

Experiences of specific IgE in asthma due to diisocyanates

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Summary

The specific IgE antibodies were studied with the Phadebas® RAST technique in 35 patients with asthma due to diisocyanates. Two had been sensitized to toluene diisocyanate (TDI), 17 to methylene diisocyanate (MDI) and 16 to hexamethylene diisocyanate (HDI). In each case the diagnosis was confirmed with a bronchial provocation test (BPT). The asthmatic reaction was immediate in 17 cases, of which three had also a late reaction (dual). Eighteen patients reacted only with a late reaction. Seven (20%) had specific IgE to diisocyanates. All RAST-positive patients had an immediate asthmatic reaction. None of the late reactors and referents had positive RASTs. RAST inhibition tests with 94–100% inhibition confirmed the specificity of the method. There was cross-reactivity between different diisocyanates, however. The patients with positive RAST to diisocyanates had a higher level of total IgE than the RAST negative group and the referents.

Introduction

Asthma due to diisocyanates is a problem in occupational medicine. In Finland it is the most usual occupational asthma caused by chemicals. The mechanism of the asthmatic reaction is not clear. The possibility of an immunological mechanism has been much investigated. Because of the great reactivity the determination of specific IgE antibodies has been difficult. Specific IgE to mono- or diisocyanates has been demonstrated; however, the specificity of the antibodies to monoisocyanates has been questioned [1–4]. In 1983 Baur reported his results on diisocyanate RAST, which were confirmed with RAST inhibition. He also found cross-reactivity between different diisocyanates [5]. Cross-reactivity has also been demonstrated between TDI, MDI and HDI in inhalation challenge tests [6]. Pezzini *et al.* reported 28 cases of asthma due to TDI or MDI; 11 of these had specific IgE to diisocyanate [7]. RAST inhibition varied

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Table 1. Sources of exposure

Exposure	No. subjects
TDI: Polyurethane (PU) foaming, TDI-cured varnish	(2)
MDI: Insulation of windows, doors and plumbing	(9)
Manufacturing of PU products	(5)
MDI-cured glue	(2)
Packing PU components	(1)
HDI: HDI-cured paints (13 car spray painters, two plate metal workers, one paint factory worker)	(16)

Table 2. Time of exposure before symptoms

No. subjects	Time
17/35	1 year or less (49%)
11/35	2-4 years (31%)
5/35	5-10 years (14%)
2/35	more than 10 years (6%)

from 30 to 99%. They also found an association between the presence of specific IgE, early symptoms and heavy exposure to diisocyanates [7].

Our material consists of 35 cases of occupational asthma due to diisocyanates.

Patients and methods

In 1983-1986 40 cases of verified asthma due to diisocyanates were diagnosed, based on asthmatic symptoms related to diisocyanate exposure and on the specific bronchial provocation tests. The sera of five cases were not available; our material thus consists of 35 cases. Twenty-eight of the patients were males and seven were females. Their age varied between 19 years and 61 years (mean 37 years). Twelve were smokers. The sources of exposure are presented in Table 1. Table 2 shows the time of exposure before the first symptoms were noted.

As exposed referents, the sera of 46 symptomless persons (30 to MDI in a refrigerator factory and 16 to HDI in three car paint shops) were used. Blood specimens were obtained from all exposed workers in these workshops. Forty sera from the Finnish blood donor pool were used as unexposed referents.

Bronchial provocation tests

The bronchial provocation tests were carried out according to a slightly modified version of the method described by Newman-Taylor and Davies [8]. In brief, tests with TDI and MDI were done with rising concentrations from 0.002 p.p.m. to 0.02 p.p.m., one test per day. As a negative control test we used polyol, the inert component of the

polyurethane foam. Tests with HDI were carried out with spray painting with HDI-cured paint. The paint without hardener was used as placebo test. The bronchial reaction was registered using a Wright's peak expiratory flow meter (PEF) during 24 hr. A 20% fall was regarded as a positive reaction. A PEF drop of 15–19% was considered positive if there were other supporting findings: wheezing rales, a dose-effect relationship or a consistent pattern of the 2-week surveillance at home and at work (two cases). The provocation test of one patient (Table 3, no. 7) was stopped when he had a 15% PEF fall because of severe urticaria, which he also had experienced at work.

The reaction was regarded as immediate when the PEF fall was deepest during the first hour after the challenge. All other reactions were late ones. Seventeen patients had an immediate type asthmatic reaction in the bronchial provocation test, three of them also a late one. Eighteen patients showed a late reaction. The type of diisocyanate did not affect the pattern of the reaction (Fig. 1).

Skin tests

Skin-prick tests with 17–30 common allergens, including animal epithelia, pollens, house dust mite and moulds, were carried out according to the method of Aas and Belin [10]. Atopy was defined as one or more reactions equal to or greater than half of the reaction to histamine, in addition to a negative reaction with the diluent.

Total and specific IgE antibodies

Total IgE values were determined by the enzyme immunoassay technique (Phadezym PRIST kit, Pharmacia Diagnostics, Uppsala, Sweden). The determination of specific IgE antibodies to diisocyanates was performed by enzyme immunoassay with commercial kits (Pharmacia Diagnostics, Uppsala, Sweden). Diisocyanate Allergo-Discs by MIAB (Knivsta, Uppsala, Sweden) were used. The results were reported as ratio values: absorbance of diisocyanate-HSA conjugate disc/absorbance of HSA disc. The ratio value 2 was the limit of positivity. Absorbance readings of less than 1/2 of the absorbance readings of the D-reference point (from the Phadezym RAST kit) were considered negative, independent of their actual ratio.

Inhibition tests

The following substances were used for the inhibition tests: TDI, a mixture of 2,4- and 2,6-toluene diisocyanate (80:20) and MDI, 4,4'-diphenyl methane diisocyanate from Merck-Suchardt (Münich, FRG) and HDI, hexamethylene diisocyanate from Fluka (Buchs, Switzerland). The diisocyanate-HSA conjugates were prepared according to Wass and Belin (Sahlgrenska Hospital, Gothenburg, personal communication [10]). Briefly, 100- μ l solutions of TDI, MDI and HDI were dissolved in 5 ml of acetonitrile, and 0.5 ml of the stock solutions were then dissolved in 4.5 ml of acetonitrile. One millilitre of the solutions was then added dropwise to 20 ml 0.05 M boric acid buffer (pH 9.4) containing 60 mg HSA (Human Albumin, Fraction V, Sigma A-1653; St Louis, MO, U.S.A.). After stirring for 30 min in an ice bath, the solutions were filtered, dialysed against 0.1 M phosphate buffered (pH 7.4) saline (0.15 M NaCl) and concentrated to 20 mg/ml HSA using ultrafiltration through a Diaflo YM-5 membrane (Amicon Corp., Danvers, MA, U.S.A.). For the inhibition studies, HSA solution was prepared by dissolving 20 mg of HSA in 1 ml of phosphate buffered saline (PBS). In order to measure the inhibitory capacity of diisocyanates, serial dilutions (20, 2, 0.2

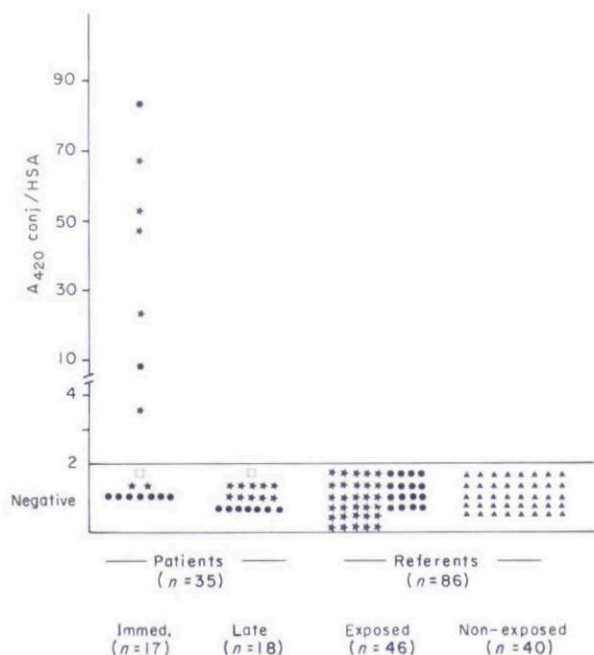


Fig. 1. Absorbance ratio of the patients (immediate and late reactors in BPT) and of referents. Exposure to (□) TDI, (★) MDI, (●) HDI, (▲) non-exposed.

and 0.02 mg/ml) of the HSA conjugates in PBS were allowed to react with the patient's serum (20 μ l of conjugate dilution + 50 μ l of serum) overnight at 4°C. The mixtures were then used for RAST determination. Phosphate buffered saline (20 μ l of serum) was used to obtain the uninhibited results. The degree of reduction for serum RAST values after the absorption was expressed as an inhibition percentage.

Results

Skin tests

Eleven patients (31%) had at least one positive skin-prick test with common allergens. Seven were immediate reactors and four were late reactors.

Total IgE and specific IgE to diisocyanates

Total IgE of all patients ranged from 2 to 560 kU/l, median (Md) 62. The immediate reactors had values from 19 to 540 kU/l, Md 90; in diisocyanate RAST-positive subjects 62 to 540 kU/l, Md 185 and in RAST-negative subjects 19–540 kU/l, Md 55. In the group with a late reaction in BPT, the total IgE ranged from 2–560 kU/l, Md 46. The median value of total IgE of all RAST-negative patients was 48. The total IgE of exposed referents was between 5 kU/l and 720 kU/l, Md 32 and of non-exposed referents 5–900 kU/l, Md 40.

The difference between total IgE values of RAST-positive and RAST-negative immediate reactors was not statistically significant, whereas there was a statistical significance between RAST-positive and all RAST-negative patients ($P < 0.02$, Mann-Whitney U -test) and also between RAST-positive patients and exposed referents ($P < 0.01$) and non-exposed referents ($P < 0.02$).

Table 3. The absorbance ratio with different diisocyanates, total IgE, atopy and smoking habits of seven patients with specific IgE

Case no.	Absorbance ratio			Total IgE (kU/l)	Atopy	Smoking
	MDI	HDI	TDI			
<i>MDI</i>						
1	65.9	28.0	18.3	540	—	—
2	52.4	16.9	21.6	250	+	—
3	18.5	18.0	9.3	62	+	+
4	43.4	23.3	36.3	256	—	+
5	23.0	5.0	7.3	90	—	—
<i>HDI</i>						
6	15.7	82.6	21.0	185	+	+
7	5.0	7.8	2.7	90	—	+

Table 4. RAST inhibition percentage with different diisocyanate conjugates and HSA

Case no.	MDI-HSA	HDI-HSA	TDI-HSA	HSA
<i>MDI</i>				
1	95	79	39	8
2	98	81	77	0
3	93	93	86	24
4	96	61	50	0
5	94	45	64	4
<i>HDI</i>				
6	67	100	60	31.5
7	88	100	60	11.4

Seven patients (20%) had a positive RAST test. Five had been exposed to MDI and two to HDI. All of them had had an immediate type asthmatic reaction in the challenge test, one a late reaction as well. None of the late reactors had specific IgE to diisocyanates. There was no positive RAST test among the exposed referents. Likewise, all the unexposed referents were RAST-negative. The results of diisocyanate RAST determinations are given in Fig. 1. Table 3 shows the absorbance ratios using three different diisocyanates (TDI, MDI and HDI). The sera of MDI asthmatics with a positive MDI RAST test gave also positive results to TDI and HDI. Likewise, the two sera of workers with HDI asthma had positive RASTs to TDI and MDI. Three of the seven RAST-positive (Table 3) and four of the 10 RAST-negative immediate reactors were atopics. Four of the seven RAST-positive patients (Table 3) and one of the 10 RAST-negative immediate reactors were smokers. No statistical significance could be found between RAST positivity, smoking habits and atopy (Mantel-Haenszel).

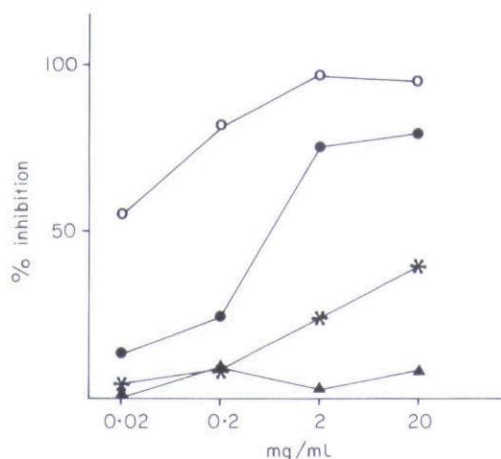


Fig. 2. Patient no. 1, RAST inhibition with different concentrations of diisocyanate conjugates. (○) MDI-HSA, (●) HDI-HSA, (*) TDI-HSA, (▲) HSA.

RAST inhibition tests

MDI-HSA caused a 93–98% inhibition when the sera of MDI asthmatics were investigated. However, TDI-HSA also gave 39–86% and HDI-HSA 45–93% inhibition. HDI-HSA inhibited the RAST tests of HDI asthmatics by 100%; TDI-HSA inhibited the tests by 60% and MDI-HSA by 67–88%. The results of all seven patients are presented in Table 4, and of patient no. 1 in Fig. 2.

RAST inhibition studies using the sera of a person with a birch pollen RAST score of 4 and total IgE of 230 kU/l gave a zero inhibition with all three diisocyanate-HSA conjugates.

RAST and time of exposure

Half of the patients had got their first symptoms during the first year of exposure (Table 2). All except one (86%) of the seven RAST-positive workers had contracted asthma in 1 year or less. The exposure time prior to symptoms of the only exception was 2.5 years. Eleven (39%) of the 28 persons with negative RAST had an exposure time of less than 1 year. The difference was not statistically significant ($P=0.1$ Hypergeometrical Distribution).

Discussion

Specific IgE was found in 20% of the cases of asthma due to diisocyanates. The corresponding result of Baur was 14% [5], whereas the RAST positivity reported by Pezzini *et al.* [7] was as high as 83% in MDI-sensitized and 27% in TDI-sensitized persons. By comparison, we found specific IgE in five (29%) out of 17 workers with MDI-induced asthma, and in two (13%) out of 16 with HDI-induced asthma. Our material comprised only two cases of TDI asthma, both of which were RAST negative. All patients with a positive diisocyanate RAST test had an immediate type asthmatic reaction in the bronchial provocation test, whereas all late reactors were RAST negative. These circumstances may be construed as further indication of IgE mediation of the immediate reactions. The immunological cross-reactivity between different diisocyanates reported previously was corroborated [5]. The RAST inhibition tests supported the specificity of IgE antibodies to MDI and HDI. The minimal binding

with HSA alone does not indicate that the protein would be the cause of the cross-reactivity.

The median value of total IgE was higher in the sera of patients with positive RAST than in that of the patients with negative RAST or the referents. This was not fully understood, as two of the four subjects with high total IgE did not display any degree of atopy in the skin-prick tests. The unspecific binding of the reactive diisocyanates with any IgE group was considered unlikely, as the birch pollen RAST test of one subject highly sensitized to birch pollen could not be inhibited at all by any of the diisocyanate-HSA conjugates.

In earlier studies of diisocyanate asthma, atopy has been considered to be of little relevance. The distribution of atopy in our material also corresponded to expected values among the general population; 31% in the whole material. Not even in the RAST-positive group of immediate reactors did atopy seem important; only three out of seven were atopic.

An association between smoking and atopy and the production of specific IgE antibodies has been claimed [12]. We could not corroborate such an association in this small sample of diisocyanate-induced asthmatics.

The presence of specific IgE antibody has been reported to be associated with early onset of symptoms [7]. We observed a similar trend; however, there was no statistical significance ($P=0.1$) between these small groups.

In conclusion, an IgE-mediated reaction explained only less than half (7/17) of the immediate reactions in the bronchial provocation test. None of the late reactors had specific IgE to diisocyanates. High total IgE was associated with RAST positivity. The RAST method seems to be reliable in determining specific IgE to diisocyanates. While the positive RAST test aids the diagnosis of asthma due to diisocyanates, a negative test by no means excludes diisocyanate-induced asthma. It is, therefore, of limited use as a substitute for inhalation challenge tests; this remains the most reliable way of confirming the diagnosis.

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