

Original article

Human airway and peripheral blood eosinophils enhance Th1 and Th2 cytokine secretion

Background: The effector function of eosinophils involves their release of toxic granule proteins, reactive oxygen species, cytokines, and lipid mediators. Murine studies have demonstrated that eosinophils can also enhance T cell function. Whether human eosinophils, in particular, airway eosinophils, have similar immunoregulatory activity has not been fully investigated. The aim of this study was to determine whether human blood and airway eosinophils can contribute to Th1 and Th2 cytokine generation from CD4⁺ T cells stimulated with superantigen.

Methods: Eosinophils were obtained from blood or bronchoalveolar lavage fluid 48 h after segmental allergen bronchoprovocation. Purified eosinophils were co-cultured with autologous CD4⁺ blood T cells in the presence of staphylococcal enterotoxin B (SEB). Cytokine levels in the supernatant fluid were determined by enzyme-linked immunosorbent assay (ELISA). Eosinophil expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules was assessed by flow cytometry before culture, 24 h after granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulation, and 24 h after co-culture with CD4⁺ T cells and SEB.

Results: Interleukin (IL)-5, IL-13, and interferon (IFN)- γ generation increased when CD4⁺ T cells were co-cultured with either blood or airway eosinophils in the presence of SEB. The ability of eosinophils to enhance cytokine generation was independent of their source (blood *vs* airway), activation by GM-CSF, or detectable expression of human leukocyte antigen (HLA)-DR, CD80, or CD86.

Conclusion: Our data demonstrate that SEB-induced generation of Th1 and Th2 cytokines is increased in the presence of human blood and airway eosinophils. Thus, eosinophils can have an immunoregulatory function in pathogen-associated allergic diseases such as atopic dermatitis, chronic sinusitis, and asthma exacerbations.

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Eosinophils are a prominent component of allergic inflammation associated with atopic asthma, rhinitis, and dermatitis. Eosinophil release of toxic granule proteins, reactive oxygen species, cytokines, and lipid mediators is proposed to contribute to tissue injury, vascular leakage, mucus secretion, and airway smooth muscle contraction (1). In addition to these effector functions, recent studies in mice suggest eosinophils possess immunoregulatory activity.

Eosinophil interactions with T cells may occur by multiple mechanisms. In mice, eosinophils can function as antigen-presenting cells (APCs; 2–5). For example, proliferation and release of interleukin (IL)-2 by *Mesocestoides corti* antigen-specific T cell clones was observed following presentation of *M. corti* by eosinophils (5). Pretreatment of eosinophils with granulocyte-macrophage colony-stimulating factor (GM-CSF) was required to upregulate major histocompatibility complex

(MHC) class II and co-stimulatory molecules. Eosinophils can also influence T cell generation of cytokines through the release of soluble mediators such as IL-4 (6) and IL-18 (7). In IL-5/eotaxin double knockout mice introduction of either eosinophils or IL-18 can restore secretion of Th2 cytokines in response to antigen challenge (7). In this model, it was suggested that eosinophils might regulate T cell generation of IL-13 via production of IL-18.

The activation of T cells occurs as a two-step process with T cell receptor (TCR) recognition of a specific epitope presented on the surface of the APC in association with an appropriate MHC molecule. A second signal is provided via the interaction between CD28 on T cells and a co-stimulatory molecule (generally a member of the B7 family, such as CD80 or CD86) expressed on the APC (8). In contrast to classical antigens, superantigens, such as staphylococcal enterotoxin B (SEB), can stabilize TCR-MHC class II interactions without antigen

specificity. This is accomplished with SEB binding to specific TCR-V β chains and the MHC class II α chain resulting in activation of approximately 30% of CD4+ T cells (9). Thus, in experimental models, SEB is a useful antigen surrogate for CD4+ T cell activation.

Although several studies have demonstrated that circulating human eosinophils can enhance T cell proliferation (10–12), their ability to regulate T cell cytokine secretion has not been reported. Our objectives were to: (i) determine if airway eosinophils differ from their circulating counterparts in their ability to regulate T cell cytokine secretion, and (ii) establish the requirements for this eosinophil immunoregulatory function, including the need for eosinophil activation and expression of MHC class II and co-stimulatory molecules.

Materials and methods

Subjects

Airway eosinophils were obtained from seven subjects with mild allergic asthma [median with range for forced expiratory volume (FEV)₁% of predicted = 95.7 (84.2–116.3) and for methacholine PC₂₀ = 1.9 (1.2–5.4) mg/ml]. Eleven additional subjects including five with allergic asthma, four with allergic rhinitis, and two normal subjects also participated in the study by donating peripheral blood only. All atopic subjects had a positive skin prick test to one or more common aeroallergens. All asthma subjects had a physician's diagnosis of asthma. Allowed asthma/allergy medications included inhaled β 2-agonists, antihistamines, and nasal steroids. No subjects had been treated with oral or inhaled corticosteroids, long-acting β -agonists or leukotriene antagonist within 6 weeks of participation. Normal, nonallergic subjects were skin test-negative, had no history of allergies or asthma and were taking no medications that would influence the outcome of this study. Informed consent was obtained from each subject prior to participation. The study was approved by the University of Wisconsin-Madison Center for Health Sciences Human Subjects Committee.

Segmental bronchoprovocation with allergen and bronchoalveolar lavage

Segmental bronchoprovocation (SBP) was performed as previously described using cat, ragweed or dust mite antigen (13). Bronchoscopy and bronchoalveolar lavage (BAL) were performed 48 h later (13) and BAL fluid was pooled for purification of airway eosinophils. Heparinized blood (120 ml) was obtained immediately before the BAL.

Eosinophil purification

Bronchoalveolar lavage cells were passaged over a two-step Percoll gradient (Amersham, Piscataway, NJ, USA) and eosinophils were collected from the 1.085/1.100 g/ml interface (13). Blood was centrifuged over Percoll (1.090 g/ml) to obtain mononuclear cells. Eosinophils were purified from the granulocyte fraction by negative depletion using anti-CD16 immunomagnetic beads (MACS system; Miltenyi, San Jose, CA, USA). Blood and BAL eosinophils were >98% viable and pure. In some experiments, eosinophil purity was further enhanced to >99% by the addition of anti-CD3 and

anti-CD14 immunomagnetic beads to remove any contaminating T cells or monocytes, respectively.

CD4+ T cell purification

Peripheral blood mononuclear cells (PBMCs) were centrifuged (200 g) for 15 min over fetal calf serum (FCS) to remove platelets. CD4+ T cells were purified by positive selection using anti-CD4-coated immunomagnetic beads (Dynal Inc., Lake Success, NY, USA). Beads were removed from the CD4+ T cells using Detachabead® (Dynal). The CD4+ T cells were >97% viable and pure as assessed by flow cytometric analysis. In a pilot study, positively selected CD4+ T cells were compared with cells isolated by negative selection (MACS system; Miltenyi).

CD4+ T cell eosinophil co-culture

CD4+ T cells were rested overnight at 37°C. Eosinophils (2×10^6 /ml) were cultured for 24 h in the presence or absence of GM-CSF (10 ng/ml). After a wash step, 1×10^6 /ml eosinophils were added to an equivalent number of autologous CD4+ T cells in a 96-well plate. Cell culture medium consisted of RPMI-1640, 10 mM HEPES, 2 mM L-glutamine, antibiotic, and antimycotic agents (100 units/ml penicillin G, 100 mg/ml streptomycin sulfate, and 250 ng/ml amphotericin B), and 5% FCS (all from Gibco BRL, Grand Island, NY, USA). Cells were cultured in the presence or absence of SEB (3 μ g/ml) for up to 6 days (as indicated). Optimal concentrations of SEB for generation of IL-5, IL-13, and interferon (IFN)- γ were determined from a dose-response curve using PBMCs. To determine the ability of eosinophils to enhance cytokine secretion in response to classical antigen, CD4+ T cells from three cat allergic donors were cultured with eosinophils in the presence of Fel d 1 (25 μ g/ml).

Flow cytometry assays

Purified eosinophils (1×10^5), BAL cells (1×10^5) or 100 μ l of whole blood were incubated for 30 min with phycoerythrin (PE)-conjugated monoclonal antibodies to CD69, human leukocyte antigen (HLA)-DR, CD80, or CD86. Propidium iodide (3 μ g/ml) was added immediately before analysis to exclude dead cells. For analysis of cells prior to purification, fluorescein isothiocyanate (FITC)-conjugated anti-CD14 and anti-CD16 were used for exclusion of neutrophils and monocytes, respectively (11). Ten thousand events were collected (BD FACScan II, San Jose, CA, USA), and data analyses were performed using the CELLQUEST software.

Cytokine assays

As previously described (14), cytokines were measured with a modified sandwich enzyme-linked immunosorbent assay (ELISA) utilizing coating antibodies and biotinylated detection antibodies (BD Pharmingen, San Diego, CA, USA). The sensitivities for IL-5, IL-13, and IFN- γ were 3 pg/ml.

Statistical analysis

Data are presented as median with interquartiles of 25% and 75% or mean $\pm$ SEM (for normally distributed data). The Wilcoxon's signed rank test (or a paired *t*-test for normally distributed data) was used to compare data within treatment pairs. The Mann-Whitney rank sum test was used to compare data between treatment

groups (blood vs BAL eosinophils). For analysis of dose-response data, multiple comparisons were performed using a Kruskal-Wallis test. For the kinetic studies, where the $n = 5$, data were normalized by log-transformation and analyzed by paired t -test. A P -value of <0.05 was considered significant. Statistical analyses were performed using the SIGMASTAT software package.

Results

Human airway and blood eosinophils enhance Th1 and Th2 cytokine generation in the presence of SEB

CD4⁺ T cells secreted low amounts of IL-5, IL-13, and IFN- γ in response to SEB compared with unstimulated

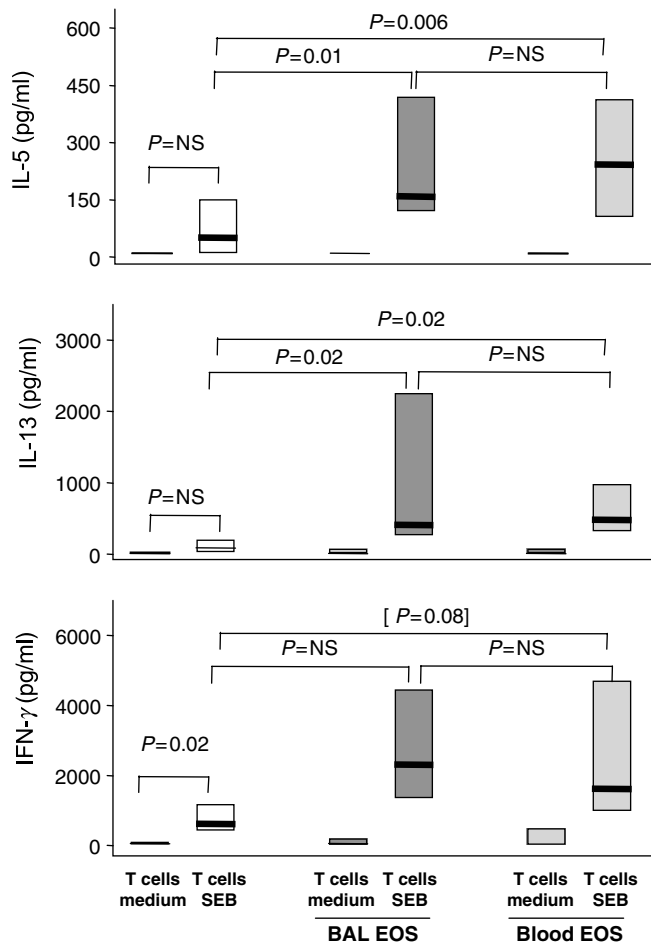


Figure 1. Both peripheral blood and bronchoalveolar lavage (BAL) eosinophils enhance staphylococcal enterotoxin B (SEB)-induced cytokine secretion. Paired samples of granulocyte-macrophage colony-stimulating factor (GM-CSF)-activated peripheral blood or BAL eosinophils were co-cultured with autologous CD4⁺ blood T cells for 6 days in the presence or absence of SEB (3 μ g/ml). Cell-free culture supernatants were collected to analyze interleukin (IL)-5, IL-13, and interferon (IFN)- γ by enzyme-linked immunosorbent assay (ELISA). Box plots represent median within 25th and 75th percentiles. $N = 7$ atopic asthma subjects.

CD4⁺ T cells (Fig. 1). Staphylococcal enterotoxin B-induced Th1 and Th2 cytokine generation was markedly enhanced by the presence of either BAL or peripheral blood eosinophils. Notably, BAL and blood eosinophils were equally efficient in this accessory function.

It should be pointed out that although PBMCs could generate IL-5 and IL-13 in response to Fel d 1, a classical antigen, CD4⁺ T cells from three cat allergic donors alone or in the presence of either blood or BAL eosinophils did not secrete measurable amounts IFN- γ , IL-5 or IL-13 (not shown).

Time course of Th1 and Th2 cytokine secretion in the presence of peripheral blood eosinophils

To determine if preferential enhancement of Th1 or Th2 cytokines occurred with different kinetics, cytokine secretion was examined at days 2, 4, and 6 following co-culture of CD4⁺ T cells and eosinophils in the presence of SEB. Concentrations of IL-5 and IL-13 significantly increased through the entire time course (day 6 > day 4 > day 2, Fig. 2). The increase in IFN- γ secretion was variable among subjects, but achieved statistical significance at days 4 and 6 compared with day 2.

Dose dependence of eosinophil-mediated enhanced cytokine secretion

To determine whether augmented cytokine secretion occurred at lower ratios of eosinophils and whether differential enhancement of Th1/Th2 cytokines would occur, the response to different numbers of eosinophils ($0.25\text{--}2 \times 10^5/\text{well}$) was examined. Upon co-culture of SEB-stimulated CD4⁺ T cells with varying numbers of blood eosinophils, there was an eosinophil dose-dependent increase in IL-5, IL-13, and IFN- γ generation (Fig. 3). There was no differential enhancement of Th1/Th2 cytokines at different concentrations of eosinophils.

GM-CSF activation of peripheral blood eosinophils does not affect enhancement of cytokine secretion

Granulocyte-macrophage colony-stimulating factor is a potent stimulus for eosinophils and can augment eosinophil expression of MHC class II (15) and the activation marker CD69 (16). To determine if GM-CSF activation affected eosinophil-mediated enhancement of cytokine secretion, CD4⁺ T cells were co-cultured with peripheral blood eosinophils that had been exposed to GM-CSF or medium. The levels of enhanced cytokine secretion were comparable between the cultures containing GM-CSF-stimulated and unstimulated eosinophils with no statistical difference for any of the cytokines (Fig. 4).

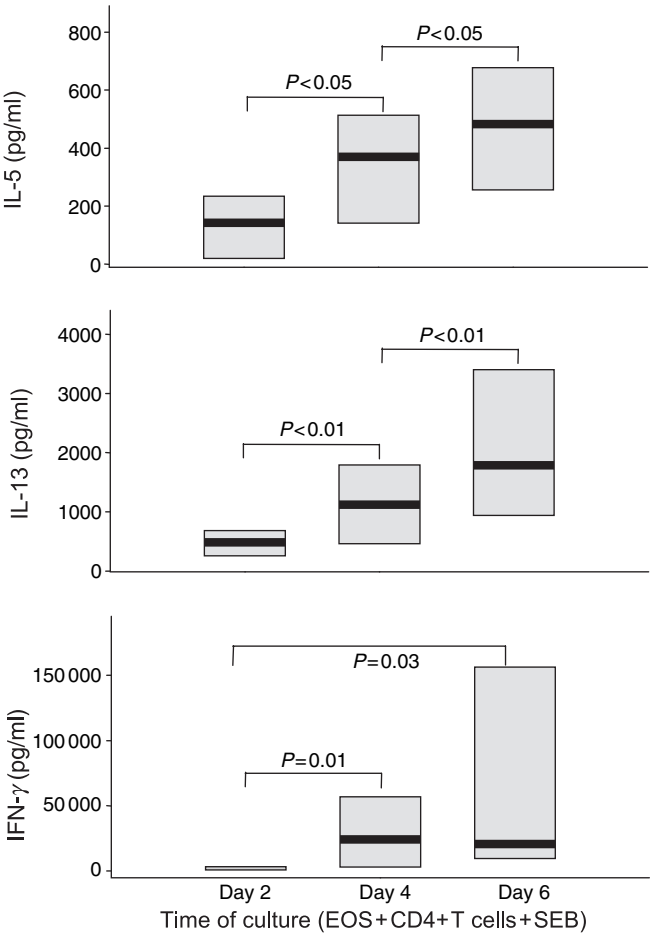


Figure 2. Kinetics of staphylococcal enterotoxin B (SEB)-induced cytokine secretion in the presence of blood eosinophils. Peripheral blood eosinophils were incubated with autologous CD4+ T cells for 2, 4, or 6 days in the presence of SEB (3 μ g/ml). Cell-free culture supernatants were collected to analyze interleukin (IL)-5, IL-13, and interferon (IFN)- γ by enzyme-linked immunosorbent assay (ELISA). Box plots represent medians within 25th and 75th percentiles ($n = 5$). Data were analyzed by a paired t -test after log-transformation.

Co-stimulatory molecules are not detectable on BAL eosinophils or GM-CSF-stimulated blood eosinophils

In addition to MHC class II, murine eosinophils can express the co-stimulatory molecules CD86 and CD80 (2, 3, 17). To determine if human eosinophils also expressed these markers, blood and airway cells were examined prior to purification and after culture with GM-CSF and/or co-culture with CD4+ T cells and SEB. BAL eosinophils were highly positive for HLA-DR and CD69, but not CD86 (Fig. 5) or CD80 (not shown). Blood eosinophils had little or no detectable HLA-DR, CD69, CD86 or CD80. Used as a positive control, blood monocytes were highly positive for CD86 (Fig. 5, panel A) and CD80 (not shown). To determine if these markers could be induced by

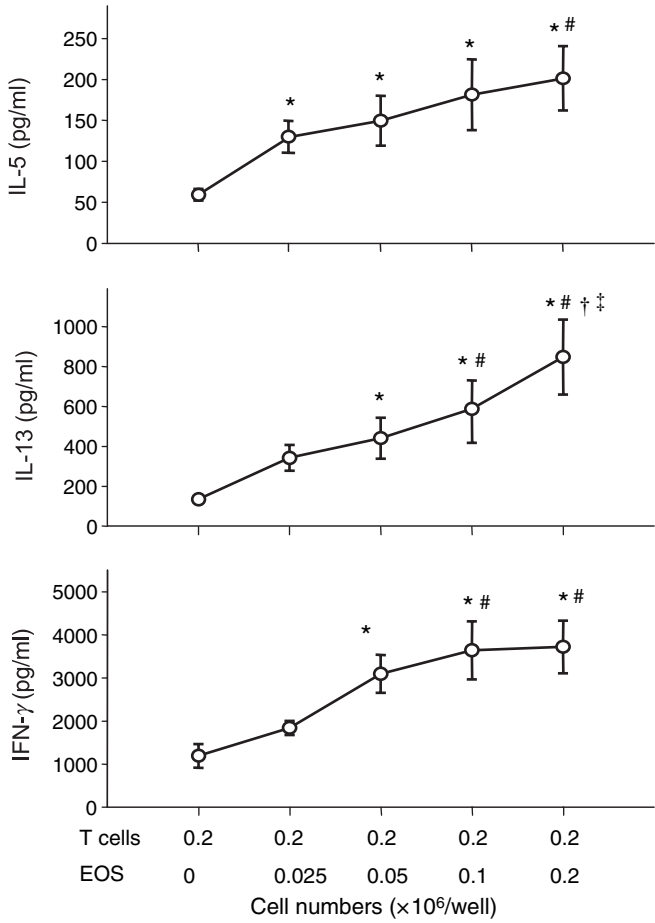


Figure 3. Eosinophils enhance staphylococcal enterotoxin B (SEB)-induced cytokine secretion in a dose-dependent fashion. CD4+ T cells (0.2×10^6 per well) were co-cultured with varying numbers of blood eosinophils for 6 days in the presence of SEB (3 μ g/ml). Cell-free culture supernatant fluids were collected to analyze cytokines by enzyme-linked immunosorbent assay (ELISA; $n = 6$). Data are presented as mean $\pm$ SEM. Multiple comparisons were analyzed using a Kruskal-Wallis test. * $P < 0.05$ vs baseline (T cells without eosinophils); # $P < 0.05$ vs T cells + 0.025×10^6 eosinophils per well; † $P < 0.05$ vs T cells + 0.05×10^6 eosinophils per well; ‡ $P < 0.05$ vs T cells + 0.1×10^6 eosinophils per well.

GM-CSF, eosinophils were analyzed 24 h after stimulation with GM-CSF (panel B). As shown in Table 1, HLA-DR was induced on blood eosinophils from only nine of 18 subjects (similar results were seen when cells were stained with a pan anti-MHC class II antibody with specificity for HLA-DR/DP/DQ). In contrast, CD86 and CD80 were not detectable on eosinophils from any subject. A marked increase in CD69 on blood eosinophils from all subjects (median channel fluorescence increased from a mean $\pm$ SD of 243 ± 282 to 998 ± 242 , $n = 18$) indicated that the cells were activated by GM-CSF. Finally, blood and BAL eosinophils were analyzed 24 h after co-culture with CD4+ T cells and SEB (Fig. 5, panel

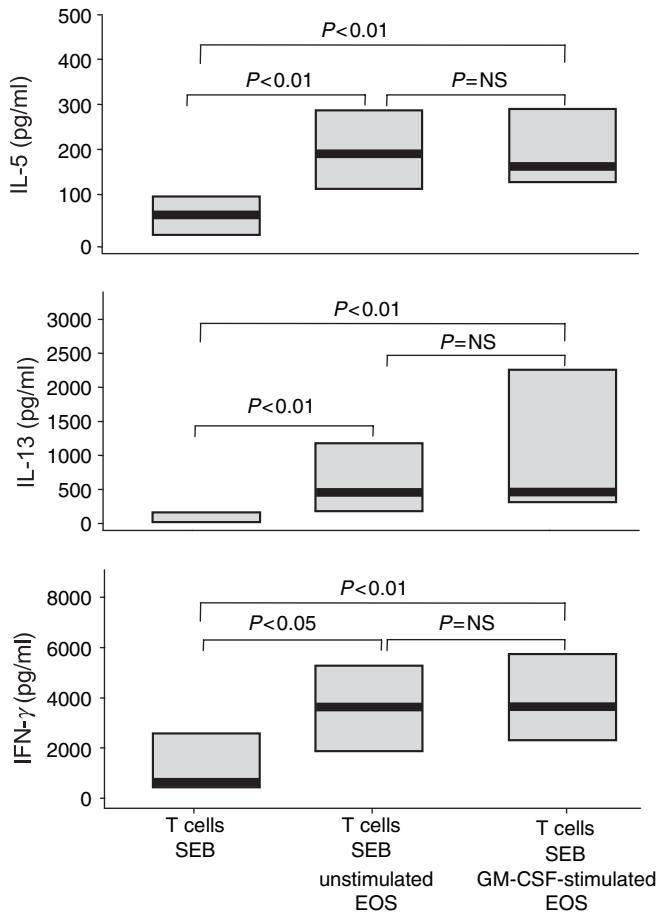


Figure 4. Effect of eosinophil activation on enhancement of staphylococcal enterotoxin B (SEB)-induced cytokine secretion. CD4⁺ T cells were co-cultured with unstimulated peripheral blood eosinophils or granulocyte-macrophage colony-stimulating factor (GM-CSF)-activated (10 ng/ml for 24 h) eosinophils (GM-EOS) for 6 days in the presence of SEB (3 µg/ml). Cell-free culture supernatants were collected to analyze interleukin (IL)-5, IL-13, and interferon (IFN)-γ by enzyme-linked immunosorbent assay (ELISA; $n = 15$). Box plots represent medians within 25th and 75th percentiles.

C). There was no further increase in HLA-DR or CD69. Moreover, CD86 and CD80 were not detectable under these conditions.

HLA-DR expression does not affect eosinophil-mediated enhancement of cytokine secretion

The variable expression of HLA-DR among subjects led us to determine whether eosinophils with high levels of HLA-DR induced a differential profile of cytokine secretion in co-culture with CD4⁺ T cells and SEB. Eosinophils that expressed little or no HLA-DR enhanced SEB-induced T cell cytokine generation as effectively as eosinophils that expressed high levels of HLA-DR (Fig. 6). There was no difference in amount of cytokines or the cytokine profile when CD4⁺ T cells

were co-cultured with HLA-DR⁺ vs HLA-DR⁻ eosinophils.

Effect of SEB on eosinophils

To further evaluate the mechanism by which eosinophils augment cytokine generation, eosinophils were stimulated with SEB in the absence of T cells. Staphylococcal enterotoxin B had no effect on eosinophil expression of CD69, HLA-DR, CD80, or CD86 nor did it induce eosinophil generation of IL-5, IL-13, or IFN-γ (mean ± SD pg/ml for cells in medium vs SEB; IL-5: 0.3 ± 0.5 vs 0.3 ± 0.05 , IL-13: 1.0 ± 2.6 vs 3.3 ± 4.2 , IFN-γ: 3.5 ± 1.3 vs 9.5 ± 3.5) or BAL eosinophils (mean ± SD pg/ml for cells in medium vs SEB; IL-5: 0.0 ± 0.0 vs 0.0 ± 0.0 , IL-13: 0.0 ± 0.0 vs 0.0 ± 0.0 , IFN-γ: 0.3 ± 0.6 vs 21.7 ± 36.7). Similar results were seen with eosinophils pretreated with GM-CSF. Finally, addition of culture supernatant fluids from SEB-stimulated eosinophils had no effect on cytokine generation by CD4⁺ T cells (not shown).

Discussion

In this study, we established that in addition to human peripheral blood eosinophils, airway eosinophils also have the ability to regulate secretion of Th1 and Th2 cytokines including IL-5, IL-13, and IFN-γ. Somewhat surprisingly, a comparison of eosinophils from the two compartments revealed no difference in their ability to promote the generation of cytokines in the presence of SEB. The ability of eosinophils to augment cytokine secretion was independent of their activation by GM-CSF, and did not appear to require detectable expression of co-stimulatory molecules or HLA-DR. Moreover, the presence or absence of demonstrable levels of HLA-DR on eosinophils had no differential effect on the profile of Th1/Th2 cytokines that were elicited in the co-cultures. These data clearly demonstrate that human eosinophils from the blood and the airway can contribute to the generation of both Th1 and Th2 cytokines in response to superantigen and support the role of human eosinophils as immunoregulatory cells.

There were several unexpected findings in this study. First, generation of both Th1 and Th2 cytokines were augmented when human eosinophils were added to cultures of CD4⁺ T cells and SEB. Secondly, although airway eosinophils expressed high levels of HLA-DR and CD69, they did not have enhanced regulatory function compared with their circulating counterparts. Finally, cytokine generation was augmented in co-cultures containing peripheral blood eosinophils that had little or no detectable MHC class II or co-stimulatory molecules.

The finding that human eosinophils augment both Th1 and Th2 cytokines is in contrast to studies in mice demonstrating a preferential induction of Th2 cytokines

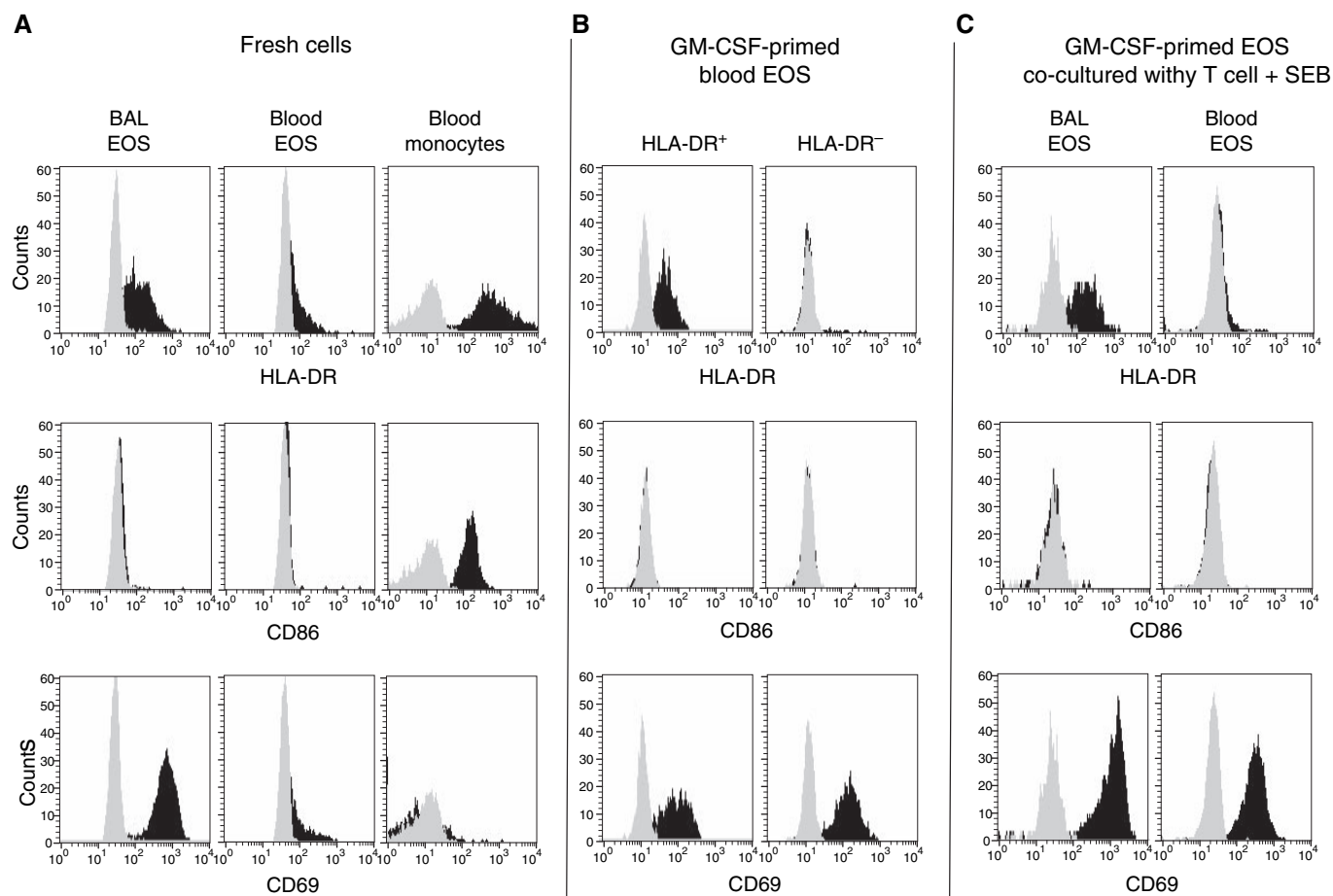


Figure 5. Analysis of human leukocyte antigen (HLA)-DR, CD86, and CD69 on blood and bronchoalveolar lavage (BAL) eosinophils. (A) Cell surface expression of HLA-DR, CD86, and CD69 was determined on nonseparated fresh BAL eosinophils or cells in lysed whole blood samples. Histograms are based on cells from a representative subject. (B) Cell surface marker expression was assessed on blood eosinophils 24 h after stimulation with granulocyte-macrophage colony-stimulating factor (GM-CSF; 10 ng/ml). Data presented are from two subjects – one with high HLA-DR expression and one with barely detectable HLA-DR expression. (C) Cell surface marker expression was assessed on blood and BAL eosinophils 24 h after co-culture with CD4⁺ T cells and staphylococcal enterotoxin B (SEB). Representative data from one subject are presented.

(4, 17). The implications of the simultaneous increase in Th1 and Th2 cytokines for allergic inflammation are unclear, but intriguing. Key features of allergic diseases such as eosinophilia, mast cell development, IgE synthesis, and mucus secretion are linked to Th2-type cytokines. While it is known that Th1 cytokines such as IFN- γ can downregulate Th2 responses, they may also contribute indirectly to allergic inflammation by increasing the expression of leukocyte adhesion molecules such as intercellular adhesion molecule (ICAM) and vascular cellular adhesion molecule (VCAM) on vascular endothelium (18). In mice, Th1 and Th2 cells can cooperate to enhance antigen-mediated inflammation (19, 20). Furthermore, we have previously demonstrated that PBMCs from subjects with asthma have an increased ability to generate both Th1 and Th2 cytokines (21) and a simultaneous increase in the frequencies of IL-4-, IL-5-,

and IFN- γ -producing T cells has been demonstrated in BAL (22) and induced sputum of subjects with atopic asthma (23). Collectively, these data suggest that the presence of both Th1 and Th2 cytokines may represent a more severe inflammatory state.

The mechanisms by which eosinophils augment SEB-induced cytokine generation are not clear. Although eosinophils have been shown to be source IL-4 (24), IL-5 (25), IL-13 (26), and IFN- γ (27), it appears unlikely that SEB directly stimulates eosinophils to produce Th1 or Th2 cytokines. We find no evidence that SEB-induced expression of cell surface activation markers or generation of IL-4, IL-5, IL-13, or IFN- γ . However, we cannot rule out the possibility that factors present in the co-cultures of eosinophils and SEB-activated T cells can stimulate eosinophil generation of Th1/Th2 cytokines. Regardless of their source, generation of Th1 and Th2

Table 1. HLA-DR and CD69 expression on eosinophils after 24 h GM-CSF stimulation

Subject	Type	HLA-DR (MCF)		CD69 (MCF)	
		Medium	GM-CSF	Medium	GM-CSF
1	AA	ND	212	ND	836
2	AA	ND	518	ND	820
3	AA	Undetectable	Undetectable	Undetectable	750
4	AR	Undetectable	Undetectable	Undetectable	808
5	AR	Undetectable	Undetectable	540	1056
6	AA	Undetectable	Undetectable	Undetectable	840
7	AA	66	312	Undetectable	652
8	AA	100	220	Undetectable	924
9	NN	Undetectable	Undetectable	120	1248
10	NN	Undetectable	Undetectable	Undetectable	788
11	AR	Undetectable	Undetectable	Undetectable	984
12	AA	Undetectable	Undetectable	Undetectable	1006
13	AA	328	738	Undetectable	1176
14	AA	140	520	Undetectable	924
15	AR	189	408	Undetectable	1548
16	AA	92	601	Undetectable	1385
17	AA	Undetectable	Undetectable	Undetectable	1292
18	AA	400	788	Undetectable	932

AA, allergic asthma; AR, allergic rhinitis; NN, normal nonallergic; MCF, median channel fluorescence; ND, not done; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA, human leukocyte antigen.

cytokines are clearly increased when CD4⁺ T cells are cultured with eosinophils and SEB.

There are a number of reports, particularly in mouse models, suggesting that eosinophils can function as APCs. For example, murine eosinophils can express MHC class II, CD80, and CD86 (2–4, 17), process antigen (3, 17), traffic to draining lymph nodes (3, 4, 17, 28) and prime CD4⁺ T cells for proliferation and cytokine generation (2–5, 17). Studies of human eosinophils have demonstrated increased T cell proliferation upon co-culture with eosinophils and stimulation with antigen peptides (10) or SEB (11, 12), or upon co-culture of rhinovirus-specific T cell lines and eosinophils preincubated with rhinovirus (29). There is, however, at least one report that murine eosinophils are not able to present antigen to naive T cells (30), and there are a number of reports demonstrating that human blood eosinophils are inefficient as APCs when classical antigens (requiring processing for presentation on MHC) are used as T cell stimuli (10–12). Our data corroborate these human studies and demonstrate for the first time that co-culture of human eosinophils and CD4⁺ T cells stimulated with SEB results in enhanced secretion of Th1 and Th2 cytokines. Moreover, we show that peripheral blood and airway eosinophils have similar accessory function and that neither cell population can support cytokine generation in response to classical antigen (Fel d 1). To our knowledge, this is the first report to evaluate the accessory function of human airway eosinophils.

Our results indicate that the eosinophil-mediated enhancement of CD4⁺ T cell cytokine secretion is

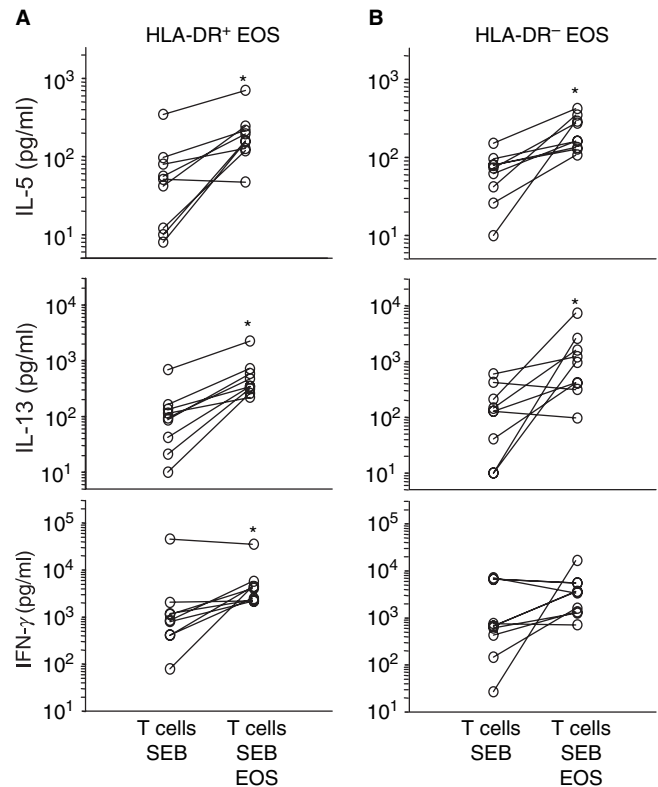


Figure 6. Effect of eosinophil expression of human leukocyte antigen (HLA)-DR on enhancement of staphylococcal enterotoxin B (SEB)-induced cytokine secretion. CD4⁺ T cells were incubated with granulocyte-macrophage colony-stimulating factor (GM-CSF)-activated HLA-DR⁺ (A) or HLA-DR[−] (B) peripheral blood eosinophils for 6 days in the presence of SEB (3 µg/ml). Cell-free culture supernatant fluids were collected to analyze interleukin (IL)-5, IL-13, and interferon (IFN)-γ by enzyme-linked immunosorbent assay (ELISA). Line graphs depict individual data from 18 subjects.

independent of detectable expression of HLA-DR and co-stimulatory molecules. This was an unexpected result given that previous study of mouse and human eosinophils showed that their ability to activate T cell proliferation correlated with expression of MHC class II molecules (2, 3, 5, 12) and could be blocked by inhibiting CD80 and/or CD86. While the level of HLA-DR, CD80, and CD86 does not appear to contribute to the accessory function of eosinophils in our study, it remains possible that very small amounts of MHC class II and co-stimulatory molecules are present on these cells and are sufficient for antigen presentation. It is also possible that co-stimulation occurs via a nonconventional mechanism such as adhesion molecules as has been described for presentation of SEB by an adenocarcinoma cell line (31). Regardless of the mechanism, it is clear from our studies that human eosinophils either provide a signal to enhance SEB-induced cytokine secretion or receive a signal from SEB-activated T cells to contribute directly cytokine generation. Additional studies will be needed to

explore the mechanisms by which these eosinophil-T cell interactions occur.

Staphylococcal enterotoxin B was utilized as a surrogate for protein allergens to activate T cells. The choice of SEB was based on its ability to interact directly with MHC class II molecules and a subset of CD4+ T cells expressing a TCR with a specific V β -region (9). Thus, SEB does not require processing, and it can nonspecifically activate a large subset (30%) of CD4+ T cells. It is unclear whether our findings with SEB can be extrapolated to complex classical antigens. Nonetheless, eosinophil augmentation of SEB-induced cytokines is likely to be relevant to diseases such as atopic dermatitis, chronic sinusitis, and nasal polyposis that are associated with eosinophilic inflammation and for which staphylococcal superantigens have been directly implicated in the disease pathogenesis (32, 33). In fact, eosinophil augmentation of SEB-induced cytokines may play a broader role in atopy. There is evidence for increased prevalence of *Staphylococcus aureus* in patients with allergic rhinitis (34–37) and there are reports that staphylococcal enterotoxins may promote development of atopy (37, 38).

Eosinophils are a significant component of allergic inflammation associated with atopic dermatitis, rhinitis, and asthma. Our results demonstrate eosinophil-mediated enhancement of Th1 and Th2 cytokine secretion in co-cultures of CD4+ T cells and superantigen and support an immunoregulatory role for eosinophils in allergic inflammation.

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References

- Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Adkinson NF, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FE, editors. Middleton's allergy: principles and practice. Philadelphia, USA: Mosby, 2003:305–332.
- Tamura N, Ishii N, Nakazawa M, Nagoya M, Yoshinari M, Amano T et al. Requirement of CD80 and CD86 molecules for antigen presentation by eosinophils. *Scand J Immunol* 1996;**44**:229–238.
- Shi HZ, Humbles A, Gerard C, Jin Z, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. *J Clin Invest* 2000;**105**:945–953.
- Shi HZ, Xiao CQ, Li CQ, Mo XY, Yang QL, Leng J et al. Endobronchial eosinophils preferentially stimulate T helper cell type 2 responses. *Allergy* 2004;**59**:428–435.
- Del Pozo V, De Andres B, Martin E, Cardaba B, Fernandez JC, Gallardo S et al. Eosinophil as antigen-presenting cell: activation of T cell clones and T cell hybridoma by eosinophils after antigen processing. *Eur J Immunol* 1992;**22**:1919–1925.
- Shinkai K, Mohrs M, Locksley RM. Helper T cells regulate type-2 innate immunity in vivo. *Nature* 2002;**420**:825–829.
- Mattes J, Yang M, Mahalingam S, Kuehr J, Webb DC, Simson L et al. Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002;**195**:1433–1444.
- Crow MK. Costimulatory molecules and T-cell-B-cell interactions. *Rheum Dis Clin North Am* 2004;**30**:175–191.
- Dellabona P, Peccoud J, Kappler J, Marrack P, Benoist C, Mathis D. Superantigens interact with MHC class II molecules outside of the antigen groove. *Cell* 1990;**62**:1115–1121.
- Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function and human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* 1993;**150**:2554–2562.
- Mawhorter SD, Kazura JW, Boom WH. Human eosinophils as antigen presenting cells: relative efficiency for superantigen and antigen-induced CD4+ T-cell proliferation. *Immunology* 1994;**81**:584–591.
- Celestin J, Rotschke O, Falk K, Ramesh N, Jabara H, Strominger J et al. IL-3 induces B7.2 (CD86) expression and costimulatory activity in human eosinophils. *J Immunol* 2001;**167**:6097–6104.
- Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H et al. Decreased expression of membrane IL-5R α on human eosinophils: I. Loss of membrane IL-5 α on eosinophils and increased soluble IL-5R alpha in the airway after antigen challenge. *J Immunol* 2002;**169**:6452–6458.
- Kelly EA, Rodriguez RR, Busse WW, Jarjour NN. The effect of segmental bronchoprovocation with allergen on airway lymphocyte function. *Am J Respir Crit Care Med* 1997;**156**:1421–1428.
- Lucey DR, Nicholson-Weller A, Weller PF. Mature human eosinophils have the capacity to express HLA-DR. *Proc Natl Acad Sci U S A* 1989;**86**:1348–1351.
- Hartnell A, Robinson DS, Kay AB, Wardlaw AJ. CD69 is expressed by human eosinophils activated in vivo in asthma and in vitro by cytokines. *Immunology* 1993;**80**:281–286.
- MacKenzie JR, Mattes J, Dent LA, Foster PS. Eosinophils promote allergic disease of the lung by regulating CD4(+) Th2 lymphocyte function. *J Immunol* 2001;**167**:3146–3155.
- Spoelstra FM, Postma DS, Hovenga H, Noordhoek JA, Kauffman HF. Interferon-gamma and interleukin-4 differentially regulate ICAM-1 and VCAM-1 expression on human lung fibroblasts. *Eur Respir J* 1999;**14**:759–766.

19. Randolph DA, Carruthers CJ, Szabo SJ, Murphy KM, Chaplin DD. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J Immunol* 1999;**162**:2375–2383.
20. Randolph DA, Stephens R, Carruthers CJ, Chaplin DD. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J Clin Invest* 1999;**104**:1021–1029.
21. Kenyon NJ, Kelly EA, Jarjour NN. Enhanced cytokine generation by peripheral blood mononuclear cells in allergic and asthma subjects. *Ann Allergy Asthma Immunol* 2000;**85**: 115–120.
22. Krug N, Madden J, Redington A, Lackie P, Djukanovic R, Schauer U et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996;**14**:319–326.
23. Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4⁺ and CD8⁺ T cells in atopic asthma. *Am J Respir Crit Care Med* 2005;**171**:224–230.
24. Melo RC, Spencer LA, Perez SA, Ghiran I, Dvorak AM, Weller PF. Human eosinophils secrete preformed, granule-stored interleukin-4 through distinct vesicular compartments. *Traffic* 2005;**6**:1047–1057.
25. Dubucquoi S, Desreumaux P, Janin A, Klein O, Goldman M, Tavernier J et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J Exp Med* 1994;**179**:703–708.
26. Schmid-Grendelmeier P, Altnauer F, Fischer B, Bizer C, Straumann A, Menz G et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 2002;**169**:1021–1027.
27. Lamkhioed B, Gounni AS, Aldebert D, Delaporte E, Prin L, Capron A et al. Synthesis of type 1 (IFN gamma) and type 2 (IL-4, IL-5, and IL-10) cytokines by human eosinophils. *Ann N Y Acad Sci* 1996;**796**:203–208 (Review 22 refs).
28. Duez C, Dakhama A, Tomkinson A, Marquillies P, Blahorn A, Tonnel AB et al. Migration and accumulation of eosinophils toward regional lymph nodes after airway allergen challenge. *J Allergy Clin Immunol* 2004;**114**: 820–825.
29. Handzel ZT, Busse WW, Sedgwick JB, Vrtis R, Lee WM, Kelly EA et al. Eosinophils bind rhinovirus and activate virus specific T cells. *J Immunol* 1998;**160**:1279–1284.
30. Van Rijt LS, Vos N, Hijdra D, de Vries VC, Hoogsteden HC, Lambrecht BN. Airway eosinophils accumulate in the mediastinal lymph nodes but lack antigen-presenting potential for naive T cells. *J Immunol* 2003;**171**:3372–3378.
31. Lamphear JG, Stevens KR, Rich RR. Intercellular adhesion molecule-1 and leukocyte function-associated antigen-3 provide costimulation for superantigen-induced T lymphocyte proliferation in the absence of a specific presenting molecule. *J Immunol* 1998;**160**:615–623.
32. Schubert MS. A superantigen hypothesis for the pathogenesis of chronic hypertrophic rhinosinusitis, allergic fungal sinusitis, and related disorders. *Ann Allergy Asthma Immunol* 2001;**87**:181–188.
33. Bunikowski R, Mielke ME, Skarabis H, Worm M, Anagnostopoulos I, Kolde G et al. Evidence for a disease-promoting effect of *Staphylococcus aureus*-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol* 2000;**105**:814–819.
34. Okano M, Takishita T, Yamamoto T, Hattori H, Yamashita Y, Nishioka S et al. Presence and characterization of sensitization to staphylococcal enterotoxins in patients with allergic rhinitis. *Am J Rhinol* 2001;**15**:417–421.
35. Riechelmann H, Essig A, Deutschle T, Rau A, Rothermel B, Weschta M. Nasal carriage of *Staphylococcus aureus* in house dust mite allergic patients and healthy controls. *Allergy* 2005;**60**:1418–1423.
36. Rossi RE, Monasterolo G. Prevalence of serum IgE antibodies to the *Staphylococcus aureus* enterotoxins (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol* 2004;**133**:261–266.
37. Shiomori T, Yoshida S, Miyamoto H, Makishima K. Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol* 2000;**105**:449–454.
38. Okano M, Hattori H, Yoshino T, Sugata Y, Yamamoto M, Fujiwara T et al. Nasal exposure to staphylococcal enterotoxin enhances the development of allergic rhinitis in mice. *Clin Exp Allergy* 2005;**35**:506–514.