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Decline in serum antibodies to methyltetrahydrophthalic anhydride after cessation of exposure. Implications for use as a biomarker

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The association between exposure intensity and serum levels of immunoglobulins E and G against low molecular weight compounds was evaluated. The decay of levels of specific IgE and IgG antibodies was studied after cessation of exposure in workers exposed to the inhalant allergen methyltetrahydrophthalic anhydride in a plant using epoxy resins. Sera have been collected in workers for 18–84 (mean value 54) months after cessation of exposure. Specific IgE and IgG was assessed by RAST and ELISA, respectively. The mean of individual half-times for IgE ($N = 10$) and IgG ($N = 8$) was 0·9 (range 0·1–1·8) and 0·4 (range 0·2–0·6) years, respectively, after total avoidance of exposure. Corresponding decreases of IgE and IgG were also observed after reduction, but not total elimination, of exposure. No correlation was seen between biologic half-times of specific IgE and total IgE, atopy, smoking habits or gender. The results indicate that the levels of specific antibodies in sensitized individuals reflect long term exposure, and may persist for years after the end of exposure.

Keywords: IgE, IgG, half-time, biomarker, exposure.

Introduction

Monitoring of air levels is a useful tool in the assessment of exposure to allergens in the work environment. The serum levels of specific antibodies in exposed individuals may also supply important information on exposure in addition to the use as a diagnostic tool (Boxer *et al.* 1987). However, a prerequisite for the proper evaluation of specific antibodies as biomarkers of exposure is a knowledge of their kinetics of formation and decay. Such information is sparse.

Occupational allergens make good models for kinetic studies of the formation and decay of immunoglobulins, since, for the occupational allergens, in contrast to many naturally occuring ones, studies can be performed before, during, and after exposure. The possibility to identify the initiation of exposure to allergens and to remove sensitized patients to environments free of exposure is unique in the work environment.

The organic acid anhydrides (OAAs) are low molecular weight reactive molecules which may induce the formation of a high prevalence of specific immunoglobulins E and G in exposed workers (Venables 1989, Welinder *et al.* 1994). Studies of exposed workers indicate an association between work-related conjunctivitis, rhinitis and asthma on the one hand, and specific IgE on the other (Nielsen *et al.* 1988, 1994a, Venables 1989). Further, an association between

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Table 1. Description of data used for the estimation of half-times of antibody decay.

	Mean	Range
Exposure time before sampling (months)	32·9	18–58
Sampling period (months)	54	18–84
Number of samples	7·3	4–12
IgE at start (% specific binding)	10·7	3·3–23
at stop	1·3	0·0–6·8
IgG at start (OD)	0·42	0·09–0·86
at stop	0·07	0·00–0·36

formation of specific IgG and exposure intensity has been observed (Nielsen *et al.* 1988, Welinder *et al.* 1994). The association between IgE and exposure is less evident. Since there is no known exposure to the OAAs in the general environment, they form an attractive model for studies of the kinetics of antibodies in occupationally exposed persons. Methyltetrahydrophthalic anhydride (MTHPA) is such an OAA causing allergic airway disease, even at low exposure levels (Welinder *et al.* 1990, Nielsen *et al.* 1992, 1994b).

Here we report results of a study of the decreases of specific IgE and IgG against MTHPA after cessation or reduction of exposure. The aim of the study is to further evaluate the association between antibody levels and exposure.

Methods

Subjects

The subjects were recruited from a plant producing and manufacturing varying plastic objects. A main product was barrels for grenade firearms, based on an epoxy resin, with a 1:1 addition of MTHPA as a hardener. After curing, the plastic barrels were processed mechanically. At the end of 1988, the workshop was reconstructed, which resulted in a significant decrease of exposure levels to MTHPA (Nielsen *et al.* 1992).

All subjects in the workforce were offered a medical and immunological investigation in 1988, which was repeated in 1989 and 1990. Thereafter, all workers handling epoxy resins have been offered yearly controls, up to 1996, of their specific antibody levels. Also, it was agreed with the company physician, that workers leaving the plant should be checked at regular intervals. Thus, it was possible to investigate the decay of antibody levels in workers after a complete cessation of exposure.

Ten workers (three females) positive in specific IgE have been included in the study, and have been tested for antibodies for up to 7·0 years after cessation of exposure. The exposure time before sampling started was between 18 and 58 months (table 1). Four persons in the group were smokers and three atopics. The mean observation period was 54 months, and an average of seven blood samples were collected from each of the workers.

In addition, four workers with high antibody titres have been tested during periods when they have been moved to work tasks within the plant with significantly reduced exposure levels to MTHPA.

Medical and immunological examinations

In each case, we recorded an occupational history and smoking habits. Venous blood samples were analysed for total serum levels of IgE and for specific IgE and IgG antibodies.

In all subjects, skin-prick tests with 13 common allergens (Allergologisk Laboratorium, Copenhagen, Denmark), and with a conjugate of MTHPA and human serum albumin (HSA; (15 mg ml^{-1}), were performed. Positive prick-test reactions were scored when the area of the wheal was at least half that induced by histamine (1mg ml^{-1}).

Subjects with a positive skin-prick test for one or more common allergens were classified as atopics.

MTHPA in air

The workers were exposed to MTHPA by inhalation of vapour released from the heated barrels. The levels of MTHPA in air have been reported elsewhere (Welinder *et al* 199

time-weighted average levels ranged from 10 to 85 μg m^{-3}. After reconstruction of the plant, the levels were reduced to about 10 % of the previous levels.

Antibody determinations

The antibody assays were performed according to methods described in detail previously (Welinder *et al* 1990). Thus, we here only present the fundamental features.

Antigen. A hapten conjugate was prepared from MTHPA (Ciba-Geigy AB, Basel, Switzerland) and HSA (Kabi, Stockholm, Sweden); 85 mg of MTHPA was added to a cooled solution of 300 mg HSA in 100 ml of 0·1 M $NaHCO_3$. The conjugate was purified from low-molecular weight compounds (< 30 000 daltons) by filtration in an Amicon ultra filtration cell (8200; Amicon Corp., Danvers, Mass., USA). The protein was lyophilized and reconstituted in 0·1 M $NaHCO_3$ when used. The hapten density of the conjugate (18 mol mol^{-1}) was determined by analysis of methyltetrahydrophthalic acid by gas chromatography after acidic hydrolysis (Welinder and Nielsen 1991, Welinder and Gustavsson 1992).

Specific IgE antibodies. The MTHPA–HSA conjugate was bound to cyanogen bromide-activated filter-paper discs for use in a radioallergosorbent test (RAST). We used the Phadebas RAST system (Pharmacia Diagnostics AB, Uppsala, Sweden), following the standard procedure. All samples were analysed in duplicate. Results were expressed as percentage specific binding (cpm of test disc – cpm of HSA reference disc) of total added radioactivity. At the end of 1992, all samples collected at that time were reanalysed in the same batch to compensate for time variations in the assays.

Specific IgG antibodies Specific IgG antibodies to MTHPA–HSA were analysed by an enzyme-linked immunoabsorbent assay (ELISA) assay. Wells of polystyrene microtitre plates (Nunc-Immuno Plate 1, Nunc, Kamstrup, Denmark) were coated by adding 100 μl of the antigen solution (150 μg ml^{-1}; 0·005 M PBS; pH 7·2). The plates were stored with blocking solution at –18 °C.

Analytical procedure: (i) Washing six times, (ii) addition of 100 μl of a 1:50 dilution (PBS) of serum, (iii) incubation for 60 min at 20 °C, (iv) washing six times, (v) addition of 100 μl of an optimal dilution of alkaline phosphatase conjugated rabbit anti-human IgG (Dakopatts, Copenhagen, Denmark), (vi) incubation for 60 min at 20 °C, (vii) washing 10 times, (viii) addition of 100 μl substrate solution (disodium *p*-nitrophenol phosphate), and (ix) incubation for 120 min at 20 °C.

The results were read at 405 nm (Titertek-Multiscan, Eflab Oy., Helsinki, Finland). All samples were analysed in triplicate and read against an HSA antigen, as a control for non-specific binding to MTHPA–HSA. All values were corrected for non-specific binding for the absorbance of positive controls on each microtitre plate. The results are expressed as the absorbance (optical density, OD) value.

The RAST and ELISA have a high specificity to specific IgE and IgG against MTHPA, as demonstrated by inhibition tests (Welinder *et al*. 1990, Welinder and Nielsen 1991).

Statistics

Individual half-times ($T_{1/2}$) of antibody decay were estimated from the model $y = A \bullet \exp(-t \bullet \ln 2/T_{1/2}) + C$ (y denotes value of IgE or IgG, t time of measurement, and A and C are constants corresponding to 'excess' level at $t = 0$ and 'background' level, respectively). Estimations were performed using non-linear regression (SPSS for Windows, SPSS Inc., Chicago, USA). Subjects were excluded from the calculations if there were less than four antibody determinations available after end/reduction of exposure, or if the value of the first determination was less than twice the upper range of referents used at our laboratory.

Spearman's rank-correlation coefficient (r_s) was used for measuring the association between variables. For comparisons of $T_{1/2}$ values between different groups, the Mann–Whitney test was used. For comparisons of (individually) paired half-time values, Wilcoxon's signed-rank test was employed. All p-values are two-tailed. 'Statistically significant' refers to $p \leq 0{\cdot}05$.

Results

Medical and immunological examinations

All the workers in the study had a long period of exposure to MTHPA before the investigation. The antibody levels in the workers were assessed for a period of up to 84 months (mean value 54 months) after complete cessation of exposure (table 1). The number of samples collected varied between 4 and 12 for the different workers. During this period the mean levels of specific IgE decreased with a factor of 8·2, while IgG decreased with a factor of 6·0. The total IgE levels varied between 10 and 211 (mean 62·5) kU l^{-1}.

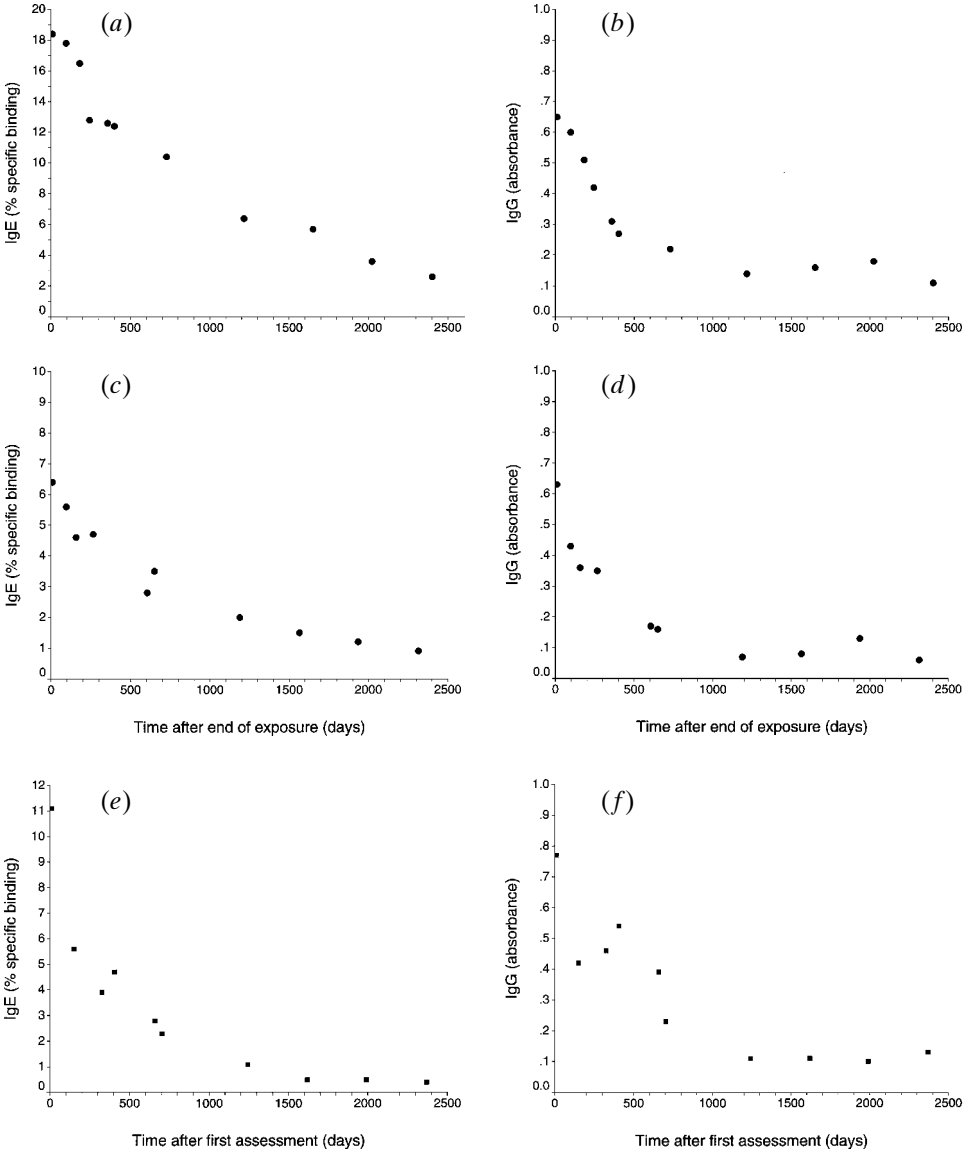

Figure 1. Levels of specific IgE and IgG to a conjugate of methyltetrahydrophthalic anhydride and human serum albumin in workers at different times after reduction or cessation of exposure. (a) IgE; cessation, (b) IgG; cessation, (c) IgE; cessation, (d) IgG; cessation, (e) IgE; reduction, (f) IgG; reduction.

The estimate of the $T_{1/2}$ values of IgE ($N = 10$) after cessation of work was 336 (range 29–655) days, and the corresponding value of IgG ($N = 8$) was 152 (range 64–235) days (table 2). Examples of the curves are shown in figure 1. All individual IgE-elimination curves implied explained variance (R^2) values > 90 %; the corresponding R^2 values for IgG were also > 90 %, except for one curve ($R^2 = 81$ %).

$T_{1/2}$ for both IgE and IgG could not be determined in all subjects; in two workers the IgG concentration was too low at cessation of exposure

Table 2. Half-time estimates of specific IgE and IgG in workers after cessation or reduction of exposure to methyltetrahydrophthalic anhydride.

	Half-times (days)			
	IgE		IgG	
Study group	*N*	Mean (range)	*N*	Mean (range)
After end of exposure	10	336 (29–655)	8	152 (64–235)
Females	3	247 (76–554)	2	70 (64–75)
Males	7	374 (29–665)	6	180 (97–235)
— $R^2 > 90$ %	10	336 (29–655)	7	164 (64–235)
After reduction of exposure	4	228 (95–475)	4	236 (99–459)

R^2, Proportion of variance explained for individual results.

individuals with available IgE *and* IgG half-time estimates ($N = 8$), the half-times of IgE were longer than the half-times of IgG ($p = 0{\cdot}01$). No clear correlation was found between $T_{1/2}$ of IgE compared with IgG ($N = 8$, $r_s = 0{\cdot}12$, $p = 0{\cdot}7$; Figure 2).

A statistically significant association was found between the initial values of IgE and IgG, respectively, and $T_{1/2}$ (IgE: $r_s = 0{\cdot}78$, $p = 0{\cdot}02$; IgG: $r_s = 0{\cdot}76$, $p = 0{\cdot}04$).

No correlation was seen between total IgE and biologic half-times of specific IgE ($r_s = 0{\cdot}18$, $p = 0{\cdot}60$). Nor was there any significant effect of atopy or smoking habits on $T_{1/2}$ of IgE ($p > 0{\cdot}4$). Gender had no significant effect on $T_{1/2}$ for IgE or IgG, but for IgG a tendency of a lower half-time was observed for females compared with males ($p = 0{\cdot}07$; table 2). However, the number of determinations as low.

For sensitized workers, who continued to work at the plant, the antibody levels varied considerably. However, significant decreases in titres were observed during periods when they were submitted to work tasks which involved reduced exposure to MTHPA. The means of individual $T_{1/2}$ estimates for IgE ($N = 4$) and IgG ($N = 4$) in workers after significant reduction -but not total avoidance- of exposure were 228 (range 95–475) and 236 (range 99–459) days, respectively (table 2). There was no significant differences in $T_{1/2}$ of IgE ($p = 0{\cdot}40$) or IgG ($p = 0{\cdot}40$) for those

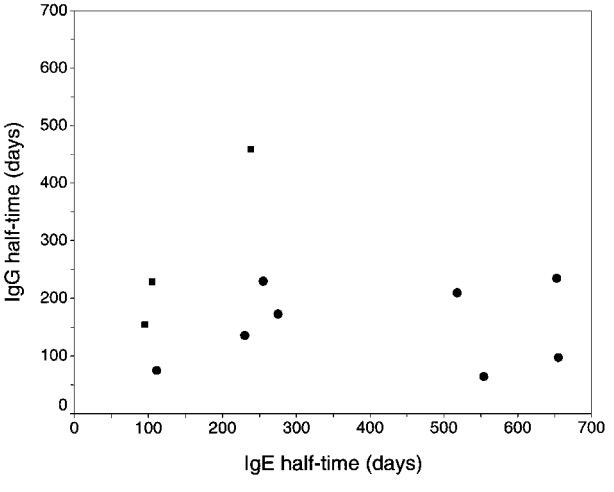

Figure 2. Correlation between biologic half-times for specific IgE and IgG in workers exposed to methyltetrahydrophthalic anhydride after reduction or cessation of exposure (● = cessation; ■ = reduction).

subjects that remained at the plant at low exposure levels ($N = 4$) compared with those who left work ($N = 10$).

Discussion

The results show a mean biologic half-time for IgE and IgG antibodies of 0·9 and 0·4 year, respectively, after total avoidance of exposure. A corresponding decrease of antibody titres could also be seen after a reduction, but not total elimination, of exposure. In this case, the mean of the individual half-times for both IgE and IgG antibodies was 0·6 year.

The decay of antibody titres was analysed as an exponential elimination of the first order. Generally, the fit was good. However, the fit of a first order exponential elimination curve to each individual data set varied to some extent. This was expected, especially since the RAST and ELISA methods are semi-quantitative, and do not give a linear relation between antibody concentrations and RAST and ELISA values, respectively, over a wide measuring range. Also, the assumption of a first order kinetics is not strictly justified by the related values of IgE and IgG at the start of the observation period and the $T_{1/2}$ values. However, the explanation of variance was > 90 % for most individuals.

Though the individual values varied considerably, the mean half-time of 0·9 year for IgE after end of exposure in the present study, is in agreement with the $T_{1/2}$ of 1 year in six tetrachlorophthalic-anhydride exposed workers after they had left their jobs (Newman Taylor *et al.*, 1987, Venables *et al.* 1987). There is, to our knowledge, no corresponding study on IgG. Grammer *et al.* (1995) made a follow-up study on workers exposed to hexahydrophthalic anhydride (HHPA) after removal from exposure. There was a decrease in antibody titres in the majority of workers, but it was not possible to calculate $T_{1/2}$ values as the follow-up time was too short. However, like in the present study, a variation in the individual elimination rates was observed. The background of this variation is not known. The difference in $T_{1/2}$ for IgE and IgG demonstrated in this study is interesting.

Atopy or total IgE did not affect the elimination kinetics. Hence, the general tendency to produce IgE seems not to be crucial. Neither was there any significant effect of smoking or gender.

The decrease of antibody levels after reduction but not complete avoidance of exposure corresponds with observations by McGrath *et al.* (1984) and Boxer *et al.* (1987) on workers exposed to trimellitic anhydride. However, the pattern is not consistent. While, in workers exposed to TMA, some antibody titres decreased, in others fluctuating titres were seen during the follow-up period (Boxer *et al.* 1987). The estimates of the biologic $T_{1/2}$ in the present study are interesting. They indicate that antibody titres may decay at the same rate as after the end of exposure, even though the exposure is not totally interrupted. It is a clinical and practical problem to decide whether a sensitized worker should be recommended to leave work or if other arrangements may be considered. The present results indicate that a replacement within the plant, or effective preventive measures, may in fact reduce antibody titres significantly.

The formation of specific IgG antibodies to low-molecular weight chemicals have been suggested as biomarkers of exposure (Biagini *et al.* 1990). An exposure–response relationship between exposure intensity and IgG formation has been demonstated in cross-sectional studies of workers exp

HHPA (Welinder *et al.* 1990, 1994), while a corresponding correlation for IgE was less evident. However, in a recent study, no statistically significant correlation was found between specific IgG and exposure intensity measured by urinary excretion of hexahydrophthalic acid in workers exposed to HHPA (Jönsson *et al.* 1997). The evaluation of the decay curves in the present study suggests that antibody titres in sensitized groups of workers may reflect long term alterations in exposure. For a more detailed evaluation, the kinetics of antibody formation should also be considered. Thus, the initiation period of antibody formation in exposed workers may be months/years (Newman Taylor *et al.* 1987). However, normally less than 50 % in exposed groups develop specific antibodies (Welinder 1990, 1994). Thus, antibody titres are of limited value as biomarkers of exposure.

In conclusion, the present results indicate that the levels of specific antibodies may persist for years after the end of exposure. The kinetics of the decrease is not affected by atopy, smoking habits or gender. A reduction in antibody titres in sensitized individuals may at a group level indicate a change in long term exposure.

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