

Specific IgE antibodies in twenty-eight workers with diisocyanate-induced bronchial asthma

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Summary

A specific IgE-mediated response was evaluated in twenty-eight workers exposed to TDI or MDI, with diagnosis of occupational asthma and positive to bronchial provocative challenge. The presence of anti-diisocyanate IgE was observed in 27% of subjects exposed to TDI and 83% of those exposed to MDI, particularly in individuals who experienced an acute massive exposure. An immediate-type response to bronchial provocative test was found in 66% of individuals with specific antibodies. Specific IgE are prevalent (91%) in subjects who developed symptoms before 6 years of exposure to isocyanates. The results suggest an association between the presence of specific IgE, early asthmatic symptoms and heavy episodic exposure.

Introduction

Toluene diisocyanate (TDI) and diphenylmethane diisocyanate (MDI) have been reported to induce an asthma-like response in as many as 5% of occupationally exposed workers, with features suggesting the involvement of the immune system (N.I.O.S.H., 1979). Studies on the pathogenesis of isocyanate asthma have yielded conflicting results (Butcher *et al.*, 1976; Davies *et al.*, 1977; Gallagher *et al.*, 1981; Danks *et al.*, 1981); however, IgE- and/or IgG-mediated immune reactions are involved at least in a subset of symptomatic exposed subjects.

We evaluated the role of humoral immune reactivity in relation to the clinical features of the respiratory syndrome induced by isocyanates in twenty-eight subjects exposed to TDI or MDI, with diagnosis of occupational asthma, by detecting serum immunoglobulin levels and specific anti-diisocyanate IgE antibodies. These data were correlated with the length of exposure, the timing of appearance of respiratory symptoms, and the type of response to specific and aspecific bronchial challenge.

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Specific IgE antibodies were detected in 39% of patients, particularly in subjects who developed respiratory symptoms after a short period of exposure.

Materials and methods

Subjects

Twenty-eight isocyanate exposed workers, eighteen males and ten females, with diagnosis of occupational asthma based on symptoms and positivity to specific bronchial provocative test (BPT), were studied. All subjects reported wheezing, chest tightness and dyspnoea when isocyanates were used in the working area; these symptoms disappeared at weekends or after short periods of absence from work. Six of them were exposed to MDI in a plant producing polyurethane foam shoe soles, while the remaining twenty-two subjects worked in various furniture plants and used two pot polyurethane varnish with activator.

Exposure time ranged from 1 to 35 years (mean 12.2 years). Symptoms started at an average of 6.4 years (range 0.5–19 years) after first isocyanate exposure. The time interval between the last exposure to isocyanate and serum collection ranged from 4 to 30 days.

Specific bronchial provocative test

Toluene-diisocyanate (TDI) bronchial provocative occupational type test was performed according to Zedda *et al.* (1976). The subjects sprayed the polyurethane varnish with TDI activator for 1 min in a cubicle of 12 m³ in which they were exposed for a maximum of 5 min. Atmospheric concentration of TDI ranged from 0.08 to 0.18 mg/m³ and was measured by the method of Marcali (1957), slightly modified.

Diphenylmethane-diisocyanate (MDI) bronchial provocative test was performed by mixing polyurethane resin with MDI. The patients were exposed in the same cubicle for 10 min to vapours released from the reacting mixture; atmospheric concentration of MDI, measured near the mouth of the patients by the same method, ranged from 0.10 to 0.18 mg/m³.

Control tests were performed with the diluent for polyurethane varnish or with resin without MDI.

All patients were studied in a symptom-free period, after stopping therapy 48 hr before testing. VC, FEV₁ and FEF_{25–75} were measured before and 15, 30 and 60 min after challenge by means of a Pulmotest Godart Spirometer. The measurements were repeated at 2, 4, 6, 8 and 24 hr thereafter. Significant airway response consisted in 20% drop in FEV₁ and/or a 35% drop in FEF_{25–75} from baseline values.

Normal subjects and asthmatic non-exposed workers with bronchial hyperresponsiveness, employed as controls, showed no significative response to BPT with isocyanates.

Bronchial hyperresponsiveness

Bronchial hyperresponsiveness was evaluated using an aerosol of bethanechol (5 mg/ml) by means of a Bird Mark 7 Nebulizer in intermittent positive pressure breathing. The number of breaths was gradually increased up to a maximum of seventy breaths (1 + 4 + 5 + 10 + 10 + 20 + 20). The procedure was stopped when a 20% drop in FEV₁ occurred, or, in the absence of any airway response, when the maximum number of breaths was reached.

This technique showed to discriminate normal subjects from patients with extrinsic non-occupational asthma (Parlanti *et al.*, in press).

Skin testing

A standard intradermal test for immediate or late response was performed with a panel of fourteen common inhalants and pollens (R. Lofarma Series). The skin-test reactions were read at 20 min for wheal and erythema and after 4–8 hr for erythema and induration. The criteria used to read intradermal skin tests were derived from Vanselow (1964); subjects showing two or more positivities were considered atopic.

Immunoglobulin evaluation

The quantitative determination of serum IgG, IgA, IgM was performed by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) employing Kallesstad immunodiffusion plates.

Total IgE values were determined by a direct radioimmunoassay technique (Phadebas PRIST kit, Pharmacia Diagnostics). Serum IgE normal range was 0–200 U/ml as determined in a population of sixty healthy subjects.

Specific anti-isocyanate IgE determination

Diisocyanates (TDI or MDI) were conjugated to human serum albumin (HSA) according to Scheel, Killens and Josephson (1964), slightly modified: equimolecular solutions of TDI (20 mg, Eastman Kodak Organic Chemicals, New York) or MDI (29 mg, a generous gift of Dr A. Cossi, Stabilimento Petrolchimico Montedison, Porto Marghera-Venezia, Italy) in 100 ml dioxane were added to a solution of 1% HSA in 0.1 M pH 9.4 borate buffer, at 0°C (molar ratio: 8 mol isocyanate added per mol HSA). The reaction mixture was brought to equilibrium by stirring and exhaustively dialysed against phosphate-buffered saline; the average number of mol of diisocyanate substituted to the protein, determined by a modified Gutmann assay (Modesto & Pesce, 1973), was 5 and 5.5 for TDI and MDI, respectively. Control solutions consisted of 100 ml dioxane similarly added to 1% HSA. Ten ml of 20 mg/ml diisocyanate-HSA or dioxane-treated HSA control solution in borate buffer was coupled to 250 mg of filter paper discs activated by the method of Ceska and Lundkvist (1972), to be used for a paper radioallergosorbent test (RAST) for specific anti-diisocyanate IgE antibodies.

Aliquots of 100 µl of test sera were added to each tube containing either antigen- or HSA-coated disc and incubated for 10 hr. After washing ($\times 3$) with saline solution additioned with Tween 20, 50 µl (35 000–40 000 cpm) anti-IgE ^{125}I (Phadebas RAST Reagent, Pharmacia Diagnostics) was added to each tube; the mixture was incubated 16–20 hr, washed and counted on a gamma counter (LKB 1270 Rackgamma II). Sera were processed in duplicate; specific IgE were evaluated as net bound radioactivity percent from the following formula:

$$\frac{\text{test bound radioactivity \%} - \text{blank bound radioactivity \%}}{\text{net bound radioactivity \% (NBR)}} =$$

mean blank bound radioactivity %: 0.48. NBR levels between 0.6 (mean plus 2 s.d. of a control group of fifty non-exposed subjects) and 0.9 were considered 'questionable' values for the presence of specific IgE, while NBR values higher than 0.9 (mean + 4 s.d.) were considered positive for specific IgE antibodies; all data were confirmed in at least two experiments. Moreover, RAST inhibition experiments with TDI-HSA or

Immunoglobulin quantitation

Immunoglobulin (IgG, IgA, IgM) quantitation showed normal values in twenty-six out of twenty-eight (93%) patients; slight variations of IgA or IgM levels were found in two individuals. Elevated total IgE were observed in five subjects: three of them also had positive skin tests suggestive of atopy, one had an isolated positivity associated with familial history of atopy.

Specific anti-diisocyanate IgE

Positivity for specific anti-diisocyanate IgE was revealed in eleven out of twenty-eight (39%) patients—i.e. in five out of six (83%) subjects exposed to MDI and in six out of twenty-two (27%) exposed to TDI. Moreover, seven out of twenty-eight (25%) subjects, all exposed to TDI, had NBR levels ranging between 0.6 and 0.9; one of the non-exposed controls showed a NBR value within the same range (Fig. 2). A highly

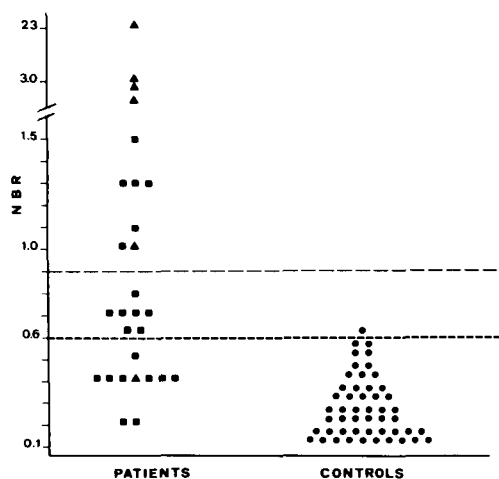


Fig. 2. Anti-diisocyanate IgE levels in twenty-eight patients with occupational asthma exposed to TDI (■) or to MDI (▲) and in fifty healthy non-exposed controls (●). NBR: net % of total radioactivity bound; (---): mean titre plus 2 s.d. of the control group; (—): mean titre plus 4 s.d. of the control group.

significant difference (exact Fisher's test, $P=0.18 \times 10^{-5}$) was observed when specific IgE-positive patients (eleven subjects with $NBR > 0.9$) were compared with non-exposed controls with $NBR < 0.6$ (forty-nine subjects). In order to evaluate a possible correlation, total IgE were plotted against NBR levels (Fig. 3) and a linear regression analysis was performed. No correlation was demonstrated neither between these parameters ($r=0.3$) nor between specific IgE, positivity to skin testing and familial history of atopy.

In order to assess the specificity of the test, RAST inhibition experiments were performed in nine out of eighteen subjects selected from positive and 'questionable' values; the results are reported in Table 2. The patients with NBR levels > 0.9 showed various degrees of inhibition, ranging from 30 to 99%.

Table 3 shows that the mean time between the first exposure to isocyanate and appearance of respiratory symptoms was 4.4 years in patients with specific IgE (3.7 years in subjects with 'questionable' results and 11.2 years in those without detectable antibodies). Moreover, the majority (ten out of eleven) of the IgE positive and of the

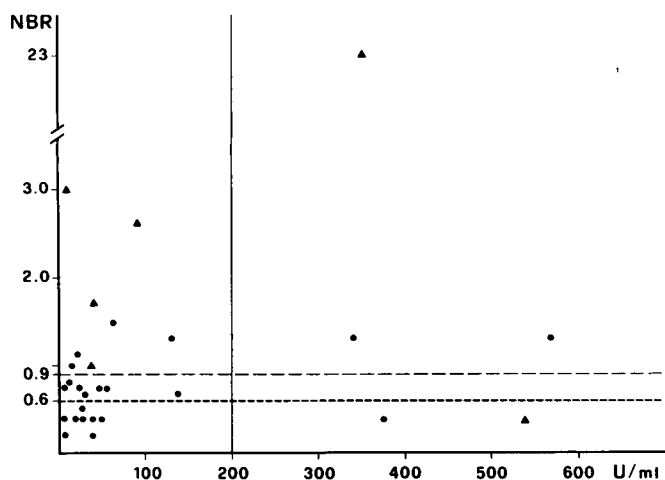


Fig. 3. Correlation between total IgE (U/ml) and net bound radioactivity % (NBR) levels in twenty-eight asthmatic patients exposed to TDI (●) or MDI (▲); (---): mean titre plus 2 s.d. of the non-exposed group (— — —): mean titre plus 4 s.d. of the non-exposed group; vertical line: upper normal value of total IgE (200 U/ml).

Table 2. Specific inhibition with TDI-HSA or MDI-HSA conjugates in nine patients with positive (1–6) or 'questionable' (7–9) NBR levels

Patient No.	Exposure	inhibition NBR	%	Total IgE (U/ml)
1	MDI	23	96	350
2	MDI	2.6	93	89
3	TDI	1.5	40	63
4	TDI	1.3	54	341
5	TDI	1.1	99	22
6	MDI	1.0	30	35
7	TDI	0.8	32	9
8	TDI	0.7	57	22
9	TDI	0.65	10	138

The mean values of two experiments are reported.

'questionable' (six out of seven) subjects became symptomatic during the first six years of exposure, while only three out of ten subjects without such antibodies showed symptoms during this period. The appearance of respiratory symptoms before 6 years of exposure, more frequent in the IgE-positive group, showed a highly significant difference ($P=0.65 \times 10^{-2}$) from the IgE-negative group.

The presence of specific IgE was associated in 66% of cases with the immediate-type response to BPT, while dual and late reactions were uniformly distributed among IgE-positive and -negative individuals. Bronchial hyperresponsiveness, tested in eight RAST-positive, seven 'questionable' and nine RAST-negative subjects, was prevalent in the latter patients (one, three and five positive individuals, respectively).

Table 3. Correlation between time of appearance of respiratory symptoms and presence of specific anti-isocyanate IgE

Appearance of symptoms	Subjects with specific IgE		
	Positive	Questionable	Negative
Before			
6 years	10	6	3
After			
6 years	1	1	7
Mean time (years)	4.4	3.7	11.2

Discussion

IgE levels indicative of specific anti-diisocyanate-HSA antibodies were detected in 11 out of twenty-eight (39%) workers with isocyanate asthma showing a positive BPT; these values are higher than those reported by some authors (Butcher *et al.*, 1980; Baur & Fruhmann, 1981; Gallagher *et al.*, 1981), whereas Karol (1980) succeeded in detecting a higher percentage of specific IgE response in a selected series of TDI asthmatic patients. However, MDI-exposed subjects gave 83% of positive results, while only 27% of the TDI-exposed group showed specific IgE antibodies, a value comparable with those found by authors (Butcher *et al.*, 1980; Baur & Fruhmann, 1981) who studied mainly subjects exposed to TDI.

Discrepancies between various laboratories may be due to differences in the assay or in the evaluation of test results. In our study, the RAST technique was performed with bifunctional isocyanate-HSA conjugates instead of the widely used monofunctional *p*-tolyl isocyanate-HSA conjugates (Karol *et al.*, 1979; Butcher *et al.*, 1980; Gallagher *et al.*, 1981), as cross-reactivity studies (Riviera & Pezzini, unpublished data) showed that, in diisocyanate (TDI, MDI)-exposed individuals, specific antibodies were best detected by bifunctional isocyanate-HSA conjugates. RAST values were considered positive only when higher than the mean + 4 s.d. of the control group, as one non exposed subject showed a NBR value close to the mean + 2 s.d. adopted as cutoff level by some authors (Karol *et al.*, 1979; Baur, 1983); RAST inhibition experiments support the specificity of the test.

The specific IgE-positive group seems characterized by an earlier onset of respiratory symptoms than the IgE-negative subjects, without correlation with the isocyanate of exposure. However, in our series, the incidence of specific IgE differs considerably in TDI- or MDI-exposed individuals; it may be suggested that MDI, a less reactive molecule than TDI, may act as a more effective hapten *in vitro* by preventing cross-linking of HSA-carrier molecules. However, previous studies (Riviera *et al.*, 1983) showed a 100% cross-reactivity between MDI-HSA and TDI-HSA conjugates in specific IgE-positive subjects exposed to MDI. Up to now there is no observation supporting the hypothesis that MDI may act *in vivo* as a more active allergen than TDI. TDI- and MDI-exposed workers of this study may differ as regards the type of environmental exposure to isocyanate; in fact, unlike TDI-exposed subjects, three out of six MDI patients experienced an accidental exposure to high

concentrations of isocyanate (because of the bursting of a MDI tank), and afterwards developed respiratory symptoms even at low concentrations of MDI; Karol (1981) also suggested that episodic heavy exposure to isocyanate may be critical for induction of IgE-mediated asthma.

Bronchial hyperresponsiveness is not a constant feature of isocyanate induced bronchial asthma (Butcher *et al.*, 1979; O'Brien *et al.*, 1979); our data are in agreement with such observations, even if the percent of bronchial hyperresponsiveness was somewhat lower than the values reported.

In conclusion, our results show a prevalence of specific immunological IgE-mediated reactions in subjects who develop asthmatic symptoms after a shorter time of isocyanate exposure and experienced an accidental acute exposure to high concentrations of isocyanate.

Acknowledgments

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