

Short communication

The association between serum levels of Th cytokines and rhinoconjunctivitis caused by methyltetrahydrophthalic anhydride

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Background There are few epidemiologic studies on the association between serum levels of T helper (Th) cytokines and allergic symptoms caused by sensitizing agents.

Methods A population of 147 workers from two condenser plants using epoxy resin with methyltetrahydrophthalic anhydride (MTHPA) underwent a questionnaire survey and serologic investigations. Total and MTHPA-specific IgE levels were measured by the Pharmacia CAP System, and serum levels of interleukin (IL)-4, IL-13, and interferon-gamma (IFN- γ) by enzyme immunoassay.

Results A significant association was found between work-related eye and nasal symptoms and a range of atopic characteristics, including the frequency of positive specific IgE, specific and total IgE levels, and IL-4 levels. A positive correlation was observed between levels of IL-4 and IL-13 ($r=0.42$). Furthermore, multiple logistic analysis revealed significant contributions of specific IgE and IL-13 to the work-related symptoms. A similar but nonsignificant association was also found for IL-4 ($P=0.07$). On the other hand, a multiple regression model with specific IgE levels as a dependent variable showed a significant association with total IgE, but not with IL-4, IL-13, and IFN- γ .

Conclusions These results suggest that work-related eye and nasal symptoms are closely related to specific IgE antibodies, and that allergic responses, mediated by IgE, to MTHPA may lead to a shift in the balance between Th1 and Th2 cells.

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The major health hazards of acid anhydrides are mucosal and skin irritation and sensitization of the respiratory tract (1). Methyltetrahydrophthalic anhydride (MTHPA), acting as a hapten after conjugation with mucosal proteins, has been also shown to stimulate the production of specific IgE antibodies which can cause occupational allergy (2–4).

Helper T (Th) cells, which modulate inflammation, may be classified into Th1 and Th2 types, according to the pattern of their cytokine production (5). Th1 cells enhance cellular immune responses, and Th2 cells favor the humoral response. A set of cytokines – interleukin-4 (IL-4),

IL-10, and IL-13 derived from Th2 cells – is central in mediating IgE production and the development of allergic diseases (6). Peripheral blood mononuclear cell (PBMC) cultures from subjects with allergic rhinitis respond with a Th2-like cytokine profile when activated *in vitro* with grass-pollen allergen (7). In atopic asthmatics with a history of exacerbations, Th2-like cytokines can be detected in sera (8) and PBMC cultures stimulated by polyclonal activators (9). These findings focused our interest on the relationship between allergic diseases caused by sensitizing agents and alterations of the balance between Th1 and Th2 cells.

In the present study, measurement was made of serum levels of Th2 cytokines (IL-4 and IL-13) and the Th1 cytokine interferon-gamma (IFN- γ) in the previous study subjects (4) to clarify Th2 cell activation in the pathogenesis of work-related eye and nasal symptoms associated with MTHPA exposure. The association of Th cytokines with MTHPA-specific IgE antibodies was also investigated.

Material and methods

Subjects and plants

We have carried out cross-sectional surveys of two populations occupationally exposed to MTHPA in 1994 and 1995. 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 workshop and exposure have been described in detail in a previous paper (4).

In plant A, 75 persons worked with MTHPA, and 73 (97%) agreed to participate in the survey. In plant B, of 103 persons, 75 (73%) agreed to take part. Altogether, 148 exposed workers were investigated. Informed consent was obtained from the study participants. However, a male worker in plant B was excluded, as his sample was not sufficient for analysis of Th cytokines. Thus, 147 workers were finally included in this study.

Medical examination

The subjects completed a questionnaire about their symptoms (from the eyes, nose, pharynx, and lower respiratory tract), smoking habits, and occupational history. Work-related eye and nasal symptoms were defined as attacks of lacrimation, pruritus and congestion of eyes, sneezing, secretion, or blockage, easing either at weekends or on holiday, and starting after first exposure to MTHPA. Similarly, occupational asthma was defined as attacks of wheezing, dyspnea, and coughing. The subjects were classified as current or former smokers or nonsmokers. A physician (K.Y.) interviewed the subjects and took a brief history, directed at the symptoms and their relation to work, without knowledge of the symptoms and smoking and occupational histories already recorded in the questionnaire. Judgment of each worker's health status was based on both the questionnaire and the physician's observations and diagnosis. After interview, venous blood samples were collected for serologic investigations. Details of work-related symptoms in the workers have been reported previously (4).

Laboratory analyses

Serum samples were collected from all the subjects and stored at -20°C until tests were done.

IgE antibody screening test. Tests for atopy were carried out with the multiallergen test, Phadiatop® (Pharmacia Diagnostics AB, Uppsala, Sweden). It includes 12 major aeroallergens such as those of house-dust mites, grass pollens, molds, and animal danders.

MTHPA-specific IgE antibodies and total IgE. Pharmacia (Pharmacia Diagnostics AB, Uppsala, Sweden) provided ImmunoCaps made from a hydrophilic polymer, to which a MTHPA human serum albumin conjugate was fixed. Specific IgE antibody to MTHPA was measured by the 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.

IL-4, IL-13, and IFN- γ . Serum cytokine levels were measured with commercially available assay kits. The detection limits were 0.05 pg/ml for IL-4 (BioSource International, Camarillo, CA, USA), 3.1 pg/ml for IL-13 (Bender & Co GmbH, Vienna, Austria), and 0.10 pg/ml for IFN- γ (Bender & Co GmbH, Vienna, Austria).

Statistical analysis

Levels of IL-4, IL-13, IFN- γ , and total and specific IgE were evaluated as log values. Differences were tested by unpaired Student's *t*-test, Mann-Whitney U-test, chi-square test, and Fisher's exact test. Pearson's correlation test was used to test the association between pairs of continuous variables.

Multiple logistic regression analysis was performed to find significant variables related to work-related eye and nasal symptoms. Plant, sex, age, atopy, current smoking, exposure time, log total IgE, log specific IgE, log IFN- γ , log IL-4, and log IL-13 were used as independent variables. Several variables were dichotomized and scored as 0 (low risk) or 1 (high risk).

Multiple linear regression analysis was also used to test the association between specific IgE levels and several variables. Variables examined included plant, sex, age, atopy, current smoking, exposure time, log total IgE, log IL-4, log IL-13, and log IFN- γ .

These analyses were conducted with the SPSS computer program (10). A *P* value of 0.05 or less was considered significant.

Table 1. Profiles of workers by presence or absence of work-related eye and nasal symptoms

	Exposed workers		P value
	Symptomatic (n=63)	Nonsymptomatic (n=84)	
Plant, A/B (n)	42/21	31/53	0.0004*
Sex, F/M (n)	25/38	32/52	0.85
Age, years, mean (range)	36.7 (19–56)	35.0 (19–58)	0.35
Atopy, n (%)	45 (71.4)	48 (57.1)	0.075
Current smokers, n (%)	24 (38.1)	30 (35.7)	0.76
Exposure time, years, mean (range)	8.1 (0.2–20)	9.8 (0.5–20)	0.097
Total IgE, IU/ml, geometric mean (range)	131 (7.48–1336)	70.3 (3.02–1402)	0.007*
Positive specific IgE, n (%)	58 (92.1)	38 (45.2)	<0.0001*
Specific IgE, U/ml, geometric mean (range) [†]	8.69 (UD–166)	1.09 (UD–174)	<0.0001*
IL-4, pg/ml, geometric mean (range) [†]	0.21 (UD–11.3)	0.12 (UD–5.55)	0.027*
IL-13, pg/ml, geometric mean (range) [†]	12.3 (UD–261)	9.73 (UD–241)	0.18
IFN- γ , pg/ml, geometric mean (range) [†]	0.50 (UD–311)	0.35 (UD–141)	0.29

* Denotes significant values; UD: undetectable. [†] Specific IgE values of <0.35 U/ml, IL-4 values of <0.05 pg/ml, IL-13 values of <3.1 pg/ml, and IFN- γ values of <0.10 pg/ml were calculated as 0.35, 0.05, 3.1, and 0.10, respectively.

Results

Forty-five of the 73 workers in plant A and 29 of the 74 workers in plant B complained of symptoms of the eyes and nose at the time of the investigation, and 63 of these complaints were diagnosed as work related. None of the subjects had previously had symptoms of work-related asthma. Table 1 shows characteristics of the population by work-related eye and nasal symptoms. A significant difference was found in the frequency of the work-related symptoms between the two plants. The frequency of positive specific IgE antibody was significantly higher in the symptomatic subjects than in the nonsymptomatic subjects. IgE levels, both total and specific, were also significantly higher in the symptomatic subjects. The symptomatic subjects had significantly higher levels of IL-4, and a similar but nonsignificant tendency was seen for IL-13 and IFN- γ levels.

IL-4 showed a weak but significant correlation with IL-13 ($r=0.42$, $P<0.001$), but did not correlate with IFN- γ ($r=0.19$). Nor was there a correlation between IL-13 and IFN- γ ($r=0.29$).

Table 2 shows the results of multiple logistic analysis for variables related to work-related symptoms. In model 1, plant and specific IgE showed a significant association with work-related eye and nasal symptoms, and none of the Th cytokines showed association with the symptoms. Since there was a positive correlation between the Th2 cytokines IL-4 and IL-13, IL-4 was used as an independent variable in model 2 and IL-13 was used in model 3. As a result, IL-4 and IL-13 were found to be associated with the symptoms in each model, but the association of IL-4 with them did not achieve statistical significance ($P=0.07$).

Table 3 shows the results of multiple regression analysis for predictors of log levels of specific IgE. Age and total IgE showed a significant association with specific IgE levels. Neither Th cytokines, atopy, nor current smoking was associated with specific IgE. Even when either IL-4 or IL-13 was used as an independent variable for the analysis, similar results were obtained.

Discussion

In the two plants studied, the frequency of positive specific IgE in currently exposed workers was 96 of 147 (65%), much higher than that (7–44%) found in previous studies of workers exposed to MTHPA (3, 11), hexahydrophthalic anhydride (12, 13), phthalic anhydride (14), tetrachlorophthalic anhydride (15, 16), or trimellitic anhydride (17). It is unlikely that this high frequency was due to selection bias, since the participation rate in workers with current exposure was adequate. Differences in the frequency of positive specific IgE could be partly attributed to higher peak or average exposures. Because of the high frequency of positive specific IgE, as explained previously (4), it is possible that the airborne MTHPA concentrations were higher before the time of the investigation. This result demonstrates that MTHPA, like the other acid anhydrides, has a high sensitizing ability.

Although the frequency of positive specific IgE in plant A was comparable to that in plant B (63% and 68%), work-related symptoms were about two times as likely in plant A workers as in plant B workers (Table 1). As previously described (4), this can be explained by the difference in the MTHPA levels between the two plants. Multiple logistic

Table 2. Results of multiple logistic regression analysis of variables relevant to occurrence of work-related symptoms. Only variables with significant odds ratio, except for log IL-4, are shown

	Model*					
	1		2		3	
	Odds ratio [†]	95% CI	Odds ratio [†]	95% CI	Odds ratio [†]	95% CI
Plant A	6.49	1.98–9.20	5.75	1.80–18.34	6.97	2.16–22.47
Log specific IgE [‡]	5.61	2.81–11.22	5.45	2.75–10.83	5.75	2.88–11.46
Log IL-4 [§]			2.09	0.93–4.68		
Log IL-13 [§]					3.08	1.11–8.52

* Both log IL-4 and log IL-13, log IL-4, and log IL-13 were entered into model 1, model 2, and model 3, respectively, as independent variable. [†] Adjusted for sex, age, atopy, current smoking, exposure time, log total IgE, and log IFN- γ . [‡] Odds ratio for 1 U/ml increase in log specific IgE. [§] Odds ratio for 1 pg/ml increase in log IL-4. [§] Odds ratio for 1 pg/ml increase in log IL-13.

analysis also confirmed a significant contribution of the plant to the symptoms (Table 2).

Nielsen et al. (2) reported that asthma was found in 10% of workers highly exposed to MTHPA (time-weighted average 20–150 $\mu\text{g}/\text{m}^3$). In the present study, however, no subject had yet developed symptoms of work-related asthma. As previously described (4), this may be explained by the low exposure levels in recent years. Alternatively, a healthy worker selection may have occurred, since our study was cross-sectional. It has been recently reported that a female worker in plant A displayed symptoms suggestive of asthma in October 1996 and was transferred to alternative work with no exposure. Follow-up studies of sensitized subjects need to be done to consider this issue.

A significant association was found between work-related eye and nasal symptoms and a range of atopic characteristics, including the frequency of positive specific IgE, specific and total IgE levels, and IL-4 levels (Table 1). A positive correlation was observed between levels of IL-4 and IL-13 ($r=0.42$). Furthermore, multiple logistic analysis

revealed significant contributions of log specific IgE and log IL-13 to the work-related symptoms (Table 2). A similar but nonsignificant association was also found for log IL-4 ($P=0.07$). These findings indicate that there is an IgE-mediated mechanism in most symptoms associated with MTHPA exposure, and atopic responses to MTHPA may tip the balance between Th1 and Th2 cells toward the Th2. The present results are in good accord with the results of previous studies (7–9) in which allergic diseases were associated with the preferential activation of Th2 cells. On the contrary, an epidemiologic survey on schoolchildren (18) revealed that cytokine profiles biased toward the Th1 type by positive tuberculin responses may lead to remission of atopic symptoms, including asthma, rhinitis, and eczema. Therefore, the imbalance in cytokine profiles may be closely related to the pathogenesis of allergic diseases.

As shown in Table 3, age was significantly associated with log specific IgE levels while controlling for exposure time, exposure type, and other independent variables. Age is a confounder to exposure time ($r=0.64$, $P<0.001$). Because age at starting to work with MTHPA displayed no correlation with exposure time ($r=0.064$, $P=0.44$), it was used as an independent variable instead of years of age. However, the new variable still significantly ($P=0.005$) contributed to log specific IgE. Thus, age is likely to be a predictor of log specific IgE levels in this cross-sectional survey of a Japanese occupational population.

Log total IgE was the most significant predictor of log specific IgE levels ($P<0.0001$), suggesting that total IgE level in a subject may represent the potential for immunoallergic responses. This agrees with the results of two longitudinal studies (19, 20) which showed that a high total IgE level results in an increased risk of sensitization to allergens. However, it is also possible that total IgE levels may be explained by sensitization to MTHPA. Therefore,

Table 3. Multiple linear regression results for predictor variables of log levels of specific IgE

	B	SEM	Beta	P value
Plant [†]	0.012	0.152	0.007	0.94
Sex [‡]	-0.207	0.146	-0.118	0.16
Age	0.024	0.008	0.295	0.005*
Atopy [§]	0.043	0.156	0.025	0.78
Current smoking [§]	0.192	0.151	0.109	0.21
Exposure time	-0.018	0.015	-0.129	0.25
Log total IgE	0.674	0.125	0.479	<0.0001*
Log IL-4	0.144	0.115	0.101	0.21
Log IL-13	-0.055	0.157	-0.030	0.72
Log IFN- γ	0.095	0.073	0.100	0.19

*Denotes significant values; [†]plant A: 1, plant B: 0; [‡]male: 1, female: 0; [§]presence of atopy: 1, absence of atopy: 0; [§]current smoking: 1, formerly smoked and never smoked: 0; B: regression coefficient; SEM: standard error of mean; beta: standardized regression coefficient.

further prospective studies, in which total IgE levels in workers are determined before their assignment to manufacturing jobs, are needed to confirm the causal relation of total IgE levels to sensitization to MTHPA.

IL-4 and IL-13 have been shown to be potent switch factors for IgE synthesis in human B cells (21, 22). Thus, one may expect that serum levels of IL-4 and IL-13 will be associated with the production of specific IgE. However, neither log IL-4 nor log IL-13 contributed to the elevation of specific IgE levels (Table 3). It may be that the increase in levels of IL-4 released by stimulated PBMCs was not observed in patients sensitized to many allergens, who are continuously exposed to allergens throughout the year (9), and that the peripheral blood of allergic subjects contains long-lived allergen-specific B cells which have already switched to IgE production, and which are not sensitive to the effect of IL-4 and IL-13 (23). In any case, serum levels of IL-4 and IL-13 may not be useful predictors of specific IgE levels.

In conclusion, IL-4 and IL-13 were found to contribute significantly to rhinoconjunctivitis caused by MTHPA, but not to the elevation of MTHPA-specific IgE. Our results suggest that allergic responses, mediated by IgE, to MTHPA may lead to a shift in the balance between Th1 and Th2 cells.

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