

IgG antibodies against polyisocyanates in car painters

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Summary

A group of thirty car painters exposed to vapours and aerosols of paint containing prepolymer and monomer of hexamethylene diisocyanate (HDI) was investigated. Specific antibodies against monomer HDI and prepolymerized HDI were analysed with RAST (IgE) and ELISA (IgG) assays after conjugation of the haptens with human serum albumin. There was no significant increase of serum IgG antibodies against HDI monomer, nor of specific IgE antibodies against HDI monomer or prepolymer. Specific IgG antibodies against prepolymerized HDI were significantly increased, as compared with non-exposed referents (medians 0.11 *vs* 0.03 absorbance (A)). Six car painters were found to have specific IgG antibodies of subclass 4 against HDI prepolymer, four also against HDI monomer. This shows an association between exposure and specific IgG antibodies. Thirteen subjects had suffered symptoms of rhinitis and/or conjunctivitis, and ten had symptoms from the bronchi (two asthma). There was no significant association between symptoms and levels of specific antibodies. Most of the symptoms were slight and unspecific, probably due to irritative effects of the exposure.

Introduction

There are numerous reports on the adverse effects of exposure to vapours of monomeric isocyanates, e.g. toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), and methylene difenyl isocyanate (MDI; vapour and aerosol). They may cause irritative effects in the mucous membranes, as well as asthma, and hypersensitivity pneumonitis [1–5].

Type 1 allergy caused by specific IgE antibodies may be the pathological mechanism behind the asthma in some sensitive subjects. In addition, specific IgG antibodies have been found [6, 7]. This is of particular interest as the hypersensitivity pneumonitis shares many features with the type 3 response.

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Polyisocyanates (prepolymerized isocyanates)

These have a wide use in many areas. Data on the effects of such exposure are scanty. It is clear, however, that they may cause both asthma and hypersensitivity pneumonitis [3, 8, 9]. The presence of specific IgE antibodies against a prepolymerized product of HDI in the serum of a single car painter has been reported [3].

Spray painting is known to produce a massive exposure to an aerosol of isocyanate, in car painting (prepolymerized aerosol of HDI and vaporous HDI [10, 11]), as well as in spray lacquering (aerosol of prepolymerized TDI and vaporous TDI [9]).

We here report on a study on specific antibodies and adverse effects in a group of thirty car painters.

Subjects and methods*Subjects and sampling*

Thirty car painters in the county of Kronoberg, known to the factory inspectorate or listed in the telephone directory, and with no access to industrial medical health care, took part in the investigation. All were men with an average age of 35 years. They had been working as painters on average for 15 years, range 1.5–40 and with isocyanate paints for 8 years, range 0.3–20.

An industrial health nurse obtained medical and occupational history using a questionnaire concerning atopy (history of hay fever, asthma, urticaria, or atopic eczema during childhood), chronic bronchitis (productive; cough during more than 3 months in more than 1 year [12]), smoking habits [13], conjunctivitis, rhinitis, and asthma (attacks of dyspnoea or chest tightness in relation to exposure). Spirometry was performed (Vitalograph®). Venous blood samples were collected for the determination of differential count and serum levels of total IgE, IgG, IgM, and IgA and specific antibodies. Subjects who had symptoms or signs indicating work-related hypersensitivity underwent a further examination by a physician and, if necessary, bronchial challenge with metacholine, lung X-ray, and skin-prick tests.

Referent samples in the antibody determinations were randomly selected from a group of twenty-two healthy workers, all men, employed in a food industry and with no history of isocyanate exposure. Data on the controls are given in Table 1.

Exposure

The painters worked in twenty-one small workshops, each with up to three employees. The paint was applied to the cars by spraying in special chambers with an air exchange rate of more than 20 times per hour. The paints were of a two-component type with isocyanates as the curing agents. The isocyanates were based on one or two oligomers of HDI (Desmodur N-100 (DN) or N-3300; Bayer AG, Leverkusen, FRG) with about 0.5% monomer HDI.

The painters took part in all working aspects of the process, including grinding, puttying and painting. The painting lasted for 0.25–4 hr/day. All but two used respiratory protection devices, but only one half were effectively protected by respirators (visors) supplied with air from a compressor; the others used mainly gas-masks with charcoal filters, without protection against respirable particles.

The exposure levels in the shops have not been investigated. Measurements in an other study of seven similar shops [14] have shown an exposure level of $< 5\text{--}89\text{ }\mu\text{g HDI}/\text{m}^3$ air and $200\text{--}8500\text{ }\mu\text{g}/\text{m}^3$ of the HDI polyisocyanate (DN) outside the respiratory

Table 1. Age, smoking, atopy, total and specific IgE and IgG antibodies in exposed workers and controls

Parameter	Exposed workers	Controls
Number (<i>n</i>)	30	22
Age (median, range; years)	35 (22–41)	43 (25–64)
Smoking (<i>n</i> ; %)	11 (37%)	16 (73%)
Atopy (<i>n</i> , %)	8 (27%)	4 (18%)
Total IgE (median; range; kU/l)	28 (2–370)	18* (3–142)
IgE-HDI-HSA (median; range; RAST ratio)	1.4 (0.6–3.1)	1.4* (0.8–1.8)
IgE-DN-HSA (median; range; RAST ratio)	1.2 (0.7–1.9)	0.9* (0.7–1.7)
Total IgG (median; range; g/l)	10 (7.4–14)	10.3† (7.6–12.4)
IgG-HDI-HSA (median; range; <i>A</i>)	0.17 (0.00–1.42)	0.15† (0.01–0.47)
IgG-DN-HSA (median; range; <i>A</i>)	0.11‡ (0.00–1.77)	0.03† (0.00–0.06)

* *n* = 10.† *n* = 12.‡ *P* = 0.01.

protection devices used. Measurements inside half gas-masks with charcoal filters gave no detectable level of HDI (<0.1 – <3 $\mu\text{g}/\text{m}^3$), but still 14–750 $\mu\text{g}/\text{m}^3$ of the polyisocyanate. Isocyanate levels inside compressed air respirators were below the detection limits (<0.5 – <3 $\mu\text{g}/\text{m}^3$). The air inside the protection devices were sampled through a teflon tube, connected to an impinger flask.

Antibody determinations

Antigens. The HDI-HSA hapten conjugate was prepared by reacting the isocyanate with human serum albumin (HSA; Kabi, Stockholm, Sweden). HDI, 225 μl in 4.5 ml acetonitrile, was slowly dropped into a cooled and stirred solution of 300 mg HSA in 100 ml 0.05 M boric acid (0.05 M KCl, pH 9.4). Thirty minutes after the addition, the solution was filtrated. The protein was then purified from low molecular weight compounds ($<30\,000$ daltons) by filtration in an ultrafiltration cell (Amicon 8200, Amicon Corp, Danvers, MA, U.S.A.). The protein was diluted with 0.1 M NaHCO_3 .

DN-HSA was synthesized by adding 40 mg Desmodur N-100 in 30 ml dry acetonitrile to a cooled and stirred solution of 150 mg HSA in 500 ml 0.05 M boric acid buffer (0.05 M KCl, pH 9.4). The reaction mixture was stirred overnight and then dialysed four times in 0.1 M NH_4NaCO_3 with a cutoff at 12 000–14 000 daltons. After dialysis the reaction mixture was filtrated and concentrated to 20 ml in an ultrafiltration cell to a concentration of 7.5 mg protein/ml 0.1 M NaHCO_3 .

Due to the small difference of absorbance between conjugated and non-conjugated HSA in the UV and visible regions of the light, the conjugates could not be

characterized by spectrometry. Instead of using spectrometry the HDI-HSA conjugate was hydrolysed in 6 N HCl at 110°C. Chemical analysis of the 1,6-hexamethylene diamine (HDA) formed proved a binding of about 8 moles of HDI per mole HSA. Corresponding results were obtained by: (i) direct analysis of HDA without derivatization in a LKB 2127 Tachophor (LKB, Bromma, Sweden) with 0.005 M BaOH, 40 mM β -alanine, 0.25% hydroxylpropyl methyl cellulose (pH 10.25) as the leading electrolyte and 20 mM TRIS and 5 mM HCl as the terminating electrolyte; (ii) analysis of the p-nitrophenylacetamide derivative of HDA with HPLC after reaction with N-Succinimidyl-p-nitrophenyl-acetate (Pierce Chemical Company, Rockford, IL, U.S.A.).

The DN-HSA conjugate has not been analysed in a corresponding way. The synthesis has been optimized to avoid precipitates. The theoretical maximal molar ratio with the procedure described is 40 moles DN per mole HSA.

Specific IgE antibodies (RAST). The HDI-HSA and DN-HSA conjugates were bound to cyanogen bromide activated filter paper discs [15] for use in RAST. We used the Phadebas RAST system (Pharmacia Diagnostics AB, Uppsala, Sweden) following the standard procedure, using 50 μ l serum and 50 μ l anti-IgE¹²⁵I solution. Results were expressed as counts per minute obtained from the antigen disc, divided by the result from a HSA control disc (RAST ratio).

Specific IgG antibodies (ELISA). Specific IgG antibodies were analysed by an ELISA assay. Wells of polystyrene microtitre plates (Nunc-Immuno Plate 1, Nunc, Kamstrup, Denmark) were coated by adding 100 μ l of an antigen solution (HDI-HSA or DN-HSA; 150 μ g/ml; 0.005 M PBS, pH 7.2). The wells were washed six times (Titertek Microplate Washer, Flow Laboratories, U.K.) with a washing solution, after storage in a refrigerator overnight, and were then incubated at 20°C with 200 μ l of blocking solution. The plates were stored with blocking solution at -18°C.

Analytical procedure. (i) Washing six times, (ii) addition of 100 μ l of a 1:50 dilution (PBS) of serum, (iii) incubation for 60 min at 20°C, (iv) washing six times, (v) addition of 100 μ l of an optimal dilution (roughly 1:1000) of alkaline phosphatase conjugated rabbit anti-human IgG (Dakopatts, Copenhagen, Denmark), (vi) incubation for 60 min at 20°C, (vii) washing ten times, (viii) addition of 100 μ l of substrate solution, (ix) incubation for 120 min at 20°C, (x) addition of 50 μ l 2 M sodium hydroxide solution, (xi) duplicate readings (Titertek-Multiscan, Flow Laboratories, U.K.) at 405 nm. The results are expressed as the absorbance value (A) for the reaction with the HDI-HSA or DN-HSA antigen, subtracted with a HSA reference antigen value.

Specific IgG4 antibodies (ELISA). The procedure for the assay of IgG subtypes were mainly the same as described for the total specific IgG antibodies. (i) Coating the wells with HDI-HSA or DN-HSA antigen, (ii) addition of diluted serum (1:50), (iii) addition of 100 μ l diluted (1:7500) mouse anti-human IgG4 subtype (Miles Scientific, Naperville, U.S.A.), (iv) addition of 100 μ l of a diluted (1:5000) solution of rabbit anti-mouse IgG conjugated with alkaline phosphatase (Sigma, St Louis, U.S.A.), (v) addition of 100 μ l substrate solution, (vi) addition of stopping solution.

Chemicals

PBS-merthiolate. This consisted of 8 g sodium chloride, 0.2 g potassium dihydrogen phosphate, 1.4 g disodium hydrogen phosphate $\times$ 12 H₂O, 0.2 g potassium chloride, and 0.01 g merthiolate per litre of distilled water.

Washing solution. This was 8 g sodium chloride, 0.2 g potassium dihydrogen phosphate, 1.4 g disodium hydrogen phosphate $\times 12 \text{ H}_2\text{O}$, 0.2 g sodium azide, and 0.5 ml Tween 20 (Sigma, St Louis, U.S.A.) per litre of water.

Blocking solution. This was 0.1% gelatine and 4% bovine serum albumin in PBS-merthiolate.

Substrate solution. This consisted of two tablets of p-nitrophenol phosphate-disodium (10 mg, Sigma, St Louis, U.S.A.) in 10 ml of a solution of 9.7 ml diethanolamine, 0.02 g sodium azide, and 0.01 g magnesium chloride per litre of water (pH 9.8).

Statistics

The χ^2 -test was employed to compare the incidence of symptoms between subgroups. When the expected number for any cell in a 2×2 table was below 5, Fisher's exact test was used. For comparisons of the distribution of age, smoking, and levels of antibodies between subgroups of exposed workers, and with controls, the Mann-Whitney *U*-test was used. Spearman's rank correlation test was used in assessing associations between antibody levels and factors such as age, smoking level and exposure time. All probability values are 2-tailed. Statistically significant refers to $P < 0.05$.

Results

Symptoms

Thirteen subjects (43%) had suffered symptoms of rhinitis and/or conjunctivitis, three (10%) had attacks of cough, and seven (23%) had attacks of dyspnoea and/or chest tightness. Three (10%) had chronic bronchitis. In total, ten (33%) had suffered symptoms from the bronchi. There was no association between exposure time or degree of protection, and symptoms. Eight subjects (27%) were atopic. They did not report more symptoms than non-atopics.

Ten subjects who had complained of symptoms from the bronchi underwent further medical examination. Two of those had a clear clinical asthma. One of those (patient 1) had suffered asthma since childhood. However, his asthma had been aggravated during the isocyanate exposure. The other (patient 2) developed asthma of late type during the time he had worked as a car painter, and it was associated with isocyanate exposure. The other eight had more unspecific symptoms without a definite association with isocyanate exposure.

Antibody findings

There were no significant differences in the total antibody levels between exposed subjects and controls (Table 1).

No significant differences were found in the RAST ratios of IgE antibodies against HDI-HSA (IgE-HDI-HSA) between exposed workers and the control subjects (car painters median 1.4, range 0.6–3.1; controls, $n = 10$, median 1.4, range 0.8–1.8); two car painters had values slightly above the highest control. Neither was there any difference as regards IgE-DN-HSA (car painters median 1.2, range 0.7–1.9; controls, $n = 10$, median 0.9 range 0.7–1.7; Table 1). Three car painters had values just above the highest control. Car painters with symptoms, including the two asthmatics, did not have elevated specific IgE values, as compared to non-symptomatics.

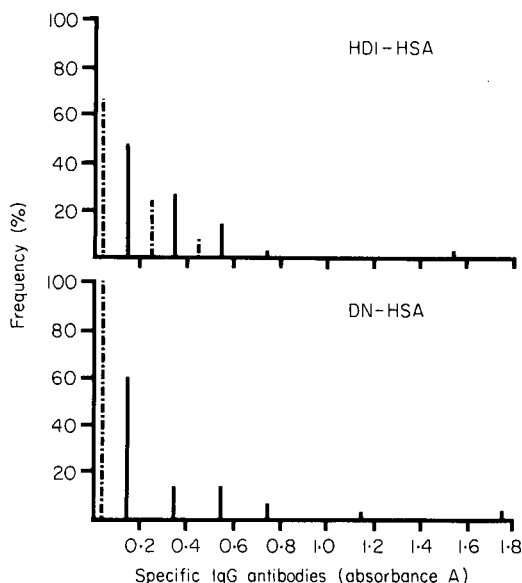


Fig. 1. Levels of specific serum IgG antibodies against HDI-HSA and DN-HSA in car painters (—) and controls (---).

IgG antibodies against HDI-HSA (IgG-HDI-HSA) were slightly, but not significantly, increased in car painters (median 0.17 A, range 0.00–1.42) as compared to controls ($n=12$, median 0.15, range 0.09–0.47; Table 1, Fig. 1); four car painters had antibody levels above the highest control.

IgG antibodies against DN-HSA (IgG-DN-HSA) were significantly increased ($P=0.01$) in car painters (median 0.11 A, range 0.00–1.77), compared with controls ($n=12$, median 0.03, range 0.00–0.06; Table 1). Patient 2 had the highest IgG-DN-HSA level (Fig. 1).

In order to study factors associated with the level of IgG antibodies against DN-HSA, the subjects were divided in two groups of fifteen subjects: high level IgG-DN-HSA and low level IgG-DN-HSA, by the median (Table 2). Age and atopy were similar in the two groups. In the high level group there were more smokers (47% *vs* 27%, not in table), and the exposure time was longer, but the differences were not statistically significant, nor were there any significant differences with regard to protection, rhinitis and/or conjunctivitis, symptoms from the bronchi (including asthma), lung function and white blood cell counts (not in table), total antibody levels (IgE, IgG, IgM, and IgA, the latter two not in table), or IgE-DN-HSA. The high level group had a significantly ($P=0.001$) higher level of IgG-HDI-HSA antibodies than the low level group.

Six persons were found to have IgG-DN-HSA antibodies of subclass 4 (IgG4-DN-HSA; median 0.49A, range 0.43–0.99), compared with controls ($n=10$, median 0.44, range 0.03–0.13; Table 2). Their smoking habits did not differ from the others. Furthermore, their median isocyanate-exposure time was 6 (1–12) years. They had thus not been exposed for a longer time than the average of the whole group, nor had they less effective protection equipment. None was atopic. There were no significant differences with regards to symptoms, lung function, white blood cells counts and total antibodies (IgE, IgG, IgM, IgA). There was, however, a significant difference in IgG-

Table 2. Age, exposure, atopy, and total and specific antibody level in car painters

Parameter	IgG-DN-HSA level		IgG4-DN-HSA‡
	Low*	High†	
Number (n)	15	15	6§
Age (median, range; years)	36 (21-61)	35 (24-48)	36 (26-46)
Exposure time (median, range; years)	6 (0.3-20)	9 (1-16)	6 (1-12)
Sufficient protection (n, %)	6 (40%)	8 (53%)	3 (50%)
Atopy (n, %)	3 (20%)	5 (33%)	0
Immunoglobulins			
Total IgE (median, range; kU/l)	25 (2-93)	32 (5-370)	27 (5-100)
IgE-HDI-HSA (median, range; RAST ratio)	1.5 (0.9-3.1)	1.4 (0.6-2.4)	1.3 (0.6-1.7)
IgE-DN-HSA (median, range; RAST ratio)	1.2 (0.7-1.6)	1.2 (0.7-1.9)	1.1 (0.7-1.9)
Total IgG (median, range; g/l)	9.9 (7.4-12)	10.1 (7.4-14)	9.9 (7.4-14)
IgG-HDI-HSA (median, range; A)	0.13¶ (0-0.32)	0.32¶ (0.09-1.42)	0.44** (0.29-1.41)
IgG-DN-HSA (median, range; A)	0.03 (0-0.10)	0.42 (0.11-1.77)	0.63 (0.47-1.8)

* IgG-DN-HSA (median, range); absorbance=0.03 (0-0.10).

† IgG-DN-HSA (median, range); absorbance=0.42 (0.11-1.77).

‡ IgG4-DN-HSA present.

§ All are included in the high level group too.

¶ $P=0.001$ (low *vs* high IgG-DN-HSA).** $P=0.0008$ (IgG4-DN-HSA *vs* all others).

HDI-HSA (median 0.4, range 0.3-1.4 *vs* 0.1, range 0.00-0.5; $P=0.008$). Also, they had higher IgG-DN-HSA (median 0.63, range 0.47-1.7, *vs* 0.07, range 0.00-0.64; $P=0.0003$). Patient 1 was negative, and patient 2 positive against IgG4-DN-HSA.

Discussion

The present study shows the presence of IgG antibodies against HDI-HSA, and especially DN-HSA. This has earlier been reported only in a single case [3]. This indicates an immunological reaction against the HDI monomer and prepolymer in the car paint.

The isocyanate exposure is very high when spraying polyisocyanate paints [9, 11, 14]. The car painters developed high titres of antibodies against DN-HSA, in spite of

the fact that they used protective equipment. This is a warning signal that urges more effective protective measures against the polyisocyanate exposure. The information that charcoal filters give poor protection against isocyanate aerosols must be given to the exposed workers.

The importance of IgG antibodies is unclear. We do not know if they, in some cases, can play a pathogenetic role, or if they just reflect exposure, or even block IgE, and thus have a protecting function. In this study, we did not find any correlation between the presence of IgG antibodies and symptoms.

It has been suggested, that IgG4 antibodies, in some cases, could be responsible for immediate type 1 allergic reactions [16, 17]. A study of phthalic anhydride-exposed persons indicate an association between work-related airway symptoms and development of IgG4 antibodies [18]. Similar results have been found in our department (J. Nielsen, pers. comm.). However, in this work, we did not find such a correlation, even though the only subject with clear-cut primary isocyanate asthma had antibodies of IgG4 type against both HDI-HSA and DN-HSA.

Most of the subjects had only slight and unspecific symptoms, probably due to irritative effects of the exposure. It is possible that a cell-mediated response could be the pathological mechanism in some cases.

The exposure to DN is two to three orders of magnitude higher than that to HDI. It is, thus, not surprising that there was a significant rise only in IgG-DN-HSA and not in IgG-HDI-HSA. At present, we are not definitely sure that antibodies against HDI make up a species distinctly separate from antibodies against DN. Further work on this issue is in progress.

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