IgG), MHC II (12-F4; isotype: mouse IgG1), Foxp3 (F-9, isotype: mouse IgG1) were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). CD4⁺ T cell and DC isolating reagent kits were purchased from Miltenyi Biotec (Auburn, CA, USA). Endotoxin levels in all reagents were detected using the Limulus assay (Limulus amoebocyte lysate QCL 1000; Bio Whittaker, Walkersville, MD, USA). The reagents used in this study contained <0.2 U endotoxin/10 μ g reagents.

Human subjects and nasal mucosal specimen collection

Thirty-five patients with AR and nasal mucosal hypertrophy, 30 patients with non-AR chronic rhinitis and 10 nasal healthy subjects were recruited into this study at the Department of Otolaryngology, Shanxi Medical University. The diagnosis of AR was based on the department routine including perennial AR case history, positive skin-prick test (SPT), with appropriate symptoms of corresponding perennial allergies. (SPT was considered positive when the resulting wheal was greater than or equal to 3 mm compared with negative controls after 15 min), and serum specific IgE levels were >10 ng/mL. All the patients were required not to use any anti-allergy drugs at least 1 month before joining this study. All AR and non-AR patients were treated with the inferior turbinectomy; some of them were also treated by sinus endoscopic surgery for their chronic sinusitis (Table 1). Specimens were taken from the surgically removed inferior turbinate tissue immediately after the surgery for further experiments. The procedures using human tissue in the study were approved by the Human Research Ethic Committee at Shanxi Medical University. Informed consent was obtained from each subject.

Nasal epithelial sample collection

With a plastic spoon (5 mm in diameter), nasal epithelial samples were scratched on the surface of the inferior turbinate from the surgically removed nasal turbinate tissue and from nasal healthy volunteers.

Immune cell isolation

Surgical removed nasal tissues were cut into small pieces (2 $\times$ 2 $\times$ 2 mm) and treated with predigestion solution (1 $\times$ Hank's balanced salt solution containing

Table 1. Demographic data of the patients

Allergic rhinitis (AR) patients			
Male	19	Specific antigen	Der p 1 and others
Female	16	Specific IgE	40.5 ± 18.2 ng/mL
Age	35.2 ± 5.6	Specific IgG1	33.4 ± 18.6 ng/mL
Case history	6.4 ± 3.4	Nasal MBP	46.8 ± 14.3 pg/mL
Surgical history	None	Nasal tryptase	107.5 ± 35.4 pg/mL
None AR patients			
Male	14	Specific antigen	None
Female	16	Specific IgE	Not detectable
Age	33.2 ± 6.3	Specific IgG1	Not detectable
Case history	5.8 ± 2.7	Nasal MBP	Not detectable
Surgical history	None	Nasal tryptase	Not detectable

Specific antigen: All AR patients were sensitized to Der p 1 as well as several other allergens. Nasal MBP: the levels of major basic protein of extracted proteins from nasal mucosa [determined using enzyme-linked immunosorbent assay (ELISA)]. Nasal tryptase: the levels of tryptase in extracted proteins from nasal mucosa (determined by ELISA).

5 mM EDTA and 1 mM dithiothreitol) at 37 °C for 30 min under slow rotation. The tissue was collected by centrifugation (800 g, 10 min) and incubated in digestion solution (dissolve 0.05 g of collagenase D, 0.05 g of DNase I and 0.3 g of dispase II in 100 mL of 1× PBS) at 37 °C for 60 min under slow rotation. Cells were filtered with a cell strainer. The mononuclear cells were isolated using gradient density centrifugation. Isolation of CD4⁺ T cells from the mononuclear cells was performed with commercial magnetic cell sorting kits. The purity of the isolated CD4⁺ T cells was over 95% as checked using flow cytometry. About 10⁷–5 × 10⁸ cells could be harvested from each gram of nasal tissue.

Generation of dendritic cells from peripheral blood mononuclear cells (PBMC)

Immature DCs were generated from PBMC. Briefly, PBMCs were prepared from healthy volunteers using gravity density centrifugation. CD11c⁺ cells were purified from the isolated PBMCs and then transferred in 12-well plates at a density of 0.5 × 10⁶ cells/mL and placed in a humidified incubator at 37 °C. The cells were grown in RPMI1640 media supplemented with 10% foetal cow serum, penicillin (100 U/mL) and streptomycin (100 µg/mL), IL-4 (10 ng/mL) and GM-CSF (20 ng/mL). The cytokine-supplemented medium was replaced weekly, and the non-adherent cells were collected and split to fresh medium. CD11c was used as an indicator of DCs. As shown by flow cytometry, over 95% cells were CD11c positive on day 6 with mild expression of CD80 and CD86 (compared with LPS-stimulated DCs; data not shown); these cells were used for further experiments.

Western blotting

Protein extracts (40 µg/well) were separated using a precast NuPAGE gel system (Invitrogen, Burlington, ON, Canada) and blotted onto nitrocellulose membrane. The membrane was incubated with primary antibody (0.05–0.1 µg/mL) in 5% nonfat milk in TBS at 4 °C overnight. This was followed by incubation with horseradish peroxidase-conjugated second antibody for 1 h at room temperature with shaking. Reactions were developed using the Pierce ECL chemiluminescence substrate kit (Thermo Fisher Scientific China, Beijing, China). Results were recorded with X-ray film.

RT-PCR

The total RNA was extracted from nasal epithelial cells using an RNeasy Mini kit (Qiagen, Shanghai, China). One microgram RNA was reverse transcribed using the iScriptTM cDNA Synthesis kit (Bio-Rad, Shanghai, China). The resulted complementary DNA was then subjected to PCR using HotStar Taq@Master Mix kit (Bio-Rad). The primers of β6 used in this study were: forward: tgcgaccatcagtgaagaag; reverse: gacaaccccgatgagaagaa (NCBI: NM_000888; start code: 1975; end code: 2191; size: 217 bp). The annealing temperature was 60 °C. Amplifying cycles: 30 cycles.

Flow cytometry

Cells were collected and incubated with primary antibodies on ice for 30 min (For the intracellular staining, cells were fixed with 1% paraformaldehyde on ice for 30 min and incubated with permeabilization reagents for 30 min). The stained cells were analysed using a BD FACS Canto flow cytometer (BD Bioscience, San Jose, CA, USA). Data were analysed using software FlowJo (Tree Star, Inc., Ashland, OR, USA). The gating technique was applied when necessary.

Enzyme-linked immunoassay (ELISA)

The levels of IL-4, IFNγ and IgE in the protein extracts from surgically removed nasal mucosa were measured using ELISA with commercial reagent kits following the manufacturer's instructions. For the measurement of Der p 1-specific IgE, the plates were coated by Der p 1 (10 µg/mL) at 4 °C overnight, then followed the manufacturer's instruction. The results were normalized to per mg protein.

Statistical analysis

Data are summarized as means ± SD. The values were analysed using the two-tailed unpaired Student's *t*-test

when the data consisted of two groups or using ANOVA when three or more groups were compared. $P < 0.05$ was accepted as statistically significant.

Results

The components of immune tolerance in the nasal mucosa are reduced in allergic rhinitis patients

Tolerogenic DC (ToIDC) and Tregs are the major components in the immune tolerant machinery that play a critical role in the maintenance of the homeostasis in the body. In this study, we observed the numbers of ToIDC and Treg in the nasal mucosa using as representative parameters of immune tolerance in the nasal mucosa. Surgically removed nasal mucosal tissue was collected from 35 patients with AR and 30 patients with non-AR chronic rhinitis. Mononuclear cells were isolated from the tissue and analysed using flow cytometry. The data showed that the frequencies of Foxp3⁺ Treg and CD11c⁺ TGFβ⁺ ToIDCs were significantly less in the AR nasal mucosal samples than that in non-AR control samples (Fig. 1). The data implicate that the immune tolerant status was compromised in the AR nasal mucosa.

We next checked the Th1/Th2 status in the nasal mucosa. In the collected nasal mucosal specimens, several immune pathological parameters were observed. The results showed that the levels of IL-4 and antigen-specific IgE were significantly higher in AR specimens than non-AR control specimens. The levels of IFNγ were slightly higher in the AR group than the control group, but the difference did not reach the significant criteria. The frequency of mast cells was assessed on

paraffin sections; the cell number of mast cells was much higher in the AR group than that in the control group. The results have defined the immune pathological type is Th2 polarization in the AR nasal mucosa. The correlation assay was performed with the data of Foxp3⁺ Treg frequency and IL-4 levels in the nasal mucosa. The results showed negative correlation ($r = -0.776$, $P < 0.01$) between Foxp3⁺ Treg number and IL-4 levels in the nasal mucosa.

Low levels of avb6 in the allergic rhinitis nasal mucosa

In another study, our data show that intestinal epithelial cell-derived avb6 plays a critical role in the development of Foxp3⁺ Tregs [15]. We thus wondered if the low frequency of Foxp3⁺ Tregs in the AR nasal mucosa had any relation to the expression of avb6 in the nasal mucosa. We therefore analysed the expression of avb6 in scratched nasal epithelial samples. As shown by Western blotting, avb6 were detectable in the nasal epithelium of healthy subjects, AR patients and non-AR patients. The levels of avb6 were much less in the AR nasal samples than that in normal subjects or non-AR nasal samples (Fig. 3).

Staphylococcal enterotoxin B levels in the allergic rhinitis nasal mucosa are correlated with local immune tolerant status

Recent publications propose that SEB has a close relation with allergic disorders. Thus, we measured the levels of SEB in collected nasal epithelial samples using ELISA. The results showed that significantly higher

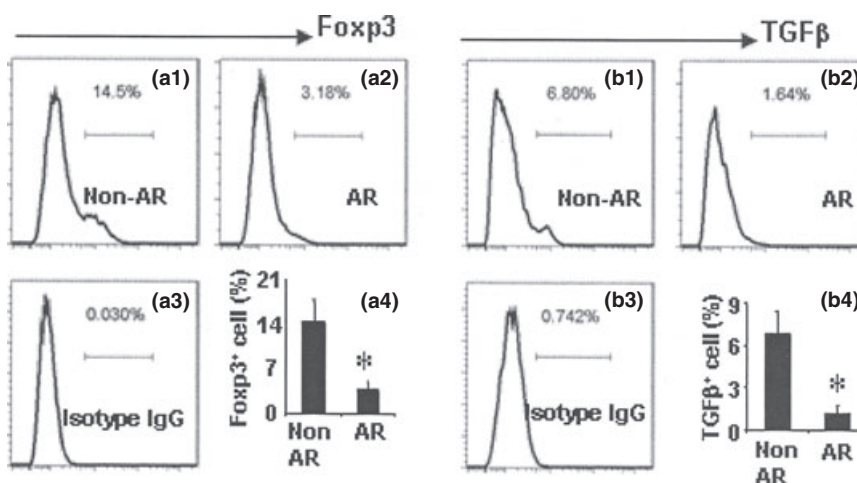


Fig. 1. Low frequency of tolerogenic dendritic cells (ToIDCs) and Foxp3⁺ Tregs in the allergic rhinitis (AR) nasal mucosa. Surgically removed nasal mucosal tissue was collected from 30 patients with chronic rhinitis (non-AR; a) and 35 AR patients with nasal mucosa hypertrophy (AR; b). CD4⁺ T cells and CD11c⁺ cells were isolated from the tissue, respectively, by magnetic cell sorting and analysed by flow cytometry. The histograms indicate the frequency of Foxp3⁺ Tregs in isolated CD4⁺ T cells (a) and the frequency of TGFβ⁺ ToIDCs in isolated CD11c⁺ cells. A3 and B3, cells stained with isotype IgG using as a control. A4 and B4, bars show summarized data of A1–2 and B1–2 (mean ± SD). * $P < 0.01$, compared with the non-AR group.

levels of SEB were detected in the AR nasal epithelium than non-AR controls (Fig. 4). Correlation assay results showed a negative correlation ($r = -0.448$, $P < 0.05$) between SEB levels and Foxp3⁺ Treg number in the AR nasal mucosa.

Avb6 promotes tolerogenic dendritic cells development

The data in Figs 1–4 imply that avb6 may play a role in the development of TolDCs and Tregs. We next observed the effect of avb6 on inducing development of TolDC. We isolated immature DCs (CD11c⁺, low levels of CD80 and CD86) from PBMC. The isolated DCs were cultured in the presence of avb6 for 7 days. Using CD11c and ALDH as indicators of TolDC (Fig. 5), the results showed that avb6 could induce TolDC development in an avb6 dose-dependent manner.

We next isolated CD4⁺ CD25[−] T cells (purity was over 99%) using magnetic cell sorting from the PBMC; the CD4⁺ CD25[−] T cells were cultured with immature DCs pulsed with or without avb6 for 6 days. As assessed by flow cytometry, abundant Foxp3⁺ Tregs were generated (Fig. 6).

Staphylococcal enterotoxin B suppresses the expression of avb6 in nasal epithelial cells

To further understand the role of SEB in the compromise of immune tolerance in the nasal mucosa, we cultured nasal epithelial cell line, RPMI2650 cells (ATCC, USA). As shown by RT-PCR and Western blotting, the expression of avb6 in nasal epithelial cells was detect-

able in nasal epithelial cells; the expression of avb6 could be suppressed by the presence of SEB in the culture; the suppressive effect on avb6 expression could be prevented by addition of anti-SEB (Fig. 7).

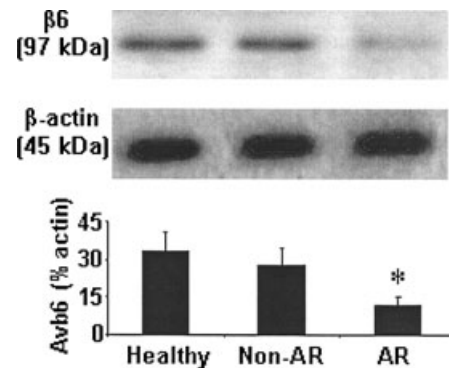


Fig. 3. Avb6 expression is suppressed in the allergic rhinitis (AR) nasal epithelium. Nasal epithelium samples were collected from 10 nasal healthy people, 10 patients with non-AR chronic rhinitis and 10 AR patients. The immune blots show contents of avb6 in the samples that were evaluated by Western blotting. Bars show the summarized data of densitometrical analysis of the blots [mean (SD, from 10 experiments)]. * $P < 0.01$, compared with healthy controls. (The 10 AR patients and 10 non-AR patients were randomly selected from the 35 AR patients and 30 non-AR patients as described in the text).

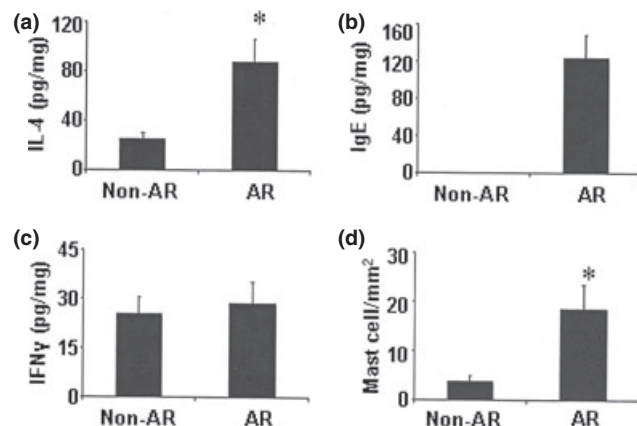


Fig. 2. Th2 polarization in the allergic rhinitis (AR) nasal mucosa. (a–c) Total proteins were extracted from the nasal tissue as described in Fig. 1 and analysed using enzyme-linked immunosorbent assay. Bars indicate the levels of IL-4 (a), OVA-specific IgE (b) and IFNγ (c). (d) A piece of nasal mucosa was taken from each sample and processed for paraffin sections. The sections were stained with 0.5% toluidine blue. Mast cells were counted under a light microscope. Bars indicate mast cell counts in the nasal mucosa. Data were presented as mean $\pm$ SD; * $P < 0.01$, compared with the non-AR group.

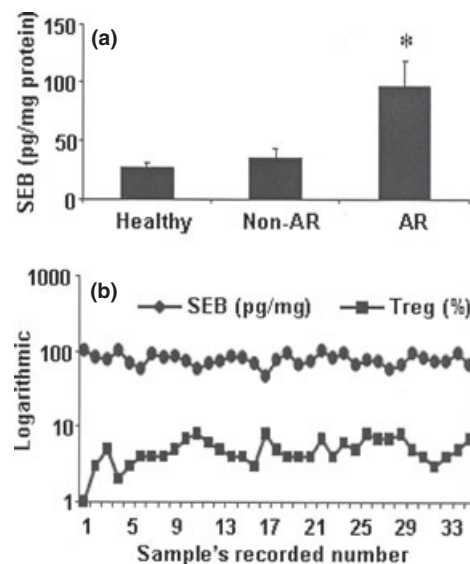


Fig. 4. Staphylococcal enterotoxin B (SEB) levels are increased in the allergic rhinitis nasal epithelium. Nasal epithelial samples were collected as described in Fig. 3. Proteins were extracted from the samples. (a) Bars indicate the SEB levels in protein extracts that were assessed using enzyme-linked immunosorbent assay. Data were presented as mean (SD, from 10 experiments). * $P < 0.01$, compared with the healthy controls. (b) Logarithmic curves show individual counter-part data point of SEB and Treg. Each dot represents a datum from one patient.

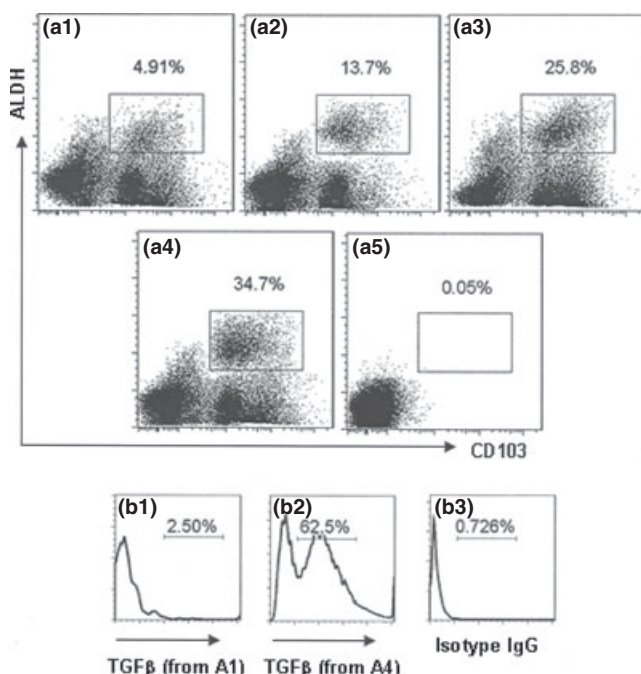


Fig. 5. Avb6 induces tolerogenic dendritic cells (TolDC) development. CD11c⁺ cells were isolated from peripheral blood mononuclear cells and cultured in RPMI1640 medium in the presence of avb6 at doses of 0 µg/mL (A1), 0.1 µg/mL (A2) and 0.5 µg/mL (A3) and 1 µg/mL (A4). Cells were collected on day 7, stained with antibodies of CD103, TGFβ, MHC II, and ALDEFLUOR[®] kit. Cells were analysed by flow cytometry. (A) Dot plots indicate CD103⁺ ALDH⁺ cells (the gated cells). (B) Histograms indicate the frequency of TGFβ⁺ (B1, B3) and MHC II⁺ (B2, B4) cells in the gated cells in panel A. Panel A5 and B5 show isotype IgG staining control. The data represent three experiments.

Discussion

It has been noted for a long time that the compromise of immune tolerance plays a critical role in allergic diseases [6]; SEB is also proposed being associated with the pathogenesis of allergic disorders [2, 13, 14]. The present data link the contamination with microbial product SEB to compromise immune tolerance in the airway mucosa.

The theory of immune tolerance proposes that the immune responses in the body are tightly regulated by a preserved system, the immune tolerance machinery [6]. Among the three components of immune tolerance, clonal deletion, T cell energy and active suppression, the active suppression catches most attention from immunologists or related scientists, as its dysfunction is associated with the pathogenesis of a large number of diseases [7]. We may also propose that, if a disease occurs, the immune tolerance machinery is dysfunction, at least in the local tissue. The present data provide evidence to support this proposal. In a group of AR patients, they have typical AR clinical symptoms based on what their physicians established the diagnosis of

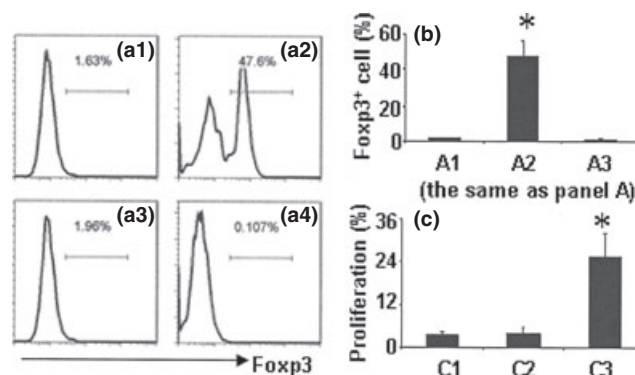


Fig. 6. Avb6-pulsed dendritic cells (DCs) induce Treg development. Tolerogenic DCs (TolDCs) were prepared as described in Fig. 5. CD4⁺ CD25⁻ T cells were isolated from peripheral blood mononuclear cells and cultured with TolDCs (T cell : DC = 10⁵ : 10⁴ cells/well) in the presence of IL-2 (20 ng/mL) for 6 days. Cells were collected and stained with an antibody of Foxp3 and analysed by flow cytometry. (a) Histograms show the frequency of Tregs. A1, naïve T cells. A2, naïve T cells/TolDCs. A3, CD4⁺ CD25⁻ T cells /CD11c⁺ DCs. A4 is an isotype control. (B) Bars show the summarized data in panel A. (C) Bars indicate the proliferation rate (the data were from flow cytometry) of Th2 cells cultured alone (C1), cultured in the presence of a specific antigen (Ag) and DC with (C2) or without (C3) the presence of generated Tregs (Th2 : DC : Treg = 10⁵ : 10⁴ : 10⁵ cells/well). The data were presented as mean ± SD from three experiments. *P < 0.01, compared with A1 (B) or C1 (C).

AR to be. The data demonstrate that the frequency of DC and Tregs, two of the major components in immune tolerance machinery, is much less than non-AR control group. As the major function of Treg in immune regulation is to suppress the 'over-function' of other effector T cells [7], the low frequency of Tregs in the AR nasal mucosa implicates impotent immune suppressor ability in local tissues, that, maybe in major part, contributes to the Th2 polarization in the nasal mucosa as we observed in the present study.

Tolerogenic DCs are another important cell population in immune tolerance [16]. The major role of TolDCs is to induce Treg development [12]. The present data indicate that the number of TolDCs is much less in the AR nasal mucosa than that in non-AR control subjects. The fact can be at least partial explanation of the low frequency of Tregs in the AR nasal mucosa. However, the causal factors involving in TolDC reduction remain obscure. Cumulative reports indicate that the microbialproduct, SEB, is associated with the pathogenesis of allergic diseases in the airways and intestine [2, 3, 5]. The present data indicate that the contents of SEB are much higher in AR nasal epithelium than that in non-AR controls and healthy control; the finding implies that SEB may interfere with the development of TolDCs in the nasal mucosa; the inference is supported by the correlation assay that the levels of SEB are negatively correlated with the number of Tregs in the nasal mucosa.

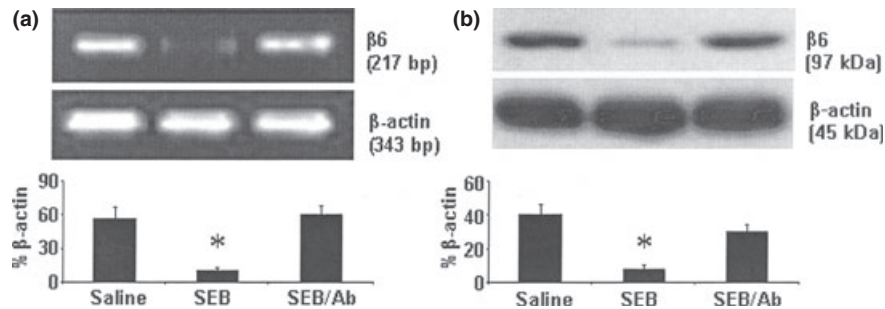


Fig. 7. Staphylococcal enterotoxin B (SEB) suppresses the expression in nasal epithelial cells. Nasal epithelial cell line, RPMI2650 cells, was cultured in the presence of saline or SEB (1 µg/mL) or anti-SEB (Ab, 1 µg/mL) and SEB (1 µg/mL) for 24 h. The gels show the expression of avb6 that was detected by RT-PCR (a) and Western blotting (b). The β-actin was used as an internal control. Bars indicate the densitometry data of the bands of mRNA (a) and protein (b). The data represent three experiments.

Our recent study indicates that epithelial cell-derived avb6 plays an important role in the induction of TolDCs and Tregs in the intestine under physiological conditions (data not shown). The present study extended the finding that by adding exogenous avb6 to cell culture, TolDCs and Tregs were generated from DCs and CD4⁺ CD25⁻ T cells. The underlying mechanism may be that avb6 converts the TGFβ precursor to the active form of TGFβ as indicated by previous studies. Indeed, the present data also show that after exposed to avb6 in culture, not only was ALDH increased but also TGFβ increased in DCs. The finding has confirmed the ability of avb6 in the generation of TolDCs; the finding also implicates that some other factors that suppress the expression of avb6 may interfere with the development of TolDCs. Similar to what we found in the intestine (data not shown), we detected the expression of avb6 in nasal epithelium in the present study. Importantly, the contents of avb6 were much less in the AR nasal samples than that in non-AR samples. The finding implicates that the decrease in avb6 in the epithelium may

be responsible for the reduction of TolDCs and Tregs in the AR nasal mucosa. Indeed, while we exposed nasal epithelial cells to SEB in cell culture, the expression of avb6 was substantially suppressed.

In summary, the present data show that the frequency of TolDCs and Tregs was much less in the AR nasal mucosa than that in the non-AR nasal mucosa. High levels of SEB and low levels of avb6 were detected in the AR nasal mucosa. Avb6 has the ability to promote the development of TolDCs and Tregs. SEB can suppress the expression of avb6 in nasal epithelial cells.

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