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Isocyanate exposure and hypersensitivity pneumonitis — report of a probable case and prevalence of specific immunoglobulin G antibodies among exposed individuals

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SELDÉN AI, BELIN L, WASS U. Isocyanate exposure and hypersensitivity pneumonitis — report of a probable case and prevalence of specific immunoglobulin G antibodies among exposed individuals. *Scand J Work Environ Health* 1989;15:234–237. A car painter experienced three episodes of a hypersensitivity pneumonitis-like disease after exposure to two-component acrylic lacquers with hexamethylene diisocyanate (HDI) as the curing agent. High titers of HDI-specific immunoglobulin (Ig) G antibodies were found in the patient's serum by means of enzyme-linked immunosorbent assay (ELISA). In the ELISA, 5 to 10 % of the sera from 455 isocyanate-exposed but asymptomatic workers were positive, depending on the criterion used for a positive test, whereas 0 % of the sera from 157 unexposed referents was found to be positive. Among 10 subjects with isocyanate-induced asthma and isocyanate-specific IgE antibodies, 50 % had specific IgG. It was concluded that the presence of isocyanate-specific IgG antibodies in serum is correlated with isocyanate exposure rather than with symptoms of isocyanate-induced disease.

Key terms: car painter, hexamethylene diisocyanate, immunology, pathogenesis.

Sporadic cases of hypersensitivity pneumonitis have been reported in association with exposure to isocyanates, ie, toluene diisocyanate (TDI) (1–4), diphenylmethane diisocyanate (MDI) (5–9), and hexamethylene diisocyanate (HDI) (1, 4, 10), although the case definition has been inconsistent. Specific serum antibodies of the immunoglobulin (Ig) G class against pertinent diisocyanates have been found in some instances (7, 9–12) but not in others. This communication reports a probable case of hypersensitivity pneumonitis after exposure to prepolymerized HDI. Serum from the patient was analyzed with regard to isocyanate-specific IgE and IgG antibodies.

However, the diagnostic significance of isocyanate-specific IgG antibodies has not been systematically evaluated. An investigation of the prevalence of such antibodies in groups of isocyanate-exposed workers, as well as in unexposed referents, was therefore included in this study.

Materials and methods

Case report. A 21-year-old man started to work as a car painter in February 1984. He was unskilled and consequently assigned to preparatory work (grinding, filling, and masking), but occasionally he spray-painted minor car details. He had no family history of atopy, but he had experienced allergic reactions in 1971–

1972, including one episode of urticaria of unknown origin. He had smoked 10–15 cigarettes a day since the age of 15 years.

On 23 May 1984 he performed his first major painting job, spraying a racing boat for about 1.5 h. Against regulations, this work was done outside the ventilated painting box. Within an hour of completing the work, he had chills, dyspnea and chest pain, followed by general malaise, fever, sweating, headache, and non-rotatory vertigo. He decided to seek medical attention at the local hospital, and upon admission transient pulmonary ronchi were noted. The patient's pulse rate was elevated (108 beats/min) concordantly with his elevated body temperature (38.6°C). His blood pressure was 115/70 mm Hg (15.3/9.3 kPa). He had a slight leucocytosis ($10.5 \cdot 10^9/l$; normal $4\text{--}9 \cdot 10^9/l$), but no differential count was obtained. Routine blood and urine tests were normal, including an erythrocyte sedimentation rate of 3 mm. Unfortunately, no pulmonary radiography, blood gas analysis, or lung function test was performed in the acute phase of the disease. The following morning, the patient's body temperature had dropped to 37.5°C. At this time, a pulmonary radiograph was normal, and the patient was discharged. He returned to work but did not paint until one day in August, when he sprayed the interior of a small car. The same evening he experienced a second episode of fever, chills, malaise, dyspnea, and chest pain. He felt alright again the next morning. A third and final episode occurred early in November when he had been watching a workmate spray painting for less than a minute.

On all three occasions, he had been exposed to a two-component acrylic lacquer with a curing agent containing 42 % (by weight) prepolymerized HDI, including

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a maximum of 0.4 % HDI monomer. He had been wearing a half-mask respirator with a charcoal filter part of the time, but he admitted that it had not been properly adjusted. On the third occasion, he used no respiratory protection at all. In addition, the exhaust ventilation had been clearly insufficient. Consequently, vapors and possibly microdroplets of the paint might have been inhaled.

The patient connected his outbreaks of illness with his work, and reported this observation to the Social Insurance Office after the second episode. When he was first seen by one of us (AS), in December 1984, he had been on sick-leave for one month, and a routine physical examination was normal. A spirometric recording (Vitalograph®) showed a vital capacity (VC) and forced expiratory volume in 1 s (FEV_{1.0}) within normal limits. The results were not significantly different from a similar spirometric examination performed at a health survey the day before the first episode of hypersensitivity pneumonitis-like symptoms.

Immunologic studies. The serological analyses started with HDI, MDI, and TDI conjugated to human serum albumin (HSA) and serum from the patient from December 1984. The conjugates had previously been optimized for the radioallergosorbent test (RAST) system with sera from isocyanate-exposed workers who had developed bronchial asthma, as well as isocyanate-specific IgE antibodies (13). The number of isocyanate molecules per carrier molecule for the MDI-HSA, HDI-HSA, and TDI-HSA conjugate had been estimated to be 6, 8, and 10, respectively. The RAST was performed according to standard procedures. The serum was also used in an immunodiffusion test.

The presence of specific IgG antibodies was investigated by means of the enzyme-linked immunosorbent assay (ELISA), employing the same conjugates as antigens as were used in the RAST. Briefly, the isocyanate-HSA conjugates were diluted in 3 mmol/l phosphate-buffered saline (PBS) solution, pH 7.2, to a final concentration of 0.05 mg/ml. The solution was added in 100 µl amounts to a polystyrene microplate (Nunc-Immuno plate I, A/S Nunc, Roskilde, Denmark), and the plate was then incubated for at least 2 h at 4°C in a humidified chamber. After incubation, the plate was washed three times with 3 mmol/l PBS with the use of a Dynatech Autowash 2000 washer/aspirator (Dynatech Laboratories Inc, Laboratory Design AB, Lidingö, Sweden). Sera were diluted with PBS containing 0.02 % Tween®. Diluted sera were added in 100 µl amounts to duplicate wells. The plate was then incubated for 1.5 h at room temperature in the humidified chamber, after which it was washed with 3 mmol/l PBS containing 0.02 % Tween, and 100 µl of peroxidase-conjugated rabbit immunoglobulins to human IgG (Dako-Immunoglobulins A/S, Copenhagen, Denmark), diluted 1:500 with PBS containing 0.02 % Tween, was then added to the plate, and the plate was incubated for 2 h. After the plate

had been washed as before, 100 µl of an orthophenylenediamine solution in 0.1 mol/l citrate buffer, pH 5.0, was added to each well. Five minutes later, 50 µl of a 2.0 mol/l sulfuric acid solution was added in order to stop the color reaction, and the absorbances were read at 450 nm by means of an MR 700 microplate reader (Dynatech Laboratories Inc).

Prevalence of immunoglobulin antibodies. The prevalence of isocyanate-specific IgG antibodies among 455 isocyanate-exposed workers (Swedish polyurethane workers and spray painters), 157 unexposed referents (industrial workers), and 10 workers with isocyanate-induced asthma and isocyanate-specific IgE antibodies (16) was determined according to two different criteria for a positive ELISA. Criterion I required at least 0.5 absorbance units when serum was diluted 20 times and at least 50 % inhibition at that dilution when 20 µg of an isocyanate conjugate was added to 1 ml of the diluted test serum prior to the analysis. Criterion II was defined as a positive test according to the first criterion plus at least 0.5 absorbance units when serum was diluted 100 times.

Results

No IgE antibodies against HDI, MDI, or TDI were detected in the patient's serum, and the total IgE concentration was 40 kU/l (normal). No precipitating antibodies could be observed when the serum was used in an immunodiffusion test.

On the other hand, the serum from the patient gave a strong positive reaction to HDI in the ELISA. Positive, but weaker, reactions were also obtained with MDI and TDI (figure 1). Approximately 75 % of the reaction could be inhibited by the addition of 20 µg of isocyanate conjugate to 1 ml of the test serum prior

Figure 1. Results of the ELISA (unbroken lines) when the patient's serum and conjugates prepared from the isocyanates hexamethylene diisocyanate (HDI), diphenylmethane diisocyanate (MDI), and toluene diisocyanate (TDI) were used and a typical result (broken line) when sera from unexposed referents were analyzed. The results for the referents were the same for all three isocyanates.

to the analysis, which was considered to support the specificity of the test system.

The RAST and ELISA analyses were later repeated with a serum sample obtained from the patient in September 1987. He had not been exposed to isocyanates during the three intervening years. Once again, the RAST was negative, and the total IgE concentration was low, 10 kU/l. A borderline positive ELISA reaction was observed for HDI but not for the other two isocyanates tested.

The antibody prevalence in the various groups is shown in table 1, where the group of exposed individuals has been subdivided according to occupation.

Discussion

In this case report, there is a lack of certain medical information to support a definite diagnosis of hypersensitivity pneumonitis (15). Specifically, there is no evidence of an impaired diffusion capacity or radiographic signs of alveolitis in the acute stage of the disease. A pulmonary radiograph with normal findings obtained some 12 h after the patient's admittance to the hospital does not preclude the diagnosis. We find the history and the symptoms, the clinical course, and the relapses upon repeated exposure to be highly suggestive of hypersensitivity pneumonitis. The offending agent seemed to be related to the somewhat uncontrolled exposure to a two-component acrylic lacquer with an isocyanate (HDI) hardener. During such work operations, concentrations of isocyanates (especially of the HDI oligomer, usually named "HDI biuret-trimer") several times higher than the Swedish occupational exposure limit have been recorded (16–19).

Attempts were made to collect immunologic evidence in support of the diagnosis. The absence of IgE antibodies against HDI was not an unexpected finding insofar as the clinical picture did not suggest an immediate type of allergic reaction. Moreover, in

isocyanate-induced asthma, IgE antibodies are an uncommon finding, although hapten sensitization of the airways by occupational exposure to various chemicals has been increasingly described.

The presence of serum IgG antibodies to an HDI-HSA conjugate followed by specific inhibition after preincubation was considered to support the conclusion that HDI was the cause of the disease. The significance of such antibodies for the case validation remained unclear, however, and we decided to investigate their presence in various groups of individuals.

As can be seen from table 1, the occurrence of specific IgG antibodies is clearly related to isocyanate exposure. The prevalence, however, is dependent on the definition of a positive test. Among the isocyanate-exposed groups, a serum dilution of only 20 in the ELISA gave positive results for up to 33 % (mean 10 %) of the samples. The analysis of serum diluted 100-fold reduced the number of positives in the exposed (but healthy) groups.

The proportion of positive tests remained unaffected among isocyanate asthmatics with specific IgE antibodies since these individuals generally had high IgG antibody titers. The high prevalence of specific IgG antibodies among these asthmatics is probably due to common factors controlling both IgE and IgG production. It should be noted that no symptoms of hypersensitivity pneumonitis had occurred in this group.

The patient was found to be positive according to both criteria when the December 1984 serum was used but only positive according to the first criterion when the serum from September 1987 was used.

The findings in the evaluation of this ELISA system are rather similar to observations on alveolitis such as farmer's lung, for which IgG antibodies are supposed to play only a minor immunopathological role (20, 21). Nevertheless, IgG antibody assays are valuable as evidence of accumulated exposure and may thus be considered supportive proof in clinically clear cases of hypersensitivity pneumonitis. From the low inci-

Table 1. Prevalence of positive tests in the enzyme-linked immunosorbent assay (ELISA) among different groups of isocyanate-exposed workers, selected patients with isocyanate-induced asthma, and unexposed referents. Two different criteria were used for a positive test. (95 % CI = 95 % confidence interval)

Group	Exposure	N	Criterion I		Criterion II	
			Percentage with positive tests	95 % CI ^a	Percentage with positive tests	95 % CI ^a
Isocyanate-exposed workers without symptoms						
Spray-painting	HDI	199	11	7—16	6	3—10
Foaming, “modern” equipment	TDI, MDI	186	5	3—9	3	1—6
Foaming, “old” equipment	TDI, MDI	39	33	21—49	15	7—30
Foaming, closed process	MDI	31	0	0—11 ^b	0	0—11 ^b
Total		455	10	7—13	5	3—7
Patients with isocyanate-induced asthma and specific Immunoglobulin E antibodies						
	MDI, HDI, TDI	10	50	24—76	50	24—76
Referents	—	157	0	0—2 ^b	0	0—2 ^b

^a 95 % confidence interval calculated from Armitage & Berry (14).

^b Upper limit of approximate 95 % CI calculated directly from the binomial distribution.

dence of this disease among isocyanate-exposed workers, it follows that the sensitivity of the test system needs to be further evaluated.

It should be noted that a specific challenge test was not performed. The Swedish rules for workmen's compensation are comparatively liberal, and the Social Insurance Office accepted our patient's disease as being of occupational origin, and he received some compensation. Thus, as the patient would not in any respect benefit from renewed exposure to a potentially harmful agent (ie, HDI), a challenge test was considered unethical.

We conclude that the demonstration of IgG antibodies in sera from patients with possible isocyanate-induced hypersensitivity pneumonitis furnishes evidence of immunostimulating hapten exposure. The correlation of the immunologic findings with the disease process seems to be of low significance, however. It is not justified to look upon these antibodies as evidence of isocyanate-induced hypersensitivity pneumonitis.

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