

The significance of specific IgG4 antibodies to methyltetrahydrophthalic anhydride in occupationally exposed subjects

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Summary

Background A definitive role for allergen-specific IgG4 as either an anaphylactic or a blocking antibody, or both, remains controversial.

Objective A population of 148 workers from two condenser plants (A and B) using epoxy resin with methyltetrahydrophthalic anhydride (MTHPA) was studied to evaluate the significance of MTHPA-specific IgG4 antibody.

Methods The workers were evaluated through questionnaire and serological investigations.

Results Ninety-seven (66%) of the currently exposed workers had positive MTHPA-specific IgE. IgE-sensitized workers in each plant had significantly more eye and nose complaints than unsensitized workers ($P < 0.03$). As the result of multiple logistic analysis, specific IgE antibodies was the most important predictor of work-related symptoms and its effect was greater than that of specific IgG4 (odds ratio 16.7 and 3.68, respectively). These indicate an IgE-mediated mechanism in most cases of work-related symptoms associated with MTHPA exposure. However, it cannot be denied that specific IgG4 is an anaphylactic antibody. Furthermore, IgE-sensitized workers in these plants displayed work-related symptoms despite the presence of specific IgG4. The frequency of positive specific IgG4 in continuously exposed workers was significantly ($P < 0.02$) higher in plant A than in plant B, reflecting the difference of the MTHPA levels between the two plants. In plant A, the frequency of positive specific IgG4 was significantly ($P < 0.002$) higher in continuously exposed workers than in intermittently exposed workers. Multiple regression analysis also confirmed that plant and exposure style contributed significantly ($P < 0.01$) to the determination of specific IgG4 levels.

Conclusion These results suggest that work-related eye and nasal symptoms are likely to be IgE-mediated, and that specific IgG4 may reflect the intensity of MTHPA exposure and may not act as a blocking antibody.

Keywords: methyltetrahydrophthalic anhydride, multivariate analysis, occupational allergy, specific IgE, specific IgG4

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Introduction

Acid anhydrides are a well recognized cause of occupational asthma and other occupational immunological respiratory

diseases [1]. Specific IgE antibodies and positive skin-prick tests have been found in workers exposed to phthalic anhydride (PA) [2,3], trimellitic anhydride (TMA) [4], tetrachlorophthalic anhydride (TCPA) [5], hexahydrophthalic anhydride (HHPA) [6,7], or methyltetrahydrophthalic anhydride (MTHPA) [8]. These data indicate an IgE-mediated mechanism, at least in some cases.

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On the one hand, specific IgG antibodies have been found in high frequency in workers exposed to MTHPA [9] or PA [2], and are associated with exposure intensity. However, it is reported that specific IgG antibodies to TMA are associated with a late respiratory systemic syndrome [4]. Nielsen *et al.* [2] have found an association between specific IgG4 antibodies to PA and work-related symptoms. Some investigators [10–12] have also attributed the activity of an anaphylactic antibody to common allergen-specific IgG4. On the other hand, the clinical effect of immunotherapy for insect venom and house dust mite hypersensitivity [13–15] is mainly attributed to the production of specific IgG4-blocking antibodies. Thus, a definitive role for specific IgG4 as either an anaphylactic or a blocking antibody, or both, remains unclear.

In this study, in order to evaluate the significance of specific IgG4 antibody, we studied the frequency or the level of specific IgG4 and IgE in 148 workers exposed to MTHPA and their relationship to work-related symptoms. The relationship between the existence of specific IgE and IgG4 antibodies was also investigated.

Materials and methods

Plants

The two plants (A and B) that were investigated manufacture condensers for electric appliances. MTHPA is used as a hardener in an epoxy resin system for electric insulation and protection. The manufacturing process in each plant consisted of assembly (Zone 1), loading and hardening (Zone 2), and inspection (Zone 3). After the condensers are assembled automatically, a small amount of epoxy resin mixture is poured into them. They pass through a curing oven at a temperature of 100°C. After curing, they are tested electrically. The main exposure originates from the curing ovens. Plant A started continuous production in 1982, and plant B in 1976. Plant A was investigated in December 1994 and February 1995, and plant B in July 1995.

Subjects

In plant A, a total of 75 employees worked with MTHPA, and 73 (97%) agreed to participate in the survey. In plant B, of 103 persons, 75 (73%) agreed to take part. They were divided into two types of MTHPA exposure categories, namely, continuous (group C) and intermittent (group I), by each plant management without knowledge of their immunological and clinical findings. In total, 30 workers in group C in these plants refused to participate for personal reasons.

Forty-five (plant A) and 66 (plant B) subjects in group C

were engaged in monitoring work in the various work zones and had continuous exposures during an 8 h work shift. In the two plants, a total of 37 subjects (group I) consisted of maintenance personnel, assistants, and laboratory personnel. They generally worked in offices or in other parts of these plants where there was no exposure to MTHPA, but they experienced intermittent, short-time exposure (15–60 min) once a day, a few times a week, or once a month.

A total of 148 exposed workers was investigated. As control subjects, 25 persons (age 21–54; three women) from other parts of the workshop and offices with little or no exposure to MTHPA were randomly selected in plant A. Informed consent was obtained from all study participants.

Medical examination

The subjects completed a questionnaire about their symptoms (from the eyes, nose, pharynx and lower respiratory tract), smoking habits, and occupational history. A subject was defined as an atopic when he or she had a history of pollinosis, asthma, atopic eczema, or urticaria during childhood or adolescence. Work-related eye and nasal symptoms were defined as attacks of lacrimation, pruritus and congestion of eyes, sneezing, secretion, or blockage, recovering either at weekends or on holiday, and starting after first exposure to MTHPA. Similarly, occupational asthma was defined as attacks of wheezing breath, dyspnoea, and cough. The subjects were classified as current or former smokers or non-smokers. A physician (KY) interviewed the subjects and took a brief history, directed at symptoms and their relationship to work, without knowledge of the symptoms and smoking and occupational histories already recorded in the questionnaire. Judgment of each worker's health state was done based on both the questionnaire and the physician's observations and diagnosis. After an interview, venous blood samples were collected for serological investigations.

Antibody determinations

The antibody determinations were performed according to procedures described and validated earlier [16]. Thus, here we describe the procedures briefly.

Antigens

A hapten conjugate was prepared from MTHPA and human serum albumin (HSA; Sigma Chemical Co., St Louis, MO, USA); 120 mg of MTHPA was added to a solution of 120 mg of HSA in 40 mL of 0.1 M sodium bicarbonate. The protein was purified from MTHPA by ultrafiltration (Millipore Co., Bedford, MA, USA) and diluted with 50 mM phosphate buffer (pH 7.2).

Specific IgE antibodies and total IgE

Pharmacia (Pharmacia Diagnostics AB, Uppsala, Sweden) provided immunocaps made from hydrophilic polymer, to which a MTHPA human serum albumin conjugate was fixed. Specific IgE antibody to MTHPA was measured by a Pharmacia CAP system. The results were expressed as U/mL, and the detection limit was 0.35 U/mL. Total IgE levels were also measured by the CAP system according to the manufacturer's instructions.

Specific IgG4 antibodies

Specific IgG4 antibody to the MTHPA-HSA conjugate was analysed by enzyme immunoassay (EIA) (Vector Laboratories Inc., Burlingame, CA, USA). Wells of microplates (ICN Biomedicals Inc., Costa Mesa, CA, USA) were coated with 100 µL of a conjugate solution (100 µg/mL; 5 mM phosphate buffer, pH 7.2). The wells were washed six times with a washing solution (5 mM phosphate buffer, pH 7.2, 0.05% Tween-20) after storage in a refrigerator overnight, and were then incubated for 2 h at ambient temperature (25 °C) with 200 µL of blocking solution (Block Ace; Snow Brand Milk Products Co. Ltd, Tokyo, Japan). The analytical procedure was as follows: washing six times, addition of 100 µL of a 1:50 dilution (Block Ace) of serum, incubation for 1 h at 25 °C, washing six times, addition of 100 µL of an optimal dilution (1:1000, Block Ace) of biotinylated anti-IgG4 antibody (Caltag Laboratories Inc., San Francisco, CA, USA), incubation for 1 h at 25 °C, washing six times, addition of 100 µL of avidin biotin-ALP 5 mM phosphate buffer, incubation for 1 h at 25 °C, washing 10 times, addition of 100 µL of substrate solution (*p*-nitrophenyl phosphate), incubation for 15 min at 25 °C, and reading at 405 nm with an autoreader (Sanko Junyaku Co. Ltd, Tokyo, Japan). All samples were analysed in triplicate and read against an HSA antigen as a control for binding not specific to MTHPA-HSA. The results were expressed as optical density (OD).

Air sampling and analysis

MTHPA levels in air had been measured, by area sampling on silica gel tubes, in the two plants every 6 months from 1993 to 1995. The sampling rate was 1 L/min, and the sampling volume 60–120 L. The anhydride analysis followed, with slight modifications, the procedure described previously [17]. The detection limit was 1.0 µg/m³ in 20 L air sample.

Statistical test

Total and specific IgE levels were evaluated in terms of their log values. Differences were tested by unpaired Student's *t*-test, Mann–Whitney *U*-test, χ^2 test and Fisher's exact test.

Pearson's correlation test was used for testing of the association between pairs of continuous variables.

Stepwise multiple linear regression analysis was used to test the association between specific IgG4 levels and several variables. The subjects were divided into the two groups according to type of MTHPA exposure (continuous or intermittent). Specific IgG4 levels was used as a dependent variable, and eight variables were used as independent variables, i.e. plant, gender, age, atopic history, current smoking, exposure style, exposure time, and log total IgE.

Multiple logistic regression analysis was also used to test the association between occurrence of work-related symptoms and several variables, including MTHPA-specific IgE and IgG4 antibodies. Several variables were dichotomized and scored as 0 (low risk) or 1 (high risk).

These analyses were conducted with the use of the SPSS computer program [18]. A *P*-value of 0.05 or less was regarded as significant.

Results*MTHPA levels in air*

The results for the air samples obtained in the various work zones in the two plants are presented in Table 1. The MTHPA levels in assembly lines were significantly ($P < 0.05$) higher in plant A than in plant B. A similar but non-significant tendency was found for inspection lines. In contrast, there was no difference in the MTHPA levels in the loading and hardening processes between these plants.

Demographic data

Table 2 shows characteristics of the population by type of MTHPA exposure in the two plants. Comparisons were performed between the groups within each plant. The proportion of female workers was significantly ($P < 0.001$) higher in group C than in group I in plant A. In plant A, group C had been exposed for a shorter period of time than group I ($P < 0.05$), and included a smaller proportion of smokers than did group I ($P < 0.02$).

Immunological data

There were no significant differences between the groups in each plant with regard to total IgE level. No significant differences were found in the frequency or the level of positive specific IgE antibody between the groups in each plant. In plant A, however, the frequency of positive specific IgG4 was significantly ($P < 0.002$) higher in group C than in group I. A similar but non-significant tendency was seen for plant B. In each plant,

Table 1. Air levels of methyltetrahydrophthalic anhydride (MTHPA) in different parts of the workshop in plants A and B during the period between 1993 and 1995

Zone	Plant A		Plant B	
	No. of samples	MTHPA ($\mu\text{g}/\text{m}^3$)	No. of samples	MTHPA; ($\mu\text{g}/\text{m}^3$);
1. assembly 1	5	30.2 (7.47–102)	4	4.93 (1.36–14.9)
assembly 2	4	63.9 (14.3–421)	—	—
2. loading and hardening 1	5	68.4 (31.2–124)	4	61.1 (23.8–107)
loading and hardening 2	4	65.9 (15.6–278)	4	56.8 (21.7–149)
3 inspection	4	25.5 (12.9–67.9)	4	5.49 (0.68–22.4)

Values are expressed as geometric mean and range.

the mean specific IgG4 level of subjects with positive specific IgG4 was higher in group C than in group I, but the difference was not significant.

All the specific IgE and IgG4 values for the 25 control subjects were <0.35 U/mL and <0.020 , respectively.

Predictor variables associated with specific IgG4 levels

The results of stepwise multiple linear regression analysis for predictors of specific IgG4 levels are shown in Table 3. Plant and log total IgE showed a relation with specific IgG4 levels in group C, and atopic history and log total

Table 2. Profiles of workers in the various MTHPA exposure groups in plants A and B

	Plant A		Plant B	
	Continuous (C) <i>n</i> = 45	Intermittent (I) <i>n</i> = 28	Continuous (C) <i>n</i> = 66	Intermittent (I) <i>n</i> = 9
Female/male (no.)	28/17 ^a	5/23	24/42	0/9
Age (years, mean, range)	34.5 (19–53)	35.0 (19–58)	37.1 (19–56)	35.3 (25–56)
Exposure time (years, mean, range)	5.2 (0.2–13) ^b	7.4 (0.5–19)	12.5 (1.3–20)	10.4 (3.3–20)
Current smokers (no., %)	5 (11.1) ^c	11 (39.3)	35 (53.0)	4 (44.4)
Atopic history (no., %)	6 (13.3)	7 (25.0)	24 (36.4)	2 (22.2)
Total IgE level (IU/mL, geometric mean, range)	59.6 (5.13–1290)	102 (6.15–895)	124 (3.02–4786)	95.7 (15.5–1002)
Positive specific IgE (no., %)*	26 (57.7)	20 (71.4)	45 (68.2)	6 (66.6)
Specific IgE level in subjects with positive specific IgE (U/mL, geometric mean, range)	8.1 (0.58–106)	7.6 (0.50–105)	8.4 (0.36–174)	4.5 (0.36–31.5)
Positive specific IgG4 (no., %)**	24 (53.3) ^d	5 (17.9)	20 (30.3)	2 (22.2)
Specific IgG4 level in subjects with positive specific IgG4 (Optical density, mean, range)	0.130 (0.020–0.333)	0.086 (0.021–0.183)	0.125 (0.028–0.452)	0.065 (0.029–0.100)

Note: Comparisons were performed between the groups within each plant.

* Values of >0.35 U/mL were considered positive.

** Values of >0.20 were considered positive on the basis of $2 \times (\text{mean} \pm 3\text{sd})$ in controls.

^{a, b, c, d} Significant vs group I in plant A (^a $P < 0.001$, ^b $P < 0.05$, ^c $P < 0.02$, ^d $P < 0.002$).

Table 3. Multiple linear regression results for predictor variables of specific IgG4 levels in 148 workers by type of MTHPA exposure (Stepwise method, $P_{in} = 0.05$, $P_{out} = 0.10$)

	Continuous ($n = 111$)				Intermittent ($n = 37$)				Total ($n = 148$)			
	B	SE	Beta	P-value	B	SE	Beta	P-value	B	SE	Beta	P-value
Intercept	-0.059				-0.050				-0.047			
Independent variables included in the model ($P < 0.05$)												
Plant [†]	0.046	0.017	0.251	0.008								
Gender [‡]												
Age (years)												
Atopic history [§]					-0.030	0.013	-0.339	0.02				
Current smoking [¶]												
Exposure style [¥]	-	-	-	-	-	-	-	-	0.041	0.015	0.215	0.006
Exposure time (years)									-0.003	0.001	-0.216	0.007
Log total IgE	0.048	0.013	0.334	0.0005	0.038	0.010	0.561	0.0004	0.044	0.011	0.334	<0.0001

Exposure style was not used as an independent variable for the analyses of groups C and I.

B, regression coefficient. SE, standard error. Beta, standardized regression coefficient.

[†] Plant A = 1, plant B = 0. [‡] Male = 1, female = 0. [§] Presence of atopic history = 1, absence of it = 0. [¶] Current smoking = 1, ex and never = 0. [¥] Continuous = 1, intermittent = 0.

IgE showed a relation with specific IgG4 levels in group I. Among all subjects, exposure style, exposure time, and log total IgE showed a relationship with specific IgG4 levels.

Associations between work-related symptoms and specific antibodies

IgE-sensitized workers in each plant had significantly more complaints from eyes and nose than unsensitized workers

Table 4. Work-related symptoms in sensitized (positive specific IgE) and unsensitized subjects among 148 factory workers with present exposure to MTHPA

	Plant A			Plant B		
	Sensitized $n = 46$	Unsensitized $n = 27$	P-value	Sensitized $n = 51$	Unsensitized $n = 24$	P-value
Positive specific IgG4 (no., %)	25 (54.3)	4 (14.8)	<0.001	22 (43.1)	0	<0.001
Work related symptoms (no., %):						
Eyes	28 (60.9)	6 (22.2)	<0.002	17 (33.3) ^a	2 (8.3)	<0.03
Nose	40 (87.0)	5 (18.5)	<0.0001	25 (49.0) ^b	4 (16.7)	<0.02
Sneezing	36 (78.3)	4 (14.8)	<0.0001	23 (45.1) ^c	3 (12.5)	<0.01
Secretion	39 (84.8)	3 (11.1)	<0.0001	24 (47.1) ^d	4 (16.6)	<0.02
Blockage	20 (43.5)	3 (11.1)	<0.005	16 (31.3)	0	<0.002
Pharynx	4 (8.7)	2 (7.4)	NS	0 ^e	0	NS
Cough	8 (17.4)	1 (3.7)	NS	5 (9.8)	0	NS
Asthma	0	0	NS	0	0	NS

a, b, c, d, e Significant vs sensitized in plant A (^a $P < 0.01$, ^b $P < 0.0001$, ^c $P < 0.001$, ^d $P < 0.0005$, ^e $P < 0.05$).

NS, not significant.

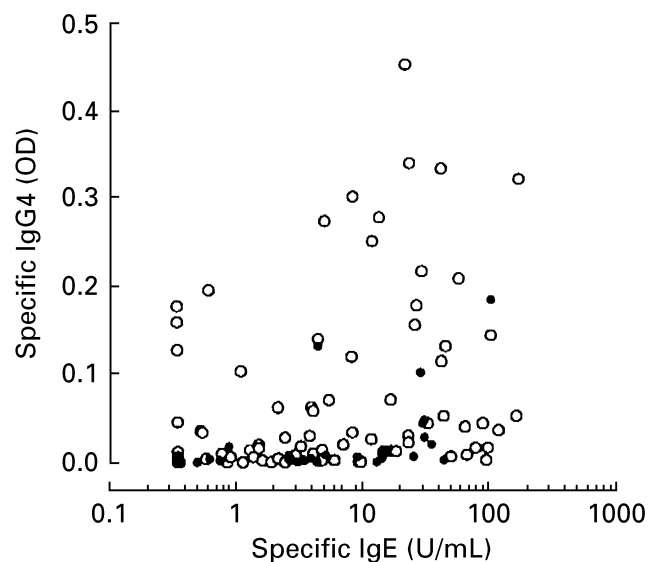
Table 5. Odds ratios of various factors for occurrence of work-related eye and nasal symptoms in 148 factory workers (multiple logistic regression analysis)

Factor	High risk	Odds ratio (95% confidence interval)
Gender	Male	0.44 (0.14–1.39)
Age (years)	≥ 35	0.99 (0.35–2.86)
Atopic history	Present	0.34 (0.12–0.94)*
Smoking habits	Current	2.58 (0.83–8.04)
Plant	A	5.54 (1.78–17.2)**
Exposure time (years)	≥ 5	0.77 (0.25–2.42)
Exposure style	Continuous	0.49 (0.14–1.65)
Specific IgE	Positive	16.7 (4.59–60.6)***
Specific IgG4	Positive	3.68 (1.33–10.2)*

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.0001$.

(Table 4; $P < 0.03$). The IgE-sensitized workers in plant A had higher frequencies for eyes, nose and pharynx than did those in plant B ($P < 0.05$). None of the subjects had yet had symptoms of work-related asthma.

As the result of multiple logistic regression analysis (Table 5), the resulting odds ratio (OR) ranged from 0.34 to 16.7. Four factors, specific IgE, plant, specific IgG4 and atopic history were significantly ($P < 0.05$) associated with work-related symptoms. Gender, age, smoking, exposure time and exposure style did not play a significant role in developing work-related symptoms.

**Fig. 1.** Scattergram of MTHPA-specific IgE and IgG4 levels from 148 workers with continuous (○) and intermittent (●) exposure to MTHPA. Specific IgE values of < 0.35 U/mL were plotted and calculated as 0.35 U/mL.

Correlation study among total IgE, specific IgE and IgG4

In the 148 workers, there was not a high correlation between specific IgE and IgG4 levels (Fig. 1; $r = 0.41$). Positive correlations were also observed between levels of total IgE and specific IgE ($r = 0.51$), and specific IgG4 ($r = 0.28$).

Discussion

The present study revealed the significance of MTHPA-specific IgG4 in occupationally exposed workers.

In the two plants studied, the frequency of positive specific IgE in currently exposed workers was 97 of 148 (66%), much higher than that (7–44%) found in previous studies of workers exposed to MTHPA [9,19], HHPA [6,20], PA [2], TCPA [21,22], or TMA [23]. It is unlikely that this high frequency is due to selection bias, since the participation rate in workers with current exposure was adequate. This result demonstrates that MTHPA, like the other acid anhydrides, has a high sensitizing ability.

The geometric mean concentrations of MTHPA were fairly low during the period between 1993 and 1995 (Table 1) and were comparable with those reported in Sweden ($10\text{--}85 \mu\text{g}/\text{m}^3$) [9]. These results can be explained by the fact that there have been continuous efforts to reduce exposure to MTHPA, such as enclosure of curing ovens and increase of exhaust ventilation, especially since 1990. The frequency of positive specific IgE in this study is much higher than that in our previous study [19] (64% and 24%) in the continuously MTHPA-exposed workers, because the exposure intensity was much lower in the latter group ($0.86\text{--}15.0 \mu\text{g}/\text{m}^3$). Because of the high frequency of positive specific IgE, it is possible that the airborne MTHPA concentrations were higher before 1993.

As shown in Table 2, the frequency of positive specific IgE in plant A is comparable with that in plant B (58% and 68%) in the continuously exposed workers (group C). By contrast, a significant ($P < 0.02$) difference was found in the frequency of positive specific IgG4 in group C between the two plants, suggesting that this may be due to the difference of the MTHPA levels in assembly and inspection lines between the two plants (Table 1). Similar results were obtained from former workers, who had been removed from MTHPA exposure for a mean of 3.5 years (range, 0.25–10 years), in our previous study [16].

Groups C and I in each plant did not differ in the frequency or the level of positive specific IgE. This finding suggests that intermittent short-time exposures may have an effect on the production of specific IgE antibodies, which is consistent with the results of a study in a factory using HHPA [20]. Our interest should therefore be focused on the exposure levels of intermittently exposed workers (group I). However, intermittent exposures have not yet

been measured. By contrast, in plant A, the frequency of positive specific IgG4 was significantly ($P < 0.002$) higher in group C than in group I. A similar but non-significant tendency was found for plant B.

As stated above, specific IgG4 levels seem to reflect the intensity of MTHPA exposure or exposure style. Multiple regression analysis also confirmed that plant and exposure style contributed significantly ($P < 0.01$) to the determination of specific IgG4 levels (Table 3).

In addition, exposure time and atopic history were isolated as factors with statistical significance ($P = 0.007$ and $P = 0.02$ in Table 3). In the model of plant A, exposure style and total IgE were isolated ($P < 0.0005$), but exposure time was not. As shown in Table 2, exposure time was longer, and the frequency of positive specific IgG4 was lower in group C in plant B than in group C in plant A. These results can explain the inverse association of exposure time with specific IgG4 levels. On the other hand, the number of intermittently exposed workers with a positive atopic history was not large enough to make possible the interpretation of a negative relation of atopic history with specific IgG4 levels.

Interleukin-4 or -13 are known to be capable of inducing the isotype switching and production of specific IgE and IgG4 [24,25]. Thus, one may expect that induction of specific IgE and IgG4 to a certain allergen would be parallel. In the present study, however, the correlation was weak between MTHPA-specific IgE and IgG4 levels (Fig. 1; $r = 0.41$). Tame *et al.* [26] have also reported that no correlation was observed between *Dermatophagoides pteronyssinus* group 2 (Der p 2)-specific IgE and IgG4 levels and that the induction of Der p 2-specific IgG4 in allergic diseases is independent to specific IgE. These results invite the speculation that mechanisms in induction of specific IgE and IgG4 may be different with each other.

Positive correlations were also observed between levels of total IgE and specific IgE, and specific IgG4. The former correlation suggests that the total IgE level in an individual may represent his or her potential for immunoallergic responses. However, it is possible that the total IgE levels may be explained by sensitization to MTHPA. Then, it is not surprising that the latter correlation was confirmed by multiple regression analysis (Table 3; $P < 0.0001$), since there was a positive correlation between specific IgE and IgG4.

We found that despite the low exposure to MTHPA, more than half the workers had specific IgE antibodies to MTHPA and suffered eye and nose symptoms (Table 4). As shown in Table 5, specific IgE antibodies was the most important predictor of work-related symptoms and its effect was greater than that of specific IgG4 (odds ratio 16.7 and 3.68, respectively). These indicate that there is an IgE-mediated mechanism in most cases of work-related

symptoms associated with MTHPA exposure. However, it cannot be denied that specific IgG4 is an anaphylactic antibody. Furthermore, the IgE-sensitized workers in plant A had higher frequencies for eyes, nose and pharynx than did those in plant B ($P < 0.05$). This seems to reflect the difference of the MTHPA levels between the two plants (Table 1). By multiple logistic analysis, it was further confirmed that plant contributed significantly ($P < 0.005$ and OR 5.54) to work-related symptoms. Additionally, atopic history was inversely significantly ($P < 0.05$ and OR 0.34) associated with work-related symptoms. This association may be due to the result in our previous study [27] that no association was found between atopic history and the production of MTHPA-specific IgE antibodies.

In the two plants, 37 (79%) of 47 IgE-sensitized workers with positive specific IgG4 had work-related symptoms, and this proportion was 60% in 50 IgE-sensitized workers without specific IgG4. These results suggest that specific IgG4 antibodies are not protective.

Nielsen *et al.* [8] reported that asthma was found in 10% of workers highly exposed to MTHPA (time-weighted average $20\text{--}150\text{ }\mu\text{g}/\text{m}^3$). In the present study, however, no subject had yet developed symptoms of work-related asthma. It is unlikely that workers denied symptoms suggestive of asthma by fear of losing their job, since workers with severe work-related symptoms can leave exposure by transferring to alternative work with no exposure. Thus, this may be explained by the low exposure levels in recent years, or might reflect the use of rigid criteria in the definition of occupational asthma. Alternatively, a healthy worker selection may have occurred, since our study is cross-sectional. To address this question, further prospective studies among IgE-sensitized subjects need to be done.

The following points were shown in the present study: eye and nasal symptoms associated with MTHPA exposure may be closely related to MTHPA-specific IgE antibodies; MTHPA-specific IgG4 may not act as a blocking antibody; and exposure style or the intensity of MTHPA exposure is the major determinant of specific IgG4 levels.

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