

An airborne mold-derived product, β -1,3-D-glucan, potentiates airway allergic responses

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Repeated inhalation of allergen leads to the down-regulation of allergen-specific IgE responses in non-atopic individuals as well as in mice. This phenomenon is named inhalation-induced IgE tolerance. In contrast, inhaled allergen causes significant IgE and allergic responses in atopic persons. The mechanisms involved in this differential regulation of airway allergen-specific immune responses remain unclear. Besides the allergen exposure of genetically susceptible individuals, environmental contamination is considered to play a role as an initiating factor for airway allergic responses. Using a murine model, we demonstrate here that airborne β -1,3-D-glucan, which exists frequently in our environment, particularly in highly humid areas, can abrogate inhalation-induced IgE isotype-specific down-regulation and promote airway eosinophil infiltration to inhaled antigen.

Key words: Allergy / Airway inflammation / Glucan / Asthma / Animal model

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1 Introduction

Allergic individuals often mount Th2 immune responses to environmental allergens, in contrast to non-allergic persons, whose allergen-specific immune reactions are mainly mediated by Th1 [1, 2]. Th2-triggered immune responses play a very important role in the mediation of allergic diseases. The nature or dose of the antigens as well as the local cytokine environment at the time of naive T cell priming have been demonstrated to be involved in the regulation of Th subset development *in vitro* [3–6].

In the lung of a non-allergic individual, the inhaled allergen can induce the suppression of allergen-specific IgE responses [7]. Repeated exposure of aerosolized protein antigens was also shown to be able to suppress antigen-specific IgE responses in mice, without suppressing the corresponding IgG production [8]. However, inhaled allergen can initiate strong IgE responses in allergic persons. These puzzling findings suggest that other factors

apart from allergen and genetics must play a very important role in initiating airway protein antigen-induced IgE allergic responses. Furthermore, the levels of mite allergen in subtropical areas, such as Taiwan, are always found to be higher than the threshold for sensitization. Therefore, the nature or the amounts of mite allergen may not be the determining factor for allergy sensitization [9].

Several epidemiological investigations have demonstrated that airborne microbial cell wall components can cause not only an inflammatory response in the airway [10, 11], but may also play an important role in chronic asthmatic diseases [12]. β -1,3-D-glucan, a glucose polymer found in the cell wall of fungi, can induce inflammatory responses in both humans and mice [13–15]. From our field measurements, the amount of indoor β -1,3-D-glucan ranged between 1 ng m⁻³ and 10 ng m⁻³ in Taipei [16]. Moreover, the β -1,3-D-glucan contents can be even higher than 100 ng m⁻³ in moldy homes [15]. In the current study, we investigated the effects of airborne β -1,3-D-glucan on the generation of antigen-specific airway allergic responses. We found that β -1,3-D-glucan can promote Th2-mediated allergic responses in mice. Thus, the airway inflammation induced by this microbial cell wall component must be regarded to be an important co-initiating factor for generating airway allergic responses.

[1 19056]

Abbreviation: C-RT-PCR: Competitive reverse transcription PCR

2 Results

2.1 The effect of β -1,3-D-glucan on murine airway antigen-induced humoral immune responses

We first conducted experiments to test the promoting effects of airborne β -1,3-D-glucan on murine airway antigen-specific immune responses. After inhalation with or without β -1,3-D-glucan and subsequently with OVA antigen, sera were collected and analyzed for OVA-specific IgE and IgG2a antibodies as measures of allergic or non-allergic immune responses, respectively. As shown in Fig. 1, mice pre-exposed to β -1,3-D-glucan mounted significantly ($p < 0.01$) higher amounts of IgE antibody responses to inhaled OVA antigen than mice sensitized with OVA alone. On the contrary, the OVA-specific IgG2a response was higher ($p < 0.05$) in only OVA-treated mice than those exposed to the combination of β -1,3-D-glucan and OVA. The mice exposed only to glucan without OVA did not show any detectable OVA-specific antibody responses (data not shown).

2.2 β -1,3-D-glucan potentiates the allergen-induced lung eosinophil infiltration

To demonstrate the airway allergic inflammation, we measured the amounts of infiltrated eosinophils in bronchoalveolar lavage after challenge with aerosolized OVA-

antigen. As shown in Fig. 2, β -1,3-D-glucan/OVA-sensitized mice mounted a significant ($p < 0.01$) increase in eosinophils in the lung after challenge with aerosolized OVA, in comparison to mice given only aerosolized OVA sensitization ($3.6 \times 10^4 \pm 3.4 \times 10^3$ vs. $0.9 \times 10^4 \pm 1.5 \times 10^3$). The numbers infiltrated cells in the lungs of OVA/glucan- and OVA-pretreated mice were $1.9 \times 10^5 \pm 3.8 \times 10^4$ and $1.9 \times 10^5 \pm 3.9 \times 10^4$, respectively. There was no significant difference in the numbers of infiltrated lymphocytes in the lungs between these two groups. The infiltration of these inflammatory cells is OVA antigen specific, because OVA- or OVA/glucan-pretreated mice did not demonstrate a significant increase in the number of eosinophils in the lung when challenged with aerosolized saline.

2.3 β -1,3-D-glucan suppresses the down-regulatory effect of airway antigen-induced IgE isotype-specific responses

We further analyzed the effect of β -1,3-D-glucan on the IgE isotype-specific down-regulation after inhalation of OVA antigen. As shown in Fig. 3, repeated OVA aerosol exposure markedly ($p < 0.01$) suppressed IgE responses to OVA administered i.p. However, OVA-specific IgG2a antibody responses were observed to increase ($p < 0.01$) in the animals after repeated OVA inhalation. This phenomenon is known as inhalation-induced IgE isotype-specific down-regulation. In contrast, the mice exposed

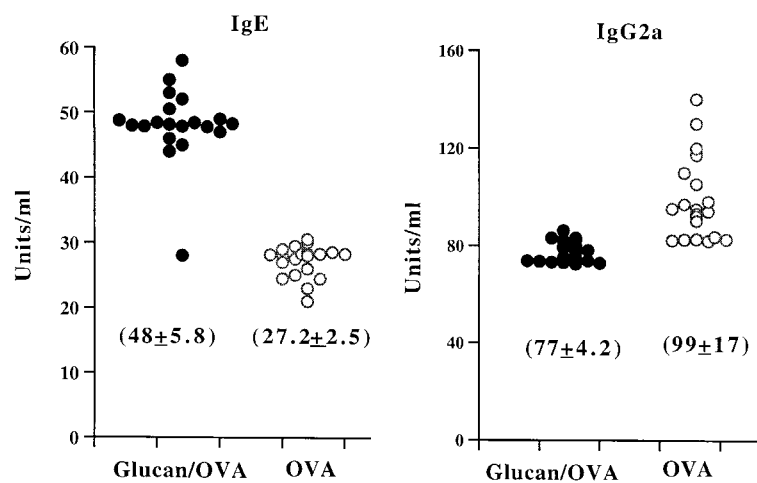


Figure 1. The effect of β -1,3-D-glucan on humoral immune responses after exposition of airway to antigen. Female BALB/cJ mice ($n = 20$) were exposed first to 400 ng/ml glucan for 40 min. After 18 h, the mice were sensitized with a daily 20-min exposure to 10 mg/ml OVA antigen for 5 consecutive days (Glucan/OVA). A group of mice ($n = 20$) were exposed to OVA only. Two weeks later, the mice were re-exposed to 10 mg/ml OVA for 20 min. After another 7 days, the sera were obtained and analyzed for OVA-specific IgE and IgG2a antibodies by ELISA. Age-matched control mice were exposed to OVA alone using the same protocol. Each symbol represents the data derived from an individual serum. The data shown are from two independent experiments.

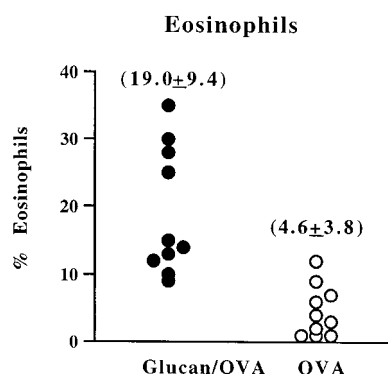


Figure 2. β -1,3-D-glucan potentiates the allergen-induced lung eosinophil infiltration. Mice ($n = 10$) were exposed to β -1,3-D-glucan and OVA antigen using the same protocol as described in Fig. 1. Seven days after the last antigen exposure, the mice were challenged with aerosolized OVA (10 mg/ml) and the lung lavage fluids were obtained 24 h later. Age-matched control mice ($n = 10$) were exposed to OVA alone using the same protocol. Eosinophils were characterized using Liu's stain. Each symbol represents the data derived from lung lavage fluid of an individual mouse.

to β -1,3-D-glucan before repeated OVA inhalation mounted higher ($p < 0.01$) amounts of IgE and lower ($p < 0.01$) amounts of IgG2a to OVA given i.p. than mice that had not received β -1,3-D-glucan treatment. Thus, the repeated OVA inhalation-induced IgE isotype-specific down-regulation was blocked by the pre-

exposure to β -1,3-D-glucan. Furthermore, Th1-related IgG2a antibody responses were found to be significantly reduced in mice after glucan/OVA inhalation.

2.4 β -1,3-D-glucan potentiates the allergen-induced lung eosinophil infiltration in mice after repeated antigen inhalation

The numbers of inflammatory cells induced by aerosolized OVA in lung lavage of β -1,3-D-glucan/OVA- or OVA-exposed mice are shown in Fig. 4. In mice exposed to β -1,3-D-glucan following repeated inhalation and i.p. immunization with OVA, there were significant ($p < 0.01$) increases in eosinophils after airway re-challenge with aerosolized OVA as compared to those treated with OVA only ($2.6 \times 10^4 \pm 1 \times 10^3$ vs. $0.08 \times 10^4 \pm 0.18 \times 10^3$). The numbers of infiltrated cells in the lungs in OVA/glucan- and OVA pretreated mice were $2 \times 10^5 \pm 2.9 \times 10^4$ and $1.8 \times 10^5 \pm 2.0 \times 10^4$, respectively.

2.5 Airborne β -1,3-D-glucan regulates the synthesis of IL-10 and IL-12 mRNA by lung wall cells

In an effort to understand the immunological mechanisms which might be responsible for the role of β -1,3-D-glucan exposure in airway OVA antigen-specific immune responses, the Th1/Th2 deviation-related pro-

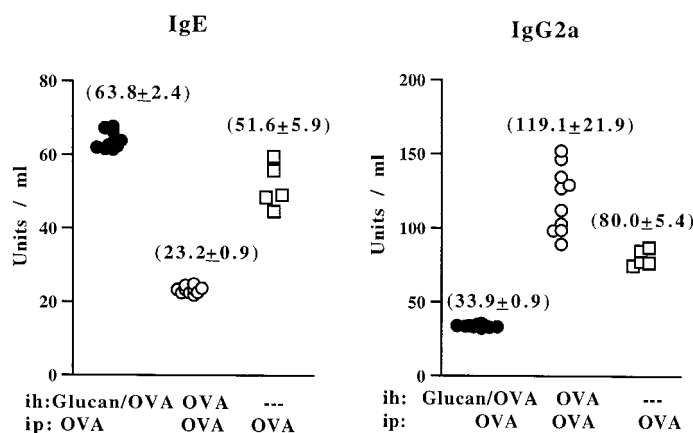


Figure 3. β -1,3-D-glucan can suppress airway antigen-induced IgE isotype-specific tolerance. Female BALB/cJ mice ($n = 10$) were exposed first to 400 ng/ml glucan for 40 min. After 18 h, the mice were sensitized with a daily 20-min exposure to 10 mg/ml OVA antigen for two 5-day periods with a 40-min glucan exposure in between during the first airway sensitization. Two weeks later, the mice were re-exposed to 10 mg/ml OVA for a 5-day period. Two weeks after the last antigen exposure, the mice were injected i.p. with 10 μ g OVA dissolved in 10 % aluminum potassium sulfate and the sera were obtained 7 days later. Age-matched control mice ($n = 5$) were injected i.p. with OVA without antigen pre-exposure. OVA-specific IgE and IgG2a antibodies were assayed by ELISA. Each symbol represents the data derived from an individual mouse serum. Data are representative of two independent experiments.

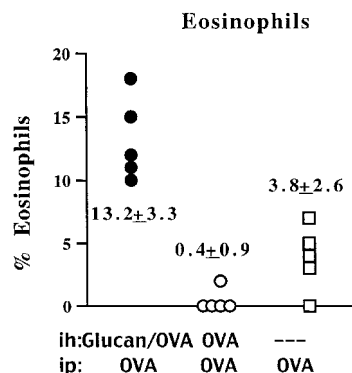


Figure 4. β -1,3-D-glucan potentiates the allergen-induced lung eosinophil infiltration in mice after repeated antigen inhalation. The mice were treated with the same protocol as described in Fig. 3. Two weeks after the last antigen exposure, the mice were challenged with aerosolized OVA (10 mg/ml) again and the lung lavage fluids were obtained 24 h later. Eosinophils were characterized using Liu's stain. Each symbol represents the data derived from the lung lavage fluid of an individual mouse. Data are representative of two independent experiments.

inflammatory cytokines, such as IL-10 and IL-12, which promote Th2 and Th1 responses, respectively, were investigated (Fig. 5). The lung-derived cells from mice after β -1,3-D-glucan inhalation were found to produce

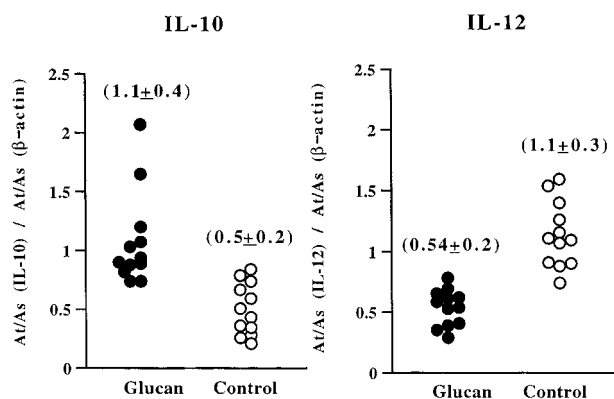


Figure 5. Airborne β -1,3-D-glucan regulates the synthesis of IL-10 and IL-12 mRNA by lung wall cells. Mice ($n = 12$) were exposed to 400 ng/ml β -1,3-D-glucan for 40 min, and the lung wall cells were harvested for mRNA preparation 18 h later. The cells of age-matched control mice were prepared using the same protocol. After competitive PCR, the products were semi-quantitated using a densitometer. The ratio of amplified target [experimental cDNA (At)] to amplified standard [PCR MIMICs (As)] PCR products was determined for each sample. The amount of cytokine PCR products is presented in dot plot form after correction by the β -actin PCR products.

higher ($p < 0.01$) amounts of IL-10 mRNA than those from control mice that had inhaled PBS. On the contrary, the production of IL-12 mRNA by lung-derived cells was lower ($p < 0.01$) in β -1,3-D-glucan-treated mice than in PBS-treated control mice.

3 Discussion

We have shown that cytokines induced by an inhaled microbial product may play an important role in determining airway antigen-specific T cell function [17]. Our present results further demonstrate that airborne β -1,3-D-glucan cannot only inhibit the down-regulation of IgE isotype-specific responses to repeated exposure of aerosolized antigen but also promote airway eosinophil infiltration in mice. Mice that had inhaled β -1,3-D-glucan and were subsequently repeatedly exposed to aerosolized OVA antigen mounted persistent antigen-specific IgE antibody responses and airway antigen-specific allergic reactions including eosinophil infiltration. In addition, we observed that spleen cells derived from the mice pre-exposed to β -1,3-D-glucan produced significantly ($p < 0.01$) higher amounts of IL-4 than those from mice sensitized with OVA only (unpublished observation).

Th cells are heterogenous with regard to the production of cytokines and their functions. Th1 cells produce IL-2 and IFN- γ [18, 19], and help the induction of IgG2a antibody synthesis in mice [20, 21]. In contrast, Th2 cells predominantly produce IL-4 and IL-5 [18, 19], and induce IgE and allergic responses [20–22]. The presence of IL-12 and IFN- γ has been shown to be important for the development of Th1 cells [3, 4, 6, 22], while the presence of IL-4 greatly favors the development of Th2 cells [5, 6, 23]. IL-12 can be produced during inflammation by macrophages in response to certain microbial agents, while IFN- γ is secreted mainly by NK cells in response to IL-12 [24, 25]. The initial factor for promoting Th2 responses is still not well documented, as none of the classical airway innate immune cells make IL-4.

From several epidemiological studies [12, 15, 26, 27], it has been suggested that organic dusts containing microbial cell wall components are potential risk factors for nonspecific airway inflammation. The airway function can be altered by microbial cell wall products reaching the lungs via inhalation [28]. In a recent study, we found that persons who had lived several years in moldy houses had a higher incidence of atopy [29]. Microbial products, such as *Listeria*-derived components and derived from bacteria, have been described to be able to induce production of IL-12 by monocytes/macrophages and promote Th1 responses [24, 25, 30]. In response to

certain microbial products, monocytes/macrophages can also secrete IL-6 and IL-10, which favor Th2 development [31, 32]. Thus, the balance between the secretion of IL-6/IL-10 and IL-12 by the monocytes/macrophages will be crucial in determining whether a Th2 or Th1 immune response will be dominant. By using a murine model, we directly demonstrated that inhaled β -1,3-D-glucan can enhance Th2 predominant allergic responses *in vivo*. The molecular mechanisms responsible remain to be investigated in detail. However, the effect may be explained by the stimulatory ability of β -1,3-D-glucan on the generation of potential Th2-promoting cytokines such as IL-6 [13] and IL-10 (Fig. 5) by airway macrophages. These results further indicated that the effect of IL-6 and IL-10 secretion during β -1,3-D-glucan-induced inflammation is prominent as compared with those of IL-12.

Repeated exposure of mice to aerosolized antigen reduced their ability to mount antigen-specific IgE responses without affecting IgG responses [7, 8]. $\gamma\delta$ T cells played a major role in mediating this type of inhalation-induced IgE isotype tolerance [8]. In our present study, the airborne β -1,3-D-glucan can somehow break this type of IgE down-regulation, and lead to the sustained production of antigen-specific IgE and the infiltration of eosinophils, which are important factors for generating early and late phases of asthmatic reactions, respectively. Whether the effect of the glucan is mediated by inhibiting the generation or the function of the airway regulatory $\gamma\delta$ T cells remains to be further investigated. In addition to the role in regulating inhalation-induced IgE tolerance, β -1,3-D-glucan can also directly enhance antigen-specific IgE production triggered by i.p. immunization (data not shown).

It has long been suggested that environmental factors, such as virus infection, are capable of overriding inhalation-induced IgE isotype tolerance and promoting airway allergic responses [26]. We demonstrated here that airborne mold-derived β -1,3-D-glucan, which exists frequently in our environments, particularly in highly humid areas, can abrogate inhalation-induced IgE isotype-specific down-regulation and promote airway eosinophil infiltration. Further investigation of the underlying mechanisms should provide important insights into the development of human airway allergic diseases and the role of airway inflammation induced by natural agents such as molds.

4 Materials and methods

4.1 Mice and antigens

Female BALB/cJ mice, aged between 6 and 8 weeks, were obtained from the animal center of our college. The glucan used in this study is a gel-forming (1,6)-branched (1,3)- β -D-glucan isolated from liquid-cultured mycelium of *Grifola frondosa* [33]. Only trace (0.00018 pg/ng) endotoxin contamination was found in our glucan preparation. The glucan was suspended and diluted in pyrogen-free water (400 ng/ml). Chicken OVA (5 $\times$ recrystallized, Sigma, St. Louis, MO) was dissolved in PBS (10 mg/ml).

4.2 β -1,3-D-glucan exposure

β -1,3-D-glucan-containing aerosols were generated by a DeVibiss 464 nebulizer (DeVibiss, Somerset, PA) as described previously [17]. In all inhalation experiments, 400 ng/ml β -1,3-D-glucan was used. This concentration can create a theoretical amount of 10 μ g/m³ in the exposure chamber which may be equivalent to that in some special working places like cotton or mill industry. The animals were exposed to β -1,3-D-glucan for 40 min.

4.3 Preparation of lung cells

The lungs were cut in slices and then suspended in plastic petri dishes containing 3 ml complete culture medium, *i.e.* RPMI 1640 supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine, 5 $\times$ 10⁻⁵ M 2-ME, 10 % heat-inactivated FBS (HyClone, Logan, UT), 0.2 mg/ml collagenase (Sigma) and 30 μ g/ml DNase (Sigma). Following 60 min incubation in a 37 °C shaking water bath, the RBC were lysed. The lung cells were harvested for cytokine mRNA determination by competitive reverse transcription PCR (C-RT-PCR).

4.4 Immunization

For airway sensitization, the mice were first exposed to PBS or 400 ng/ml β -1,3-D-glucan for 40 min and subsequently sensitized daily for 20 min with 10 mg/ml aerosolized OVA for 5 consecutive days. Fourteen days later, the mice were re-exposed once to 10 mg/ml aerosolized OVA for 20 min. The sera were obtained 1 week later and stored at -20 °C until analysis. For inhalation-induced tolerance studies, groups of naive or β -1,3-D-glucan-pre-exposed BALB/cJ mice were repeatedly treated by daily 20-min inhalation of 10 mg/ml OVA antigen for 10 days with 2 days without antigen-inhalation in between. The mice were rested for 2 weeks and treated with glucan for 40 min and subsequently with OVA daily for 5 consecutive days again. Three weeks after the last aerosol exposure, the animals were challenged by i.p. injection with 10 μ g OVA dissolved in 10 % aluminium potassium sulfate, and bled 7 days later.

4.5 Preparation of bronchoalveolar lavage cells

The mice were injected slowly with 1.5 ml complete culture medium into the bronchi. After each instillation the fluid was withdrawn and collected in tubes. The cells were centrifuged, collected, and typed using Liu's stain and counted.

4.6 C-RT-PCR assays

Mice were exposed to aerosolized β -1,3-D-glucan, and the amounts of IL-10 and IL-12 mRNA expressed by lung-derived cells were measured using C-RT-PCR as described previously [17]. In brief, total RNA was isolated by acid guanidine thiocyanate-phenol-chloroform extraction. RT was performed using Superscript R/T enzyme (Gibco-BRL, Grand Island, NY). The cDNA samples and the constant amount of cytokine specific competitors (Clontech, Palo Alto, CA) were amplified by PCR with β -actin and cytokine-specific primers for 35 cycles. β -actin: 5' primer, GTGGGC-CGCTCTAGGCACCAA and 3' primer, CTCTTTGATGTCA-CGCACGATTTC; IL-10: 5' primer, TCCTTAATGCAGGACTT-TAAGGGTTACTTG, and 3' primer, GACACCTTGGTCTT-GGAGCTTATTTAAATC, IL-12 p40: 5' primer, CGTGCTCAT-GGCTGGTGACCCG, and 3' primer, CTTTCATCTGCAAGTT-CTTGGGC. The PCR was performed in an automatic DNA thermal cycler (Perkin Elmer, Norwalk, CT) as follows: denaturation 94 °C, 40 s, annealing 55 °C, 60 s, extension 72 °C, 60 s. The amplification products were analyzed by gel electrophoresis, and semi-quantitated using a densitometer. The amount of cytokine PCR products was corrected by β -actin PCR products.

4.7 ELISA assay

The methods for detection of antigen-specific IgE and IgG2a have been previously reported [17]. In brief, for determining IgE levels, 96-well vinyl microtiter plates (Costar, Cambridge, MA) were coated with 50 μ g/ml OVA diluted in NaHCO₃ buffer, pH 8.2. After overnight incubation at 4 °C, plates were washed twice with 0.1 M Tris-Tween-20 (0.05 %, vol/vol) and blocked with Tris-BSA (1 %, wt/vol) buffer, pH 9.6, for 2 h at 37 °C. Serum samples were diluted 1:20 in 0.1 M Tris-BSA-1 % Tween-20 buffer, added to the plates and incubated overnight at 4 °C. Plates were then washed, and alkaline phosphatase-conjugated rat anti-mouse IgE mAb (PharMingen), diluted in Tris-BSA-1 % Tween buffer (1:1000), was added for an additional 2 h. The reaction was developed with an amplification system (Life Technologies, Gaithersburg, MD). Plates were read in a microplate reader (Meter-tech) at 490 nm. For IgG2a detection, the plates were freshly coated with OVA at a concentration of 10 μ g/ml in NaHCO₃ buffer, pH 8.2. The plates were developed using the alkaline phosphatase-conjugated rat anti-mouse IgG2a mAb system (PharMingen). The plates were read in a microplate reader (Metertech) at 405 nm. The purified mouse IgE (PharMingen) or IgG2a (PharMingen) antibodies were directly coated on the plates and used as internal standard.

The antibody titers of the sample were calculated by comparison with the absorbance values of internal standards. One ELISA unit is equivalent to the response of 1 ng/ml of purified isotype standard antibody.

4.8 Statistical analysis

The statistical significance of differences between groups was evaluated using Student's *t*-test and *p* < 0.05 was taken as the level of significance.

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5 References

- 1 **Field, E. H., Noelle, R. J., Rouse, T., Goeken, J. and Waldschmidt, T.**, Evidence for excessive Th2 CD4 subset activity *in vivo*. *J. Immunol.* 1993. **151**: 48–59.
- 2 **Holt, P. G.**, Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol. Today* 1994. **15**: 484–489.
- 3 **Gajewski, T. F., Joyce, J. and Fitch, F. W.**, Antiproliferative effect of IFN- γ in immune regulation. III. Differential selection of Th1 and Th2 murine helper T lymphocyte clone using rIL-2 and rIFN- γ . *J. Immunol.* 1989. **143**: 15–22.
- 4 **Manetti, R., Parronchi, P., Giudizi, M. G., Piccinini, M. P., Maggi, E., Trinchieri, G. and Romagnani, S.**, Natural killer cell stimulatory factor (IL-12) induces Th1-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* 1993. **177**: 1199–1204.
- 5 **Abelsira-Amar, O., Gilbert, M., Jolij, M., Theze, J. and Jankovic, D. L.**, IL-4 plays a dominant role in the different development of Th0 into Th1 and Th2 cells. *J. Immunol.* 1992. **148**: 3820–3829.
- 6 **Seder, R. A. and Paul, W. E.**, Acquisition of lymphokine-producing phenotype by CD4 T cells. *Annu. Rev. Immunol.* 1994. **12**: 635–673.
- 7 **Holt, P. G. and McMenamin, C.**, Defence against allergic sensitization in the healthy lung: the role of inhalation tolerance. *Clin. Exp. Allergy* 1989. **19**: 255–262.
- 8 **McMenamin, C., Pimm, C., McKersey, M. and Holt, P. G.**, Regulation of IgE responses to inhaled antigen in mice by antigen-specific $\gamma\delta$ T cells. *Science* 1994. **265**: 1869–1871.
- 9 **Li, C. S., Hsu, C. W. and Lin, R. H.**, House dust mite allergens (Der p1 and Der p5) within domestic environments of atopic and control children. *Arch. Environ. Health* 1997. **52**: 208–212.

- 10 Kilburn, K. H., Lynn, W. S., Tres, L. L. and McKenzie, M. N., Leukocyte recruitment through airway walls by condensed vegetable tannins and quercetin. *Lab. Invest.* 1973. **28**: 55–59.
- 11 Rylander, R. and Nordstrand, A., Pulmonary cell reactions after exposure to cotton dust extract. *Br. J. Ind. Med.* 1974. **31**: 220–223.
- 12 Peden, D. B., Mechanisms of pollution-induced airway disease: *in vivo* studies. *Allergy* 1997. **52** (suppl 38): 57–58.
- 13 Adachi, Y., Okazaki, M., Ohno, N. and Yadomae, T., Enhancement of cytokine production by macrophages stimulated with β -1,3-D-glucan, grifolan, isolated from *Grifola frondosa*. *Biol. Pharm. Bull.* 1994. **12**: 1554–1560.
- 14 DiLuzio, N. R., Update on the immunomodulating activities of glucans. *Springer Semin. Immunopathol.* 1985. **8**: 387–393.
- 15 Rylander, R., Persson, K., Goto, H., Yuasa, K. and Tanaka, S., Airborne β -1,3-D-glucan may be related to symptoms in sick buildings. *Indoor Environ.* 1992. **1**: 263–267.
- 16 Wan, G. H. and Li, C. S., Indoor endotoxin and glucan in association with airway inflammation and systemic symptoms. *Arch. Environ. Health* 1999. **50**: in press.
- 17 Lin, J. Y., Wang, L. F. and Lin, R. H., The association between lung innate immunity and differential airway antigen-specific immune responses. *Int. Immunol.* 1996. **8**: 499–507.
- 18 Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. and Coffman, R. L., Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986. **136**: 2348–2357.
- 19 Mosmann, T. R. and Coffman, R. L., Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 1989. **7**: 145–173.
- 20 Snapper, C. M. and Paul, W. E., IFN- γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* 1987. **236**: 944–947.
- 21 Stevens, T. L., Bossie, A., Sanders, V. M., Fernandez-Botran, R., Coffman, R. L., Mosmann, T. R. and Vitetta, E. S., Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature* 1988. **334**: 255–258.
- 22 Finkelmann, F. D., Holmes, J., Katona, I. M., Urban, J. F., Beckmann, M. P., Park, L. S., Schooley, K. A., Coffman, R. L., Mosmann, T. R. and Paul, W. E., Lymphokine control of *in vivo* Ig isotype selection. *Annu. Rev. Immunol.* 1990. **8**: 303–333.
- 23 Le Gros, G., Ben-Sasson, S. Z., Seder, R., Finkelman, F. D. and Paul, W. E., Generation of IL-4-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J. Exp. Med.* 1990. **172**: 921–929.
- 24 Hsieh, C. S., Macatonia, S. E., Tripp, C. S., Wolf, S. F., O'Garra, A. and Murphy, K. M., Development of Th1 CD4 T cells through IL-12 produced by Listeria-induced macrophages. *Science* 1993. **260**: 547–549.
- 25 D'Andrea, A., Rengarayu, M., Valgint, N. M., Chemini, J., Kibin, M., Aste, M., Chan, S. H., Kobayashi, M., Young, D. and Nichbarg, E., Production of natural killer cell stimulatory factor (IL-12) by peripheral blood mononuclear cells. *J. Exp. Med.* 1992. **176**: 1387–1398.
- 26 Holt, P. G., Vines, J. and Bilyk, N., Effect of influenza virus infection on allergic sensitization to inhaled antigen in mice. *Int. Arch. Allergy Appl. Immunol.* 1988. **86**: 121–123.
- 27 Michel, O., Ginanni, R., Duchateau, J., Vertongen, F., Bon, B. L. and Sergysels, R., Domestic endotoxin exposure and clinical severity of asthma. *Clin. Exp. Allergy* 1991. **21**: 441–448.
- 28 Henson, P. M., Riches, D. W. H., Worthen, G. S., Parsons, P. E. and Doherty, D. E., LPS and cytokine in the lung injury. In Brigham, K. L. (Ed.) *Endotoxin and the lungs*. Marcel Dekker, New York 1994. pp 267–285.
- 29 Thorn, J. and Rylander, R., Airway inflammation and glucan in a rowhouse area. *Am. J. Respir. Crit. Care Med.* 1998. **157**: 1798–1803.
- 30 Yoshimoto, T., Nagai, N., Ohkusu, K., Ueda, H., Okamura, H. and Nakanishi, K., LPS-stimulated SJL macrophages produce IL-12 and IL-18 that inhibit IgE production *in vitro* by induction of IFN- γ production from CD3intIL-2R β T cells. *J. Immunol.* 1998. **161**: 1483–1492.
- 31 D'Andrea, A., Aste-Amezaga, M., Valiante, N. M., Ma, X., Kubin, M. and Trinchieri, G., IL-10 inhibits human lymphocyte interferon γ -production by suppressing natural killer stimulatory factor/IL-12 synthesis in accessory cells. *J. Exp. Med.* 1993. **178**: 1041–1048.
- 32 Rincon, M., Anguita, J., Nakamura, T., Fikrig, E. and Flavell, R. A., IL-6 directs the differentiation of IL-4-producing CD4 T cells. *J. Exp. Med.* 1997. **185**: 461–469.
- 33 Nono, I., Ohno, N., Masuda, A., Oikawa, S. and Yadomae, T., Oxidative degradation of an antitumor (1-3)- β -D-glucan, grifolan. *J. Pharmacobiodyn.* 1991. **14**: 9–19.

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