

Journal of Endotoxin Research

<http://ini.sagepub.com/>

β -Glucans in standardized allergen extracts

Malcolm A. Finkelman, Steven J. Lempitski and Jay E. Slater

Journal of Endotoxin Research 2006 12: 241

DOI: 10.1177/09680519060120040501

The online version of this article can be found at:

<http://ini.sagepub.com/content/12/4/241>

Published by:



<http://www.sagepublications.com>

On behalf of:

[International Endotoxin & Innate Immunity Society](#)

Additional services and information for *Journal of Endotoxin Research* can be found at:

Email Alerts: <http://ini.sagepub.com/cgi/alerts>

Subscriptions: <http://ini.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

Citations: <http://ini.sagepub.com/content/12/4/241.refs.html>

>> [Version of Record](#) - Aug 1, 2006

[What is This?](#)

β -Glucans in standardized allergen extracts

Malcolm A. Finkelman¹, Steven J. Lempitski¹, Jay E. Slater²

¹Associates of Cape Cod, East Falmouth, Massachusetts, USA

²Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, Maryland, USA

Background: Allergen extracts contain variable quantities of bacterial endotoxin. Recent studies have suggested that (1 $\rightarrow$ 3)- β -D-glucans (β -glucans), also microbial cell wall components, may have adjuvant properties that could affect allergen immunotherapy.

Objective: To determine the quantities of β -glucans in standardized allergen extracts.

Materials and Methods: Ninety-four lots of 13 standardized allergen extracts were tested for β -glucan content by GlucateLL assay, and for endotoxin content by a specific, chromogenic formulation of the *Limulus* amoebocyte lysate test.

Results: Standardized allergen extracts contain variable quantities of endotoxins and β -glucans. As in our previous work, endotoxin activity was greatest in cat pelt and *Dermatophagoides farinae*, and least in the pollens. There was no correlation between endotoxin and β -glucan levels ($r = 0.1887$; $P = 0.07$). β -Glucan content was highest for grass pollen (median content, 10.6 ng/ml; range, 0.4–41.8 ng/ml), ragweed pollen (32.9 ng/ml; range, 6.5–41.2 ng/ml), and cat pelt (25.5 ng/ml; range, 16.7–41.1 ng/ml), and lowest for cat hair (4.9 ng/ml; range, 1.2–10.3 ng/ml), *D. farinae* (1.2 ng/ml; range, 0.4–5.2 ng/ml) and *Dermatophagoides pteronyssinus* (1.8 ng/ml; range, 0.4–6.7 ng/ml).

Conclusions: β -Glucans are present in standardized allergen extracts. The effects of these quantities of β -glucans on allergen immunotherapy and allergen skin testing require further study.

Keywords: β -Glucan, endotoxin, allergen immunotherapy, allergen extracts, allergen standardization

INTRODUCTION

Allergic sensitization is typically influenced by several factors other than the allergen(s) to which the individual is exposed. Host factors such as major histocompatibility phenotype are critical, as are incidental features like concurrent viral infection, age at exposure, and frequency of exposure. Experimental and epidemiological data have suggested that concurrent exposure to microbial adjuvants such as endotoxins and (1 $\rightarrow$ 3)- β -D-glucans (β -glucans) may modulate the immune response to allergens as well. The possible effects of endotoxin exposure on allergic responses have been reviewed exten-

sively.^{1,2} Like endotoxin, β -glucan is a common contaminant of the indoor environment and has been implicated as a modulator of allergic responses in murine^{3–5} and human studies.^{6–10} Like endotoxin, β -glucan has been associated with both Th1^{9,11–13} and Th2^{4,5,14} responses.

Allergen extracts are produced by a relatively unselective aqueous extraction of complex biological plant and animal source materials, and are used widely for the diagnosis and treatment of allergic disease. In 2003, we examined the presence of endotoxin in 14 standardized allergen extracts by *Limulus* amoebocyte lysate (LAL) assay, and found a broad range of endotoxin content that was of uncertain clinical significance.¹⁵ Among the extracts tested, cat pelt had significantly more endotoxin activity than cat hair, and *Dermatophagoides farinae* extracts had more activity than *Dermatophagoides pteronyssinus*. Grass and ragweed pollen extracts contained less endotoxin than all of the extracts other than *D. pteronyssinus*. β -Glucan content was estimated indirectly, and appeared to be significant (> 10% of LAL reactivity) in only three – ragweed pollen and two grass pollen extracts.

Received 2 June 2005

Revised 2 December 2005, 13 January 2006

Accepted 17 January 2006

Correspondence to: Jay E. Slater MD, CBER/FDA (HFM-422), 1401 Rockville Pike, Rockville, MD 20852, USA
Tel: +1 301 496 4575; Fax: +1 301 480 4103;
E-mail: jay.slater@fda.hhs.gov

The injection of graded doses of allergen extracts has been used successfully to modulate immune responses to allergens for nearly a century, but allergen immunotherapy is neither uniformly effective nor free of risk.¹⁶ Before the effects of potent immunomodulatory adjuvants such as endotoxin and β -glucan in allergen extracts can be understood, the presence of these compounds must be ascertained and quantified. In this study, we examined the β -glucan content of standardized allergen extracts using a direct and validated test, re-examined the endotoxin content by an endotoxin-specific LAL assay, and analyzed the relationship between these two immunomodulatory compounds.

MATERIALS AND METHODS

Allergen extracts

Ninety-four lots of 13 standardized allergen extracts representative of US licensed products were tested. The lots included extracts derived from 7 grass pollen species, *D. farinae*, *D. pteronyssinus*, short ragweed, ragweed mix, cat hair, and cat pelt. Thirty-seven lots were prepared in glycerin (50%, v/v) alone, one in aqueous solution with phenol (0.4%, w/v), and 52 in both glycerin and phenol. One extract was alum-precipitated in aqueous buffer containing 0.4% phenol, and three were acetone-precipitated in buffer containing 50% glycerin.

Bacterial endotoxin analysis

The analysis was performed using a bacterial endotoxin-specific, chromogenic formulation of LAL (ES-50M, Seikagaku Corporation, Tokyo, Japan). The analysis was performed according to the manufacturer's instructions, with a slight modification. Briefly, a standard curve was constructed using Control Standard Endotoxin (Associates of Cape Cod, Inc., East Falmouth, MA, USA), calibrated against USP endotoxin standard lot G1, using a log-log analysis of time of onset (number of seconds required to reach a pre-determined absorbance at 405 nm) versus endotoxin concentration. A standard curve was performed with each analysis and all correlation coefficients exceeded 0.999. Allergen samples were diluted 100-fold in LAL Reagent Water prior to measurement, 50 μ l each of sample and LAL were added to each well. All measurements were performed in duplicate. Samples with endotoxin values exceeding the upper limit of the standard curve were diluted to bring them within the standard curve limits. All incubations were at 37°C. Analysis was performed using SoftMax[®] Pro software (Molecular Devices Corporation, Sunnyvale, CA, USA).

(1 $\rightarrow$ 3)- β -D-glucan analysis

β -Glucan content was measured using GlucateLL[®], a β -glucan-specific modification of the LAL, according to manufacturer's instructions (Associates of Cape Cod, Inc.) using the time of onset mode described above. In this assay, 25 μ l of sample plus 100 μ l of GlucateLL reagent were added to each well and the reactions were monitored kinetically. Each sample was run in duplicate. A linear standard curve was constructed using a β -glucan standard (Pachyman, Associates of Cape Cod, Inc.). Samples with β -glucan values exceeding the upper limit of the standard curve were diluted (1:100 or 1:200) with glucan-free water (supplied with the kits) to bring them within the standard curve limits.

In order to determine whether allergen extracts interfere with the measurement of β -glucans, spike recovery was evaluated with 20% of the samples and found to be within the acceptable range for an LAL-based reagent (50–200%). Similarly, no inhibitory effects of glycerin and phenol were noted (data not shown). Prior evaluations of the GlucateLL[®] assay confirm that it is non-reactive with other carbohydrates and with endotoxin.^{17–19}

Statistical analyses

The statistical significance of the differences between the corrected endotoxin or β -glucan activities of the different classes of products was determined using the Kruskal-Wallis rank-sum statistic. This non-parametric method was used because the activity data are not normally distributed. Dunn's test was used to assess the differences in rank-sums between each pair of extract type (for six extract types, there are $n(n-1)/2 = 15$ pairs). The correlation between the β -glucan and endotoxin data was assessed using the Spearman rank-order correlation. Analyses were performed using the Prism software program (v. 3.03, GraphPad Software, Inc.). For graphical presentation, data were log transformed and presented with 95% confidence intervals of the log values.

RESULTS

As we observed in 2003,¹⁵ the mean endotoxin content of the 94 extracts tested in this study varied greatly, from undetectable to over 10,000 EU/ml (Fig. 1). As in the previous study, grass pollen, ragweed pollen and *D. pteronyssinus* allergen extracts had the lowest endotoxin contents (median content, 4 EU/ml, range, 0–453 EU/ml; median content, 27 EU/ml, range, 6–284 EU/ml; and median content, 3 EU/ml, range, 0–1556 EU/ml, respectively). Cat pelt, cat hair, and *D. farinae* allergen extracts had the highest endotoxin contents (median content, 150 EU/ml, range, 66–4171 EU/ml; median content, 128 EU/ml, range,

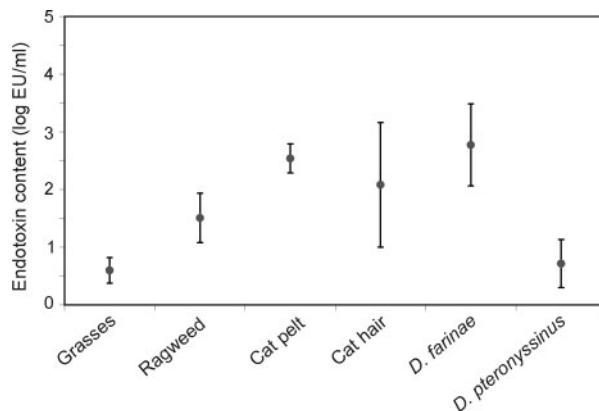


Fig. 1. Endotoxin content of standardized allergenic extracts, mean (log value) $\pm$ 95% confidence interval. Number of independent samples evaluated: grasses, 41; ragweed, 8; cat pelt, 3; cat hair, 8; *D. farinae*, 9; *D. pteronyssinus*, 25. Results were significantly different by Kruskal-Wallis test ($P < 0.0001$). Paired comparisons using the Dunn's test were significantly different for the following four pairs: grasses or *D. pteronyssinus* compared to *D. farinae* (each at $P < 0.001$); cat hair to *D. pteronyssinus* or grasses ($P < 0.01$). The other eleven paired comparisons were not significantly different.

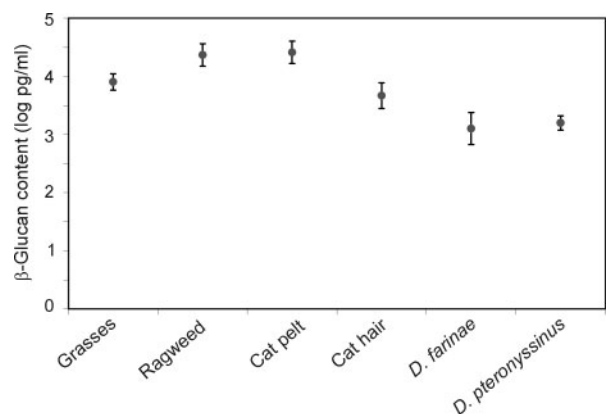


Fig. 2. β -Glucan content of standardized allergenic extracts, mean (log value) $\pm$ 95% confidence interval. Number of independent samples evaluated were as indicated in Figure 1. Results were significantly different by Kruskal-Wallis test ($P < 0.0001$). Paired comparisons using Dunn's test were significantly different for the following six pairs: ragweed compared to *D. farinae* or *D. pteronyssinus*; grasses to *D. pteronyssinus* (all at $P < 0.001$); *D. farinae* to grasses; and cat pelt to *D. farinae* or *D. pteronyssinus* ($P < 0.01$). The other nine paired comparisons were not significantly different.

45–559 EU/ml; and median content, 233 EU/ml, range, 23–10 304 EU/ml, respectively). Once again, the difference between the endotoxin content of the two mite extracts was notable and statistically significant ($P < 0.001$).

β -Glucan content was also variable but with a different pattern (Fig. 2). Grass pollen, ragweed pollen, and cat pelt allergen extracts had the highest β -glucan content (median content, 10.6 ng/ml, range, 0.4–41.8 ng/ml; median content, 32.9 ng/ml, range, 6.5–41.2 ng/ml; median content, 25.5 ng/ml, range, 16.7–41.1 ng/ml, respectively), while cat hair, *D. farinae* and *D. pteronyssinus* allergen extracts had the least (median content, 4.9 ng/ml, range, 1.2–10.3 ng/ml; median content, 1.2 ng/ml; range, 0.4–5.2 ng/ml; median content, 1.8 ng/ml, range, 0.4–6.7 ng/ml, respectively).

There was no correlation between the endotoxin and β -glucan values (Spearman $r = 0.1887$; $P = 0.07$).

DISCUSSION

Endotoxins are normal components of Gram-negative bacterial cell walls, and their intense pro-inflammatory effects are part of the complex interplay between the outer envelope of highly dangerous pathogens and an innate immune system designed to mitigate the danger as quickly as possible. The dramatic clinical consequences of parenteral endotoxin exposure – chills, fever, hypotension, organ failure, death^{20,21} – simulate the symptoms of Gram-negative sepsis, and most parenteral drugs and biologicals are required to undergo rigorous testing to assure that they contain no more than a specified quantity of endotoxin, based on dosing instructions.²² For practical

reasons, certain biologicals, among them allergen extracts, are not required to undergo evaluation for the presence of endotoxin (21CFR 610.13(b)). Our report in 2003¹⁵ that some standardized allergen extracts contained endotoxin was not surprising; this had been noted previously in non-standardized products.^{23,24} However, our observation that *D. farinae* allergen extracts contained significantly greater quantities of endotoxin than *D. pteronyssinus* extracts was novel. This observation has now been confirmed in the current study, using different extracts and a more specific technique. The biological basis for this observation may be the presence of an endosymbiotic Gram-negative organism in *D. farinae*.²⁵

The amounts of β -glucan present in the pollen extracts (10.6 ng/ml and 32.9 ng/ml) suggest that only a small portion of β -glucans are extracted intact from the pollen grain surface. In one study,⁸ pollens were found to contain between 79–1800 ng β -glucan/10⁶ grains. Based on an estimated dry pollen mass of 10⁻¹–10⁻² g/10⁶ grains (R. Esch, personal communication), a typical 1:20 (w/v) extraction of ragweed pollen would contain 0.5 g/ml, or 5 $\times$ 10⁶ to 5 $\times$ 10⁷ pollen grains/ml. Thus, the calculated β -glucan content available for extraction would be approximately 5 $\times$ 10²–10⁵ ng/ml. Our data further indicate that the endotoxin and β -glucan levels in allergen extracts are independent. Since they derive from different sources – endotoxins from the cell walls of Gram-negative bacteria, β -glucans from the cell walls of plants and some fungi – their independence is to be expected. Likewise, the presence of elevated levels of β -glucan in the pollen extracts would be anticipated as well, and is consistent with our previous findings.¹⁵ However, the elevated levels

in the cat pelt extracts were unexpected. It is possible that manufacturer's process equipment, such as cellulosic depth filters, may contribute to the presence of β -glucan in these products.²⁶

In our previous work,¹⁵ we measured endotoxin content by the standard gel-clot LAL, and inferred the β -glucan content by depletion analysis. The methods which we applied to the current study are more specific and direct: in the β -glucan assay, only the factor G pathway is active in the LAL preparation to minimize interference from endotoxin. The endotoxin assay is likewise highly specific. Thus, the improved techniques used in this study confirm and expand our previous analyses.

As with our previous findings of elevated levels of endotoxin in some allergenic extracts, the clinical implications of these data are uncertain. Oral administration of substances rich in β -glucan appears to be devoid of adverse consequence, and may be beneficial.^{27,28} Inhaled β -glucan has been reported to be associated with wheezing,^{7,10} and experimental inhalation of β -glucans induces peripheral lymphocytosis²⁹ and eosinopenia.³⁰ The presence of elevated levels of β -glucans in broncho-alveolar lavage fluid from patients with acute eosinophilic pneumonia suggests that the inhalation of molds or their β -glucan components may be pathogenic.³¹ In contrast, experimental nasal application of β -glucans appears to have no inflammatory effects.³²

Parenteral administration of β -glucans may be a relatively common event: blood products processed through cellulose depth filters appear to contain elevated levels of (1 $\rightarrow$ 3)- β -D-glucans.^{26,33,34} As serum levels of β -glucan have been used as a diagnostic test for certain systemic mycoses, this contamination appears to have been associated with false-positive diagnostic testing using this technique. Pure cellulose consists exclusively of (1 $\rightarrow$ 4)- β -D-glucan linkages, which are not detected by the GlucateLL[®] assay,¹⁹ so the appearance of (1 $\rightarrow$ 3)- β -D-glucan in these products is presumably due to residual (1 $\rightarrow$ 3)- β -D-glucan from the plant material used to produce these filtration products. It is not clear that these doses of β -glucan cause any physiological effects. However, highly variable doses of (1 $\rightarrow$ 3)- β -D-glucan, administered by intravenous, intraperitoneal, and oral routes, can up-regulate cell-mediated cytotoxicity, particularly against tumors.^{28,35,36}

Nonetheless, the effect of β -glucans on the efficacy of allergen immunotherapy should be considered in future studies. The use of allergenic extracts as treatment for allergy is based upon a subtle, but durable, effect of gradually increased parenteral doses of allergen on T-cell responses to inhaled allergens. Clinically successful allergen immunotherapy is associated with the induction of circulating IL10⁺CD4⁺CD25⁺ T cells, an increase in mucosal interferon- γ -secreting T-cells, a decrease in mucosal IL-5-secreting T-cells, increased allergen-specific IgG, and a

decreased seasonal rise in allergen-specific IgE.^{37–40} When performed with well-characterized allergens, the clinical effects of allergen immunotherapy on diseases such as allergic rhinitis, allergic asthma, and hymenoptera-induced anaphylaxis are dramatic and sometimes curative.^{41–45} Among the standardized allergen extracts examined in this study, the pollen extracts – which contain the greatest amounts of β -glucan – are associated with highly successful immunotherapy treatment regimens. A typical immunotherapy dose (1.0 ml of a mix consisting of timothy (10,000 BAU/ml), short ragweed (1:100), mites (2500 AU/ml), and cat pelt (5000 BAU/ml); see Nelson⁴⁶) might contain as much as 30 ng of β -glucan. This amount is comparable to the amount delivered by only 7 ml of one of the albumin solutions described by Usami *et al.*³⁴ While this dose is unlikely to produce adverse events, its effect on the efficacy of immunotherapy is uncertain and needs to be addressed. In addition, allergen extracts are used increasingly in mucosal challenge studies to determine the efficacy of interventions and establish the mechanisms of airway pathology. Under such circumstances – at a minimum – investigators should be aware of the β -glucan content of their reagents, consider possible confounding effects, and incorporate adequate experimental controls in their study designs.

ACKNOWLEDGEMENTS

The views expressed in this article are the personal opinions of the authors and are not the official opinion of the U.S. Food and Drug Administration or the Department of Health and Human Services.

REFERENCES

1. Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. *J Allergy Clin Immunol* 2002; **109**: 379–392.
2. Michel O. Role of lipopolysaccharide (LPS) in asthma and other pulmonary conditions. *J Endotoxin Res* 2003; **9**: 293–300.
3. Instanes C, Ormstad H, Rydjord B, Wiker HG, Hetland G. Mould extracts increase the allergic response to ovalbumin in mice. *Clin Exp Allergy* 2004; **34**: 1634–1641.
4. Ormstad H, Groeng EC, Lovik M, Hetland G. The fungal cell wall component beta-1,3-glucan has an adjuvant effect on the allergic response to ovalbumin in mice. *J Toxicol Environ Health A* 2000; **61**: 55–67.
5. Wan GH, Li CS, Guo SP, Rylander R, Lin RH. An airborne mold-derived product, beta-1,3-D-glucan, potentiates airway allergic responses. *Eur J Immunol* 1999; **29**: 2491–2497.
6. Gehring U, Douwes J, Doekes G *et al.* Beta-(1 $\rightarrow$ 3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. *Environ Health Perspect* 2001; **109**: 139–144.
7. Douwes J, Zuidhof A, Doekes G *et al.* (1 $\rightarrow$ 3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. *Am J Respir Crit Care Med* 2000; **162**: 1348–1354.
8. Rylander R, Fogelmark B, McWilliam A, Currie A. (1 $\rightarrow$ 3)-beta-D-glucan may contribute to pollen sensitivity. *Clin Exp Immunol* 1999; **115**: 383–384.

9. Rylander R, Holt PG. (1 $\rightarrow$ 3)-beta-D-glucan and endotoxin modulate immune response to inhaled allergen. *Mediators Inflamm* 1998; **7**: 105–110.
10. Thorn J, Rylander R. Airways inflammation and glucan in a rowhouse area. *Am J Respir Crit Care Med* 1998; **157**: 1798–1803.
11. Murata Y, Shimamura T, Tagami T, Takatsuki F, Hamuro J. The skewing to Th1 induced by lentinan is directed through the distinctive cytokine production by macrophages with elevated intracellular glutathione content. *Int Immunopharmacol* 2002; **2**: 673–689.
12. Inoue A, Kodama N, Nanba H. Effect of maitake (*Grifola frondosa*) D-fraction on the control of the T lymph node Th-1/Th-2 proportion. *Biol Pharm Bull* 2002; **25**: 536–540.
13. Suzuki Y, Adachi Y, Ohno N, Yadomae T. Th1/Th2-Balancing immunomodulating activity of gel-forming (1 $\rightarrow$ 3)-beta-glucans from fungi. *Biol Pharm Bull* 2001; **24**: 811–819.
14. Fogelmark B, Thorn J, Rylander R. Inhalation of (1 $\rightarrow$ 3)-beta-D-glucan causes airway eosinophilia. *Mediators Inflamm* 2001; **10**: 13–19.
15. Trivedi B, Valerio C, Slater JE. Endotoxin content of standardized allergen vaccines. *J Allergy Clin Immunol* 2003; **111**: 777–783.
16. Bernstein DI, Wanner M, Borish L, Liss GM. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990–2001. *J Allergy Clin Immunol* 2004; **113**: 1129–1136.
17. Takaki Y, Seki N, Kawabata SS, Iwanaga S, Muta T. Duplicated binding sites for (1 $\rightarrow$ 3)-beta-D-glucan in the horseshoe crab coagulation factor G: implications for a molecular basis of the pattern recognition in innate immunity. *J Biol Chem* 2002; **277**: 14281–14287.
18. Odabasi Z, Mattiuzzi G, Estey E *et al.* Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004; **39**: 199–205.
19. Tanaka S, Aketagawa J, Takahashi S, Shibata Y, Tsumuraya Y, Hashimoto Y. Activation of a *Limulus* coagulation factor G by (1 $\rightarrow$ 3)-beta-D-glucans. *Carbohydr Res* 1991; **218**: 167–174.
20. Wolff SM. Biological effects of bacterial endotoxins in man. *J Infect Dis* 1973; **128**: 259–264.
21. Miller MA, Hjelle JT. Endotoxins and their clinical significance. In: Prior RB. (ed) *Clinical applications of the Limulus amoebocyte lysate test*. Boston, MA: CRC Press, 1990; 1–14.
22. Guideline on the validation of the *Limulus* Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices. Rockville, MD: FDA, 1987 <www.fda.gov/Cber/gdlns/lal.pdf>.
23. Siraganian RP, Baer H, Hochstein HD, May JC. Allergenic and biologic activity of commercial preparations of house dust extract. *J Allergy Clin Immunol* 1979; **64**: 526–533.
24. Smith TF, Morrison DC. Lack of interference in skin tests by endotoxin in allergen extracts. *Ann Allergy* 1986; **57**: 410–412.
25. Valerio C, Arlian LG, Slater JE. Bacterial 16S ribosomal DNA sequences isolated from house dust mites. *J Allergy Clin Immunol* 2005; **115**: S163.
26. Nagasawa K, Yano T, Kitabayashi G *et al.* Experimental proof of contamination of blood components by (1 $\rightarrow$ 3)-beta-D-glucan caused by filtration with cellulose filters in the manufacturing process. *J Artif Organs* 2003; **6**: 49–54.
27. Bell S, Goldman VM, Bistran BR, Arnold AH, Ostroff G, Forse RA. Effect of beta-glucan from oats and yeast on serum lipids. *Crit Rev Food Sci Nutr* 1999; **39**: 189–202.
28. Hong F, Yan J, Baran JT *et al.* Mechanism by which orally administered beta-1,3-glucans enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. *J Immunol* 2004; **173**: 797–806.
29. Beijer L, Thorn J, Rylander R. Effects after inhalation of (1 $\rightarrow$ 3)-beta-D-glucan and relation to mould exposure in the home. *Mediators Inflamm* 2002; **11**: 1–53.
30. Thorn J, Beijer L, Rylander R. Effects after inhalation of (1 $\rightarrow$ 3)-beta-D-glucan in healthy humans. *Mediators Inflamm* 2001; **10**: 173–178.
31. Kawayama T, Fujiki R, Honda J, Rikimaru T, Aizawa H. High concentration of (1 $\rightarrow$ 3)-beta-D-glucan in BAL fluid in patients with acute eosinophilic pneumonia. *Chest* 2003; **123**: 1302–1307.
32. Beijer L, Rylander R. (1 $\rightarrow$ 3)-beta-D-Glucan does not induce acute inflammation after nasal deposition. *Mediators Inflamm* 2005; **2005** (1): 50–52.
33. Ohata A, Usami M, Horiuchi T, Nagasawa K, Kinoshita K. Release of (1 $\rightarrow$ 3)-beta-D-glucan from depth-type membrane filters and their in vitro effects on proinflammatory cytokine production. *Artif Organs* 2003; **27**: 728–735.
34. Usami M, Ohata A, Horiuchi T, Nagasawa K, Wakabayashi T, Tanaka S. Positive (1 $\rightarrow$ 3)-beta-D-glucan in blood components and release of (1 $\rightarrow$ 3)-beta-D-glucan from depth-type membrane filters for blood processing. *Transfusion* 2002; **42**: 1189–1195.
35. Yan J, Vetvicka V, Xia Y *et al.* Beta-glucan, a ‘specific’ biologic response modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). *J Immunol* 1999; **163**: 3045–3052.
36. Hong F, Hansen RD, Yan J *et al.* Beta-glucan functions as an adjuvant for monoclonal antibody immunotherapy by recruiting tumoricidal granulocytes as killer cells. *Cancer Res* 2003; **63**: 9023–9031.
37. Creticos PS, Van Metre TE, Mardiney MR, Rosenberg GL, Norman PS, Adkinson Jr NF. Dose response of IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 1984; **73**: 94–104.
38. Francis JN, Till SJ, Durham SR. Induction of IL-10⁺CD4⁺CD25⁺ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; **111**: 1255–1261.
39. Wachholz PA, Nouri-Aria KT, Wilson DR *et al.* Grass pollen immunotherapy for hayfever is associated with increases in local nasal but not peripheral Th1: Th2 cytokine ratios. *Immunology* 2002; **105**: 56–62.
40. Wilson DR, Nouri-Aria KT, Walker SM *et al.* Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J Allergy Clin Immunol* 2001; **107**: 971–976.
41. Lowell FC, Franklin W. A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Engl J Med* 1965; **273**: 675–679.
42. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978; **299**: 157–161.
43. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995; **151**: 969–974.
44. Durham SR, Walker SM, Varga EM *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**: 468–475.
45. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; **102**: 558–562.
46. Nelson HS. Preparing and mixing allergen vaccines. In: Lockey RF, Bukantz SC. (eds) *Allergens and Allergen Immunotherapy*. New York: Marcel Dekker, 1999; 401–422.