

IgG1 and IgG4 antibody responses to the dust mite *Lepidoglyphus destructor* in a naturally exposed farming population

Johansson E, Härfast B, Johansson SGO, van Hage-Hamsten M. IgG1 and IgG4 antibody responses to the dust mite *Lepidoglyphus destructor* in a naturally exposed farming population.

Allergy 1995; 50: 473–477. © Munksgaard 1995.

The natural humoral immune response to the dust mite *Lepidoglyphus destructor* was assessed by comparing the IgG1 and IgG4 responses elicited in allergic ($n = 44$) and healthy ($n = 16$) individuals in a farming population chronically exposed to this allergen. With the aid of an immunoblotting technique and ELISA, the sera were analyzed for anti-*L. destructor* antibodies. While the majority of sera from the allergy group displayed several bands for both IgG1 and IgG4, the nonatopic healthy group was negative as analyzed in immunoblotting. When they were analyzed in ELISA, there was a significantly higher response in the allergy group than in the healthy group for IgG4, but not for IgG1. Taken together, these results imply that the immune system of individuals spared from allergic reactions to *L. destructor* not only lacks IgE antibodies but also seems largely to “ignore” these allergens/antigens despite exposure.

E. Johansson, B. Härfast,
S. G. O. Johansson, M. van
Hage-Hamsten

Department of Clinical Immunology, Division of
Laboratory Medicine, Karolinska Hospital,
Stockholm, Sweden

Key words: atopic patients; IgG1; IgG4;
immunoblotting; *Lepidoglyphus destructor*;
mites.

Eva Johansson, Dr. Med. Sc.
Department of Clinical Immunology
Karolinska Hospital
S-171 76 Stockholm
Sweden

Accepted for publication 2 December 1994

The atopic state is characterized by a specific IgE antibody response to common allergens. Allergic asthma and allergic rhinitis are immediate hypersensitivity reactions caused by mediators released from mast cells after interaction of IgE with the allergen. Studies on the natural specific antibody response to allergens from other immunoglobulin classes and subclasses are sparse in atopics and almost entirely lacking in nonatopics. However, in the 1970s, Chapman & Platts-Mills demonstrated that atopics had increased levels of antigen-specific IgG and IgA (2, 15).

Cytokines, produced largely by specific T cells, determine immunoglobulin class and subclass production. *In vitro* experiments show that interleukin-4 (IL-4) causes a large fraction of B cells to switch to either IgE or IgG4 (4, 9). Furthermore, repeated antigen stimulation has been reported to be required for IL-4 production. In certain individuals, chronic stimulation by antigens/allergens has been postulated to select for high-IL-4-producing T cells that in turn could explain the switch to an IgE response.

We have previously extensively studied the IgE response to the dust mite *Lepidoglyphus destructor* (18) in a farming population heavily exposed to this

species in their working environment (19). Approximately 6% of 2578 farmers studied on the island of Gotland (Sweden) have IgE to *L. destructor* allergens, and atopy was calculated to be present in 18.6%. In the present study, we specifically compared the IgG4 and IgG1 subclass responses, believing that analyses of the natural immunoresponses from individuals chronically exposed to inhaled allergens would give information on differences in response between atopics and nonatopics.

Material and methods

Mite extract

L. destructor extracts were prepared from whole mite cultures obtained from Allergon, Ängelholm, Sweden, as previously described (6). Briefly, extracts were prepared in 0.15 M NaCl as 1/10 (w/v) for 18 h at room temperature. The extracts were then centrifuged at 6000 g for 10 min, and the supernatants were filtered through an 0.8- μ m Millex-HA filter (Millipore, Bedford, MA, USA). For use in immunoblotting, the extract supernatants were desalted on prepacked Sephadex G-25 columns, PD 10 (Pharmacia, Uppsala, Sweden) and lyophilized.

Sera

Serum samples from 60 farmers included in a previous epidemiologic survey (18) of respiratory allergy were used. They were divided into three groups according to symptoms. First, sera from 16 asymptomatic farmers who were negative in RAST to *L. destructor* made up the healthy group. The second group comprised sera from 25 farmers with asthma and rhinoconjunctivitis and a positive RAST to *L. destructor* (the asthma group). The third group included 19 sera from farmers with rhinoconjunctivitis only, and IgE antibodies to *L. destructor* (the rhinitis group).

SDS-PAGE and immunoblotting

SDS-PAGE was performed in a Mini-PROTEAN[®] II Dual Slab Cell (Bio-Rad, Richmond, CA, USA) according to the operating instructions. The separation slab gel was cast as a gradient (7.5–20%), and on top the stacking gel was molded (6%). The mite extract was resuspended in a nonreducing sample buffer, and 800 µg of protein was loaded into a preparative well (74 × 1.0 mm). Low-molecular-weight standard proteins (LMW, Electrophoresis Calibration Kit, Pharmacia) were loaded into a reference well (3 × 1.0 mm). After electrophoresis, the proteins were transferred onto nitrocellulose (Trans-blot[®] NC membrane; pore size 0.45 µm; Bio-Rad) in a Mini Trans-Blot[®] Electrophoretic Transfer Cell (Bio-Rad) according to the manufacturer's instructions. The membrane with standard proteins was stained with AuroDye forte[™] (Janssen Biotech NV, Olen, Belgium). The membrane with mite proteins was blocked for 60 min at 37°C with 0.5% Tween 20 in 0.05 M phosphate buffer, pH 7.4, also containing 0.15 M NaCl, 0.2% bovine serum albumin, and 0.02% NaN₃. After blocking, the membrane was cut into narrow strips and incubated for 16–18 h with 500 µl of serum diluted 1/4 in blocking buffer. Next, after washing, 500 µl of either mouse anti-human IgG1 (clone-6001 Sigma, St Louis, MO, USA), diluted 1/1000 in blocking buffer, or anti-human IgG4 (clone-6023 Sigma) (1/4000) was added, and the strips were incubated for 2 h. The next incubation was with 500 µl of alkaline-phosphatase-conjugated rabbit antimouse IgG (Dakopatts, Glostrup, Denmark) diluted 1/4000 in blocking buffer for 2 h, followed by 1 ml of enzyme substrate (Alkaline Phosphatase Conjugate Substrate Kit, Bio-Rad) for 30 min. The molecular weight of each mite component was determined by comparing its relative mobility with that of LMW standard proteins.

ELISA

In brief, microtiter plate wells were coated with 200 µl of a solution of *L. destructor* extract (1 µg/ml) in 0.05 M carbonate buffer, pH 9.6, overnight at room temperature. Phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin and 0.1% Tween 20 was used as a diluent. Next, 100 µl of serum diluted 1/50 (or 1/10 for IgG4 analysis) was added and allowed to incubate overnight. In the following step, either mouse antihuman IgG1 (1/1000) or antihuman IgG4 (1/1000) (Sigma) was added and incubated overnight. Thereafter, ALP-conjugated rabbit antimouse IgG (Dakopatts) diluted 1/1000 was added and incubated for 2 h. Finally, the substrate *p*-nitrophenyl phosphatase disodium (Sigma) was diluted in 1 M diethanolamine buffer, pH 9.8, and added. The absorbance at 405 nm was measured with an ELISA plate reader after 45-min incubation for IgG1 and after 2 h for the IgG4 determinations. The test values were corrected for the absorbance obtained when sera were omitted. The cutoff limit was set at 0.12 and 0.26 for the IgG4 and IgG1 assays, respectively. These absorbance values correspond to twice the mean values obtained with six sera from nonatopic individuals living in the Stockholm area.

Inhibition ELISA

For assessment of the specificity of the assays, sera were either preincubated with various concentrations (0.01–10 µg/ml) of *L. destructor* extract or with its culture medium for 1 h. These assays were subsequently completed as above.

Results

Immunoblotting

Table 1 gives the percentage of sera from the two symptom groups, rhinitis and asthma, recognizing the major 15-kDa allergen of *L. destructor* by IgG1 and IgG4. The IgG1 responses for the two groups were 42% and 52%, respectively. The corresponding values for IgG4 were 95% and 65%. None of

Table 1. Binding of IgG1 and IgG4 to *L. destructor* components in immunoblotting by sera from farmers with rhinitis or asthma

Sera	Percentage of sera binding to major allergen of 15 kDa	
	IgG1	IgG4
Rhinitis ¹ (n=19)	42	95
Asthma ¹ (n=25)	52	65
Healthy ² (n=16)	0	0

¹ Sera from farmers found to be RAST positive to *L. destructor*.

² Sera from farmers found to be RAST negative to *L. destructor*.

Table 2. Number of components detected in immunoblotting by sera from farmers with rhinitis or asthma

		Percentage of sera binding to different numbers of components of <i>L. destructor</i>								
		No. of components								
		0	1	2	3	4	5	6	7	8
Rhinitis										
IgG1		21	42	16	11	11	0	0	0	0
IgG4		5	47	32	0	5	5	0	5	0
Asthma										
IgG1		20	20	48	0	4	0	4	4	4
IgG4		28	32	24	12	4	4	0	0	0

these differences were significant as evaluated with Fisher's test. All 16 sera from the healthy group were negative for IgG1 as well as for IgG4 against the 15-kDa component. As for the two symptom groups, the frequency of sera reacting with only an IgG4 response was higher in the rhinitis group than in the asthma group: 58% vs 24% of sera; $P < 0.05$; Fisher's test. While 1/4 of the sera from the asthma group lacked both IgG1 and IgG4 responses to 15 kDa, none of the sera from the rhinitis group did so: $P < 0.05$; Fisher's test. The majority of sera detected either one or two components (Table 2) for both IgG1 and IgG4 (range 0–8 for IgG1 and 0–7 for IgG4). Furthermore, in the asthma group about 1/4 of the sera lacked a detectable IgG4 response, while the corresponding value was only 5% in the rhinitis group (1/19 sera), which was not significant in Fisher's test.

ELISA

The anti-*L. destructor* IgG1 (Fig. 1A) and IgG4 (Fig. 1B) antibody levels in the two symptom groups

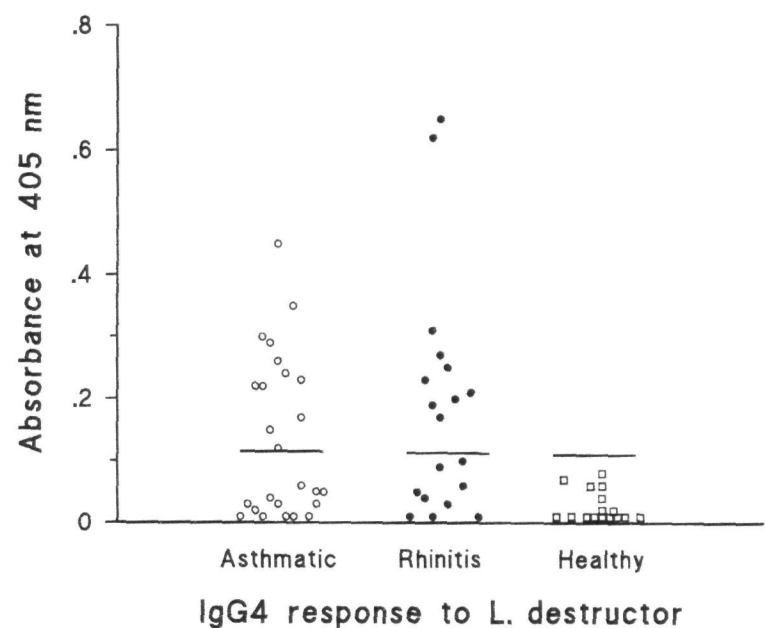


Fig. 1B. Detection in ELISA of anti-*L. destructor* IgG4 response from sera of asthmatic farmers (open rings), farmers with rhinitis (filled rings), and healthy farmers (squares). Cutoff limit (—).

and in the healthy group were analyzed with ELISA. The IgG4 response of the allergy group (the two symptom groups) was significantly higher than that of the healthy group ($P < 0.05$; Mann-Whitney test). In contrast, the IgG1 response did not differ significantly between the allergy group and the healthy group.

Inhibition ELISA

To ascertain that the binding of IgG1 and IgG4 was to mite extract and not to the culture medium, we preincubated a serum with either of these preparations (Figs. 2A and B). The culture medium failed to inhibit the binding of IgG1 or of IgG4. In contrast, *L. destructor* extract completely inhibited the ELISA

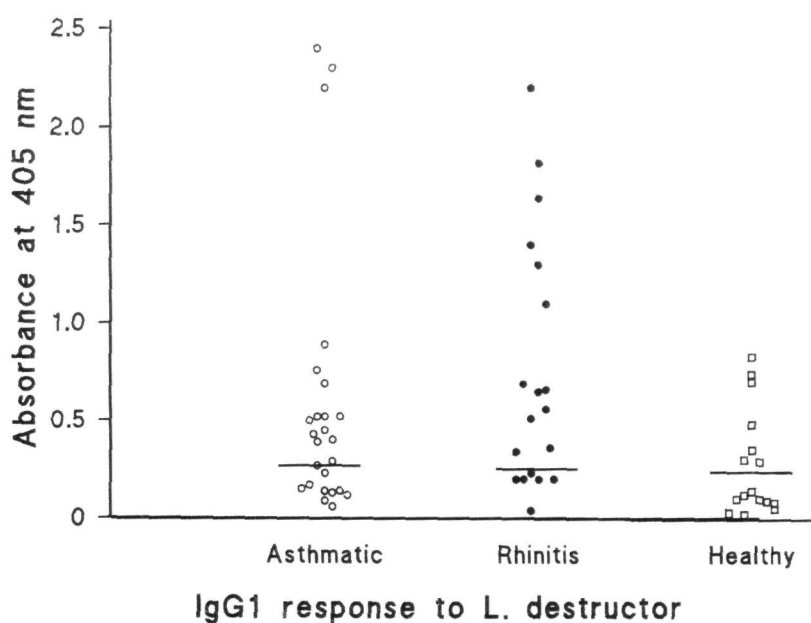


Fig. 1A. Detection in ELISA of anti-*L. destructor* IgG1 response from sera of asthmatic farmers (open rings), farmers with rhinitis (filled rings), and healthy farmers (squares). Cutoff limit (—).

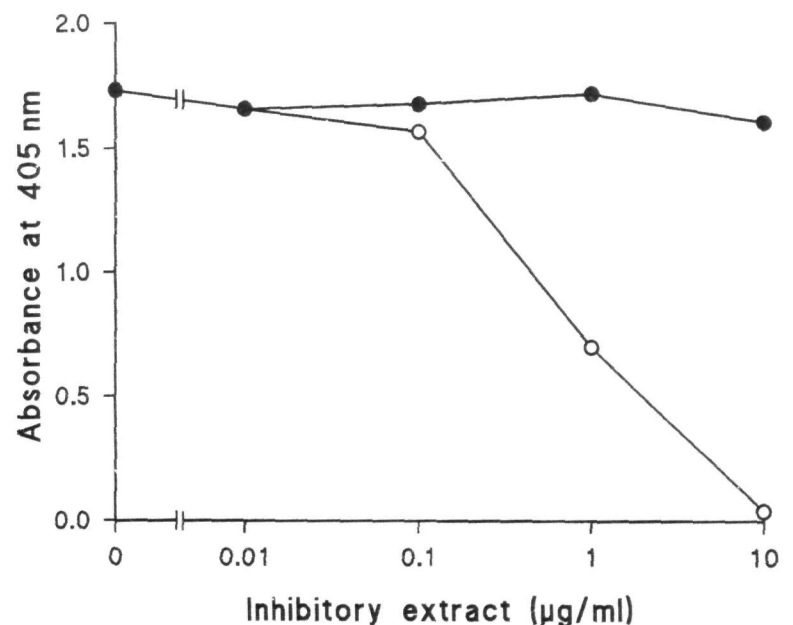


Fig. 2A. Ability of various concentrations of either *L. destructor* extract (open rings) or mite culture medium (filled rings) to inhibit binding of IgG1 to *L. destructor* in ELISA.

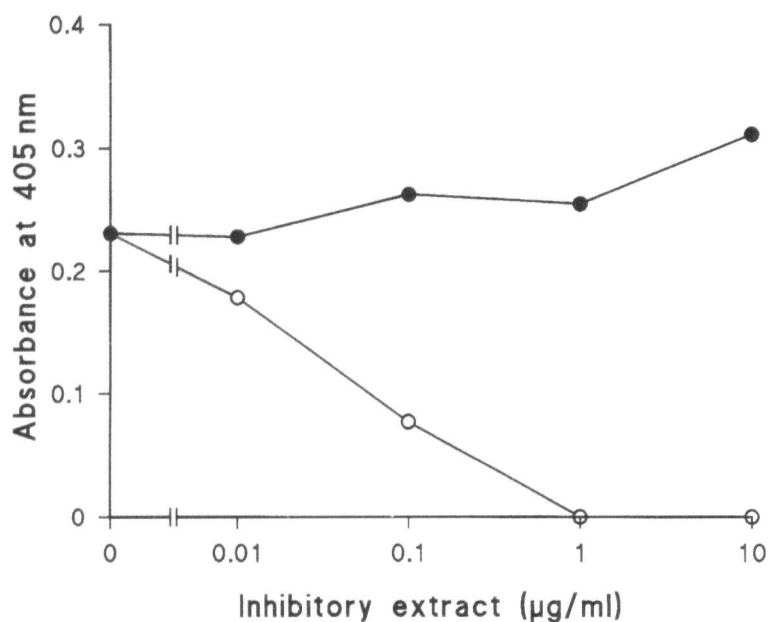


Fig. 2B. Ability of various concentrations of either *L. destructor* extract (open rings) or mite culture medium (filled rings) to inhibit binding of IgG4 to *L. destructor* in ELISA.

reaction. The IgG4 response required less inhibiting extract than did the IgG1 response.

Discussion

Mites are notorious allergy-inducing agents. In a previous epidemiologic study, we analyzed the IgE response to the dust mite *L. destructor* in an agricultural population where mites were the predominant allergen (18). Mites were found on all 16 farms studied, eight of which belonged to farmers with respiratory symptoms related to barn dust and eight to healthy farmers (19). As these studies leave no doubt that all farmers studied were "chronically" exposed to *L. destructor* in their daily work but "only" some (6.2%) developed allergy to mites, we thought it would be of interest to investigate further and compare the pattern of the humoral response to *L. destructor* among healthy and *L. destructor*-allergic individuals. Our previous analysis showed the allergy group to display IgE reactivity to *L. destructor*, while the healthy group did not. Therefore, in the present work, we compared the IgG subclass response to *L. destructor* between these two groups. We thought that this approach should have a bearing on the discussion and hypothesis about the role played by various subclasses of IgG in regard to development of, or protection from, allergic reactions. We focused on the role played by IgG1 and IgG4. Several reports state that there is an increase of allergen-specific IgG4 following allergen desensitization (1, 3, 11, 14). In the present investigation, the IgG1 and IgG4 responses to *L. destructor* allergens and antigens were analyzed with the aid of immunoblotting and ELISA techniques. The main finding was that the "healthy" farmers did not express either an IgG1 or an IgG4 response to *L. destructor* as assessed by

immunoblotting. In contrast, a large majority of the farmers with symptoms displayed IgE and IgG4 as well as IgG1 to *L. destructor*. The logical conclusions from these results are twofold. The first is that healthy nonallergic individuals do not have IgG antibodies; thus, IgG antibodies cannot prevent them from getting symptoms. Instead, our observation seems to suggest that the healthy farmers are non/low-responders to *L. destructor* and that this is an advantage. Second, in the allergic farmers, the IgG1 and IgG4 anti-*L. destructor* antibodies are not, or are at best partially, protective as these subjects do display symptoms. Comparison of the immunoblotting results between our "asthma" group and our "rhinitis" group showed that the immune response shifted toward stronger IgG4 production in the latter. Whether this has any bearing on the symptoms shown is at present unknown.

The ELISA results were not as clear-cut as the immunoblotting data. The IgG4 response was significantly higher in the allergy group than in the healthy group, while the IgG1 was not. For *L. destructor*, recombinant allergens are not yet available. In studies with crude extract, we consider that immunoblotting greatly improves our ability to analyze the reactivity pattern of patient sera as compared to studies using ELISA alone.

Immunoblotting has been used in a few investigations studying IgG-binding components in *Dermatophagoides farinae* and *D. pteronyssinus*. Oshika et al. (13), using crude extract, found that sera from asthmatic patients mainly detected seven bands. Among these, *Der p* II was detected by 72.5% of the asthmatics, and 81% of these patients were positive in RAST to *D. pteronyssinus*, while only 20% of the controls identified this band (*Der p* II). Nakanishi & Shimokata (12), analyzing the IgG4 response to *D. farinae*, found 7/30 sera from asthmatic subjects to be positive, while the controls were negative. Lin et al. (8) studied the IgG and IgE responses following immunotherapy. They used the immunoblotting technique and found parallel expression of these classes of antibodies to several components of *D. pteronyssinus*.

Soliman & Rosenstreich (16) measured mite-specific IgG in ELISA. They found 62% of the asthmatic sera to be positive compared with only 10% of the control sera. Kemeny et al. (7) also measured mite-specific IgG using ELISA and found most of both nonatopics and atopics to be positive for IgG1 as assessed against crude house-dust mite extract. IgG4 was somewhat more frequent in the atopic group than in the nonatopic (66% vs 53%). The same authors also found IgG1 response to *D. pteronyssinus* extract during the first year of life (10).

According to the prevailing view, the cytokine profile determines the direction of the immuno-

response. Where there is excessive IL-4 production, the response is switched to IgG4 and IgE, while interferon-gamma favors IgG1. Thus, according to this notion, it would be expected that the nonatopics should display a strong IgG1 response against allergens. This seems to be at variance with the view of Chapman & Platts-Mills (2) that factors governing inhalant allergy act on antibody responses in general and not only on the IgE response. In line with the latter notion, our study suggests that a strong IgG response to inhaled antigens could be itself viewed as an aberrant event. One hypothesis could be that the immune system might be designed to handle inhaled, noninfectious antigens by other means than specific immune response; e.g., by phagocytosis. Naturally, it could be reasoned that if the specific immune system is indifferent to the *L. destructor* antigens there will be no IgE antibodies produced. The outcome of the natural immune response to an inhaled allergen when it enters via the nasal mucosa seems, in any case, to be fundamentally different in the asymptomatic group and the allergic group. The specific immune system in the former group seems to "ignore" inhaled allergens while the latter responds. The reason for this is not clear. One possible reason could lie in the function of the antigen-presenting cells. These cells could be too efficient at presenting antigens (allergens) in the allergic group.

Although the notion of suppressor cells is not in vogue, the possibility that the healthy group had become tolerant to these antigens is not yet excluded. In experimental animal systems, induction of antigen-specific tolerance (5), as well as nonspecific tolerance, has been reported (17). We do not know whether such mechanisms exist naturally in man. Prospective studies could possibly shed light on the question of whether the asymptomatic group has a transient IgG response.

Acknowledgments

Financial support for this work was received from the Swedish Association against Asthma and Allergy, the Swedish Work Environmental Fund, the Swedish Medical Research Council (grant 00105), the Hesselman Foundation, the Hedlund Foundation, Sigurd and Elsa Goljes Minne, the Konsul Th. C. Bergh Foundation, and the Karolinska Institute.

References

1. AALBERSE RC, VAN DER GAAG R, VAN LEEUWEN J. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J Immunol* 1983; 130: 722–6.
2. CHAPMAN MD, PLATTS-MILLS TAE. Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen-binding assay, using a partially purified fraction of mite extract (F₄P₁). *Clin Exp Immunol* 1978; 34: 126–36.
3. EINARSSON R, DREBORG S, HAMMARSTRÖM L, LÖFKVIST T, SMITH CIE, SVENSSON G. Monitoring of mite *Dermatophagoides farinae* allergen-specific IgG and IgG subclass distribution in patients on immunotherapy. *Allergy* 1992; 47: 76–82.
4. GASCAN H, GAUCHAT JF, RONCAROLO MG, YSSEL H, SPITS H, DE VRIES JE. Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4+ T cell clones. *J Exp Med* 1991; 173: 747–50.
5. HOLT PG, McMENAMIN C. Defence against allergic sensitization in the healthy lung: the role of inhalation tolerance. *Clin Exp Allergy* 1989; 19: 255–62.
6. JOHANSSON E, BORGÅ Å, JOHANSSON SGO, VAN HAGE-HAMSTEN M. Immunoblot multi-allergen inhibition studies of allergenic cross-reactivity of the dust mites *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1991; 21: 511–18.
7. KEMENY DM, URBANEK R, EWAN P, et al. The subclass of IgG antibody in allergic disease. II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals. *Clin Exp Allergy* 1989; 19: 545–9.
8. LIN KL, WANG SY, HSIEH KH. Analysis of house dust mite-specific IgE, IgG4 and IgG antibodies during immunotherapy in asthmatic children. *Ann Allergy* 1991; 67: 63–9.
9. LUNDGREN M, PERSSON U, LARSSON P, et al. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. *Eur J Immunol* 1989; 19: 1311–15.
10. MARIANI I, PRICE JF, KEMENY DM. The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of the immune system following natural exposure. *Clin Exp Immunol* 1992; 22: 29–33.
11. MOSS RB, HSU YP, KWASNICKI JM, SULLIVAN MM, REID MJ. Isotypic and antigenic restriction of the blocking antibody response to ryegrass pollen: correlation of rye group I antigen-specific IgG1 with clinical response. *J Allergy Clin Immunol* 1987; 79: 387–98.
12. NAKANISHI K, SHIMOKATA K. Immunoblot analysis of *Dermatophagoides farinae* antigen. *Ann Allergy* 1990; 64: 219–23.
13. OSHIKA E, KUROKI Y, SAKIYAMA Y, MATSUMOTO S. Measurement of IgG subclass antibodies to the group II antigen of *Dermatophagoides pteronyssinus* (*Der p* II) in sera from children with bronchial asthma. *Ann Allergy* 1992; 69: 427–32.
14. PENG Z, NACLEIRO RM, NORMAN PS, ADKINSON NF. Quantitative IgE- and IgG-subclass responses during and after long-term ragweed immunotherapy. *J Allergy Clin Immunol* 1992; 89: 519–29.
15. PLATTS-MILLS TAE. Local production of IgG, IgA, and IgE antibodies in grass pollen hay fever. *J Immunol* 1979; 122: 2218–25.
16. SOLIMAN MY, ROSENSTREICH DL. Natural immunity to dust mites in adults with chronic asthma. *Am Rev Respir Dis* 1986; 134: 962–8.
17. THEPEN T, McMENAMIN C, OLIVER J, KRAAL G, HOLT PG. Regulation of immune response to inhaled antigen by alveolar macrophages: differential effects of *in vivo* alveolar macrophage elimination on the induction of tolerance vs immunity. *Eur J Immunol* 1991; 21: 2845–50.
18. VAN HAGE-HAMSTEN M, JOHANSSON SGO, HÖGLUND S, TULL P, WIRÉN A, ZETTERSTRÖM O. Storage mite allergy is common in a farming population. *Clin Allergy* 1985; 15: 555–64.
19. VAN HAGE-HAMSTEN M, JOHANSSON E, WIRÉN A, JOHANSSON SGO. Storage mites dominate the fauna in Swedish barn dust. *Allergy* 1991; 46: 142–6.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.