

Diphenylmethane diisocyanate (MDI)-induced asthma: evaluation of the immunologic responses and application of an animal model of isocyanate sensitivity

K. C. CHANG and M. H. KAROL

Department of Allergy and Clinical Immunology, Kaiser-Permanente Medical Center, Portland, Oregon, and Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.

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Summary

A worker developed two episodes of severe asthma 90 min after cutting a polyurethane plate made of diphenylmethane diisocyanate (MDI) using a rapidly turning carbide blade. Intradermal skin testing with MDI-human serum albumin (MDI-HSA) and *p*-tolyl isocyanate-HSA (*p*-TMI-HSA) were positive at 0.002 mg/ml. Control subjects showed no reaction at 2 mg/ml. Bronchial provocations of the worker with MDI-HSA and *p*-TMI-HSA, made 1 year after the occupational asthmatic episode, were negative at 10 mg/ml. Bronchial reactivity to methacholine decreased toward normal during a 2-year follow-up. RAST using MDI-HSA or *p*-TMI-HSA were strongly positive when compared with binding by sera from atopic controls which contained the same amount of total IgE. RAST titres decreased during a 1-year follow-up. In the MDI-HSA RAST, inhibition studies indicated specificity of antibodies for MDI-HSA. In the *p*-TMI-HSA RAST, *p*-TMI-HSA was a very effective inhibitor whereas MDI-HSA was not. These results indicated the formation of at least two distinct groups of IgE antibodies: those reactive with MDI, and those reactive with *p*-TMI determinants. Guinea pigs immunized with MDI formed antibodies with specificities similar to those of the patient. We conclude that the worker had occupational asthma accompanied by the formation of specific IgE antibodies of the specificities. The causal relationship of the antibodies to the occupational asthma remains uncertain.

Introduction

Sensitivity to organic isocyanates has been recognized for many years (Bruckner *et al.*, 1968; Fuchs & Valade, 1951). Most reports of isocyanate-induced asthma describe reactions to toluene diisocyanate (TDI), a highly reactive low-molecular-weight chemical used in the manufacture of polyurethane. More recent reports have described

Correspondence: Dr Kuo C. Chang, Department of Allergy and Clinical Immunology, Kaiser-Permanente Medical Center, 3414 N. Montana Avenue, Portland, OR 97227, U.S.A.

asthmatic responses upon exposure to other isocyanates, including hexamethylene diisocyanate, naphthylene diisocyanate, and diphenylmethane diisocyanate (MDI) (O'Brien *et al.*, 1979; Tanser, Bourke & Blandford, 1973; Harries *et al.*, 1979; Zeiss *et al.*, 1980). There is much disagreement on the immunologic and/or pharmacologic pathogenesis of isocyanate sensitivity (Davis *et al.*, 1977; Karol, Ioset & Alarie, 1978a; Karol & Alarie, 1980; Butcher *et al.*, 1980; Baur & Fruhmman, 1981). In a recent report, evaluation of two patients with MDI sensitivity revealed IgE antibodies reactive with MDI-human serum albumin conjugate (MDI-HSA) in a patient with immediate-onset asthma. IgE as well as IgG antibody specific for MDI-HSA was detected in a second patient who presented with symptoms of hypersensitivity pneumonitis (Zeiss *et al.*, 1980).

One of the authors (MHK) has developed animal models for pulmonary hypersensitivity to organic isocyanates. Guinea pigs were sensitized to TDI by inhalation (Karol *et al.*, 1980), as well as by topical (Karol *et al.*, 1981), intraperitoneal or intradermal exposure (Karol, 1980). Animals responded with pulmonary reactions upon bronchial provocation with TDI (Karol *et al.*, 1981) or with tolyl isocyanate antigens (Karol *et al.*, 1980; Karol *et al.*, 1981). The animal model was developed to establish concentration-response relationships and to enable the prediction of exposure levels that would preclude sensitization in industrial workers. In addition, the model has been particularly useful for identifying isocyanate-conjugate antigens effective in detection of isocyanate specific antibodies.

The current study was undertaken to evaluate the antibody response to MDI in an occupationally sensitized individual and to compare the specificities of such antibodies with those produced in guinea pigs as a result of deliberate immunization with MDI. Antigens found effective in detecting antibodies in the patient were used additionally for skin testing and for bronchial provocation.

Case report

The patient, R.K., a 50-year-old white male and an asymptomatic 30 pack-year ex-smoker, had worked as a grain elevator operator/repairman for 5 years. He was healthy until March 1980 when he developed two episodes of asthma 90 min after cutting a polyurethane plate ('Rhinohyde'), containing MDI using a rapidly rotating carbide blade. The second episode was severe and required hospital treatment for 4 days. The asthma was most severe 4-8 hr after the exposure. There was no fever. Chest X-ray showed hyperinflation without infiltrate. Arterial blood gas revealed: pH 7.47, PaO₂ 58 mmHg, PaCO₂ 30 mmHg and BE 0. Sputum smear showed 30% eosinophils. R.K. had cut Rhinohyde on at least three occasions in the previous 2 years without experiencing any problems. In the subsequent 2 years he had avoided cutting Rhinohyde plate and had been doing well except for occasional chest congestion at night, occurring primarily in the 1st year of follow-up. Pulmonary function tests 10 days after his last asthmatic reaction revealed a FVC 6.03 l (127% of predicted), FEV₁ 3.76 l (102% of predicted), FEV₁/FVC 62% (observed), FEF₂₅₋₇₅ 2.35 l/sec (66% of predicted). Residual volume 3.01 l (130% of predicted), total lung capacity 8.61 l (123% of predicted), RV/TLC 35% (observed). No change of pulmonary function was observed after the inhalation of a bronchodilator, and no change was noted during 2 years follow-up. Prick skin tests using stock solutions of forty common inhalant allergens and grain dust were all negative. Intradermal skin tests with the above allergens, up to a concentration of 1:1000 were all negative except for a 2+ reaction for

Dermatophagoides farinae at the concentration of 1:10 000 and a 2+ reaction for *Aspergillus fumigatus* at the concentration of 1:1000. RAST results were similar: *Dermatophagoides farinae* was positive and *Aspergillus fumigatus* was borderline positive. House dust, *Alternaria tenuis*, cladosporium and ryegrass, which were negative by skin testing, were also negative by RAST. Immunodiffusion for *Aspergillus* mix was negative.

Materials and methods

Subjects

In addition to the patient, two atopic and three non-atopic controls were examined by skin testing. Sera from another two atopic individuals, each with total serum IgE levels of approximately 1500 u/ml served as controls for RAST and enzyme-linked immunosorbent assay.

Animals

Female, English, smooth-haired guinea pigs (Hilltop Lab Animals, Scottsdale, PA) weighing 250–350 g were used for sensitization and passive cutaneous anaphylaxis (PCA) assays.

Immunization procedure

Six guinea pigs were immunized by intradermal injection of 50 μ l of MDI (Mondur MR, 50% MDI monomer, with the remainder higher molecular weight MDI polymers, Mobay Chemical Corporation, Pittsburgh, PA). No adjuvant was used in these immunizations (Karol, 1980). Fourteen days later, animals were bled from the nailbed and the sera evaluated for antibodies.

Antigens

Isocyanate antigens were prepared by dropwise addition of isocyanates to a rapidly stirred solution of HSA or guinea-pig serum albumin (GSA) in borate buffer at pH 9.4 as previously described (Karol *et al.*, 1978b). Reaction time was varied to achieve coupling of 30–40 mol of isocyanate hapten per mol of serum albumin. The following isocyanates were used as haptens: toluene diisocyanate (TDI, Mondur TD₈₀, Mobay Chemical Corporation), hexamethylene diisocyanate (HDI, Mondur HX, Mobay Chemical Corporation), diphenylmethane diisocyanate (Mondur MR, Mobay Chemical Corporation), *p*-tolyl isocyanate (*p*-TMI), phenyl isocyanate (PHI, Eastman Chemicals) and dicyclohexylmethane diisocyanate (HMDI, Mobay Chemical Corporation). The degree of haptenic substitution of protein by aromatic isocyanates was determined by spectrophotometric analysis (Karol, 1980). Haptenic substitution by aliphatic isocyanates was determined by the trinitrobenzene sulfonic acid method (Snyder & Sobocinski, 1975).

Bronchial provocation test and skin test

Methacholine bronchial provocation test was carried out using the method of Chai *et al.* (1975). Aerosol was generated by a DeVilbiss 646 nebulizer driven by a Maximyst air compressor. The results were expressed as PC₂₀ (Ryan *et al.*, 1981). A standardized procedure was used for antigen bronchial provocation testing (Chai *et al.*, 1975) with modifications. Because the patients's asthma developed 90 min after cutting Rhino-

hyde plate but was most severe 4–8 hr later, the interval between the increment of concentration was 1 hr, and only two to three steps were carried out each day. Three days were required to complete the bronchial provocation test for each antigen. Serial pulmonary functions were monitored for 72 hr.

A standardized procedure was used for skin testing (Norman, 1976). Results were expressed as the mean diameter of the weal and flare reactions.

Serologic assays

Total serum IgE. The total amount of IgE in serum samples was determined by the PRIST method using a commercial test kit (Pharmacia Diagnostics).

RAST. Specific IgE antibodies in human sera were assayed by RAST using isocyanate-HSA antigens covalently bound to paper discs (Karol *et al.*, 1978a). RAST was performed by incubating 50- μ l serum with an antigen-coated disc for 16 hr at ambient temperature. After thorough washing of discs, 50 μ l I¹²⁵-rabbit anti-human IgE (RAST reagent, Pharmacia Diagnostics) were added and discs incubated an additional 16 hr at room temperature. Discs were counted for 10 min in a Packard γ counter. Results were expressed as Ct/min, or percent of added I¹²⁵ reagent bound to discs (Karol, 1981a). Replicate determination differed by 10% or less.

RAST inhibition. The ability of antigens to inhibit RAST reactions was determined using a modification of a standard procedure (Gleich *et al.*, 1974). Sera (25 μ l) were incubated with increasing amounts of isocyanate-conjugate antigen (1–100 μ g) dissolved in 25- μ l buffer (PRIST diluent, Pharmacia Diagnostics). Control tubes contained serum and 25- μ l buffer. After 2 hr at 37°C, an antigen-coated disc was added to each tube. Samples were incubated an additional 16 hr, then washed and incubated with radiolabelled rabbit anti-IgE as described above. The percent of RAST inhibition was calculated as follows:

$$\% \text{ inhibition} = 100 \left(1 - \frac{Ci - Cb}{Cs - Cb} \right),$$

where Ci = Ct/min bound by serum in the presence of inhibitor, Cs = Ct/min bound by serum in the absence of inhibitor, and Cb = non-specifically bound radioactivity.

Enzyme-linked immunosorbent assay (ELISA). Polystyrene plates were coated with antigen as previously described (Karol, Ioset & Alarie, 1979). IgG antibodies were detected using peroxidase-labelled goat anti-human IgG (Miles-Yeda). The titre was defined as the highest serum dilution which yielded a visible blue-black colour reaction (Karol *et al.*, 1979).

Guinea-pig sera

Passive cutaneous anaphylaxis (PCA). To detect cytophilic antibodies, 0.1 ml serum (diluted as indicated with saline) were injected intradermally into the shaved backs of guinea pigs. Following a 6-hr latent period, 0.5 ml of solution containing 2.5 mg isocyanate-conjugate antigen and 2.5 mg Evans blue dye were injected intravenously. Reactions were read 30 min later. Titres were expressed as the reciprocal of the highest serum dilution which yielded a blue reaction of at least 5 mm diameter. Control sera, drawn from animals prior to immunization, gave negative results with all antigens tested.

Results

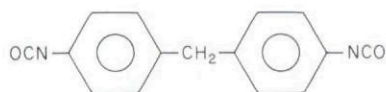
Sequence of clinical and immunologic evaluation

The patient's last occupational asthmatic response was in March 1980. The first blood sample was drawn in June 1980. Subsequent samples were obtained in September 1980, January 1981 and April 1981. Methacholine provocation was performed in June 1980, April 1981, and again in July 1982. Antigen bronchial provocation tests and skin tests were performed 1 year after the asthmatic attack (April and July 1981).

IgE antibodies

The total serum IgE was 1760 u/ml in June 1980 and 1660 u/ml in September 1980. Values remained between 1660 and 1470 u/ml during the subsequent year.

The chemical structure of MDI and *p*-TMI are shown in Fig. 1. Respective RAST bindings using MDI-HSA and *p*-TMI-HSA discs showed 48 and 42% in June 1980; 44 and 41% in September 1980; and 21 and 28% in April 1981. Titres declined substantially during the year.



Diphenylmethane diisocyanate (MDI)



p-tolyl isocyanate (*p*-TMI)

Fig. 1. Chemical structure of diphenylmethane diisocyanate (MDI) and *p*-tolyl isocyanate (*p*-TMI).

The presence of high titres of IgE antibody reactive with *p*-TMI-HSA as well as with MDI-HSA, prompted more thorough investigation of the reactivity of the patient's serum with other isocyanates. In serum drawn 6 months post-symptomatic exposure, RAST binding was observed with several antigens (Fig. 2). Undiluted sera from atopic controls, which contained 1400–1600 u/ml total IgE bound a maximum of 10–12% radioactivity with all antigen-coated discs as indicated by the shaded area (Fig. 2).

Patient R.K.'s serum showed highly significant binding ($P < 0.001$, ZM test) to MDI-HSA and *p*-TMI-HSA discs. The serum also showed reactivity with TDI-HSA and PHI-HSA. No reactions were noted with discs containing HMDI-HSA or HSA.

Inhibition of MDI-HSA RAST

The specificity of binding in the MDI-HSA RAST was investigated by RAST inhibition using several isocyanate-conjugate antigens as inhibitors. Results are given in Table 1. MDI-HSA was the most potent inhibitor of reaction. TDI-HSA was 200 times less potent. The other antigens tested were essentially non-inhibitors of this reaction since comparable inhibition required 2000 times the amount of inhibitor. Calculation of the slopes of the inhibition data indicated no significant difference in

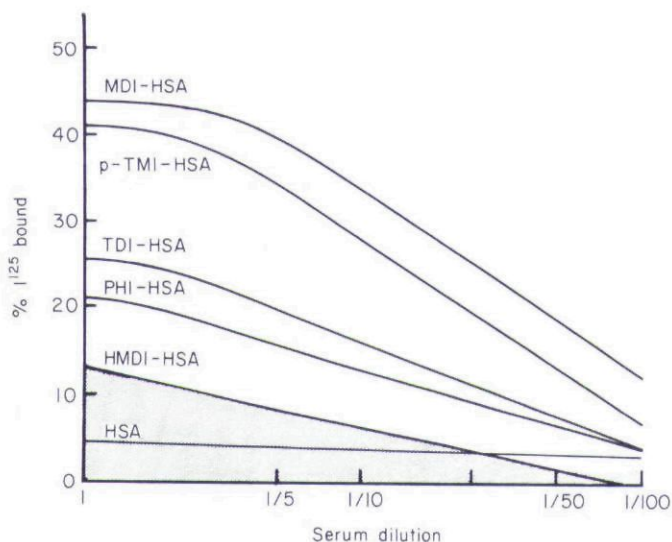


Fig. 2. RAST assays of the patient's serum (September 1980) using several RAST systems. The shaded area indicates the maximum non-specific binding by two control atopic sera observed in all RAST assays.

slope between MDI-HSA and TDI-HSA inhibition lines. This finding has been interpreted to indicate that both inhibitors recognized the same antigen-binding site on the antibody (Hunter, 1978). By contrast, inhibition of MDI-HSA RAST by *p*-TMI-HSA was poor and the slope was significantly less than those obtained with TDI-HSA and MDI-HSA.

Inhibition of p-TMI-HSA RAST

RAST inhibition was used to evaluate the specificity of RAST binding by R.K. serum to *p*-TMI-HSA discs. In this system, *p*-TMI-HSA was the most effective inhibitor of reaction (Table 2). Fifty percent inhibition was achieved by the addition of 27 μ g *p*-TMI-HSA. More than 100 times this amount of MDI-HSA, and greater than 3000

Table 1. Inhibition of MDI-HSA RAST

Inhibitor	μ g required for 50% inhibition*
MDI-HSA	1.9
TDI-HSA	383
<i>p</i> -TMI-HSA	5 200
HDI-HSA	> 10 000
PHI-HSA	> 10 000
HMDI-HSA	> 83 000
HSA	> 100 000

* The amount of inhibitor required to effect 50% inhibition of binding was determined from concentration-response lines.

Table 2. Inhibition of *p*-TMI-HSA RAST

Inhibitor	μg required for 50% inhibition*
<i>p</i> -TMI-HSA	27
MDI-HSA	3000
PHI-HSA	7300
HDI-HSA	> 100 000

* The amount of inhibitor required to effect 50% inhibition of binding was determined from concentration-response lines.

times this amount of HDI-HSA, was required to achieve comparable inhibition of reaction. In addition, the slopes of inhibition obtained with the three test antigens listed in Table 2 differed from that shown by *p*-TMI-HSA.

ELISA

MDI-HSA was bound to polystyrene plates for use in ELISA to detect specific IgG antibodies. Serum drawn from the patient in June 1980 yielded a titre of 8. Control sera showed no reaction (titre=0). This titre represents a minimal amount of antibody (Karol *et al.*, 1979) to MDI-HSA.

Skin testings

A prick test 1 year following the occupational asthmatic reaction with MDI-HSA and *p*-TMI-HSA, each at 10 mg/ml, gave a weal/flare reaction of 4/18 mm and 3/8 mm, respectively. Using an intradermal method, weal/flare reactions of 5/20 mm and 6/18 mm were obtained to MDI-HSA and *p*-TMI-HSA, respectively, at a concentration of 0.002 mg/ml. The patient gave no reaction to HSA at 2 mg/ml. Intradermal skin tests to MDI-HSA and *p*-TMI-HSA made on two atopic and three non-atopic controls were negative at 2 mg/ml.

Methacholine bronchial provocation test

The PC₂₀ was 1.39, 0.93 and 13.0 mg/ml 3 months, 1 year and 2 years respectively after the last asthmatic reaction.

Bronchial provocation to MDI-HSA and *p*-TMI-HSA

R.K. and one atopic control were challenged with MDI-HSA in April 1981 and with *p*-TMI-HSA in July 1981. No responses were obtained using antigens at concentrations up to 10 mg/ml.

PCA assay in guinea pigs

Four guinea pigs with highest PCA titres to MDI-GSA were selected for further PCA analyses (Table 3). Animals immunized with MDI formed antibodies reactive with both MDI-GSA and with *p*-TMI-GSA. Titres to *p*-TMI-GSA were typically one dilution less than those to MDI-GSA. By contrast, sera were much less reactive with TDI-GSA. No reactions were noted when GSA was used for intravenous challenge.

Table 3. PCA titres of animals immunized with MDI

Challenging antigen	Antibody titre*			
	Animal No.			
	1	2	3	4
MDI-GSA	256	32	128	32
<i>p</i> -TMI-GSA	128	16	64	32
TDI-GSA	<4	4	32	4
GSA	0	0	0	0

* Reciprocal of highest serum dilution which yielded a positive reaction.

Discussion

The purpose of this study was to evaluate the immunologic response to isocyanates in a patient who had experienced asthmatic reactions after cutting a Rhinohyde plate with a rapidly turning carbide blade.

Rhinohyde is a 1 cm thick plate of MDI-based polyurethane polymer reinforced with steel wire. Cutting Rhinohyde with the carbide blade produces heat. Since isocyanates are regenerated from heated polyurethanes (Encyclopedia of Chemical Technology, 1967), it was suspected that MDI was released during the Rhinohyde cutting operation.

The patient's asthmatic reactions occurred 90 min following the cutting operation and were most severe 4–8 hr following exposure. Serologic evaluation of the patient 3 months after symptomatic exposure revealed a high titre of MDI-specific IgE antibody with only a minimal titre of MDI-specific IgG antibody. These findings are similar to those reported by Zeiss *et al.* (1980) and by Malo and Zeiss (1982). In three cases of occupational pulmonary hypersensitivity attributed to MDI exposure one, diagnosed with immediate-onset asthma, was found to have much IgE antibody to MDI-HSA. The other two cases, diagnosed as hypersensitivity pneumonitis, had high titres of IgG antibody.

Zeiss *et al.* (1980) noted that the sera of the patient with MDI sensitivity cross-reacted with TDI-HSA antigen. A similar finding was made in the study reported here. Patient R.K.'s serum bound 25% of the radiolabel in TDI-HSA RAST compared with 48% binding in MDI-HSA RAST (Fig. 2). Moreover, TDI-HSA was 200 times less potent in MDI RAST inhibition than was MDI-HSA (Table 1).

Patient R.K.'s serum had a high titre of IgE antibody reactive with *p*-TMI-HSA (Fig. 2). These antibodies reacted with *p*-TMI since RAST inhibition revealed that *p*-TMI-HSA was not an effective inhibitor of the MDI-HSA RAST system (Table 1), and antibodies reactive with *p*-TMI-HSA were not effectively inhibited by MDI-HSA (Table 2). Interest in *p*-TMI-HSA arose as a result of studies (Karol *et al.*, 1978a; Karol *et al.*, 1980; Butcher *et al.*, 1980; Karol, 1981a; Baur *et al.*, 1980; White *et al.*, 1980) which demonstrated that this conjugate antigen was particularly effective in detecting antibodies to TDI. Effectiveness was attributed to the monofunctional, as opposed to

bifunctional nature of the isocyanate hapten (Karol *et al.*, 1978a). Being monofunctional, *p*-TMI could not cause cross-linking or polymerization of protein. The result was an isocyanate-protein conjugate which contained exposed hapten groups.

Several lines of evidence suggested an association between antibodies to *p*-TMI-HSA, and occupational exposure: (i) antibody titres to *p*-TMI-HSA (as well as to MDI-HSA) decreased in successive serum samples collected during the year following symptomatic exposure. (ii) MDI was shown to be antigenic in guinea pigs and the antibodies reacted with *p*-TMI-GSA. The stimulus for production of antibodies to *p*-TMI-HSA remains speculative. It is possible that the patient was exposed to *p*-TMI released during thermal decomposition of the MDI-based polyurethane. However, this explanation cannot be offered for the anti-*p*-TMI response in guinea pigs. The possibility of *p*-TMI as an impurity in MDI was ruled out by thin-layer chromatographic analysis of MDI (Dunlap, Sandridge & Keller, 1976). Failure to detect any *p*-TMI in a 300- μ g MDI sample indicated MDI purity >99.8%. The possibility of *in vivo* formation of *p*-TMI resulting from metabolism of MDI must be considered.

The formation of 'new antigenic determinants' (NAD) as a result of exposure to isocyanates has been suggested (Bernstein, 1982). Such determinants are thought to represent altered protein which would stimulate production of antibody against the altered protein. No evidence of NAD was obtained in the current study, from analysis of either human or guinea-pig sera. If such antibodies were produced, one would have expected reaction with HMDI-HSA in RAST (Fig. 2) and reaction of guinea-pig immune sera with TDI-GSA rather than with *p*-TMI-GSA (Table 3). Such reactions were not apparent. A remaining possibility is that MDI exposure stimulated formation of two groups of antibodies—i.e. those specific for MDI determinants, and those with specificity toward *p*-TMI.

In a previous study of a patient with MDI asthma, and another with MDI hypersensitivity pneumonitis, skin testing with MDI-HSA produced negative results (Zeiss *et al.*, 1980). Patient R.K. was skin-tested 1 year after the last asthmatic reaction, by intradermal injection with MDI-HSA and *p*-TMI-HSA. Strong sensitivity was detected to both antigens, whereas no response occurred to HSA injection. Skin-testing control subjects produced negative results to each of the three antigens.

Bronchial provocation tests of the patient with MDI-HSA and *p*-TMI-HSA performed 1 year after the asthmatic reaction were negative in spite of positive skin tests and RAST at the same time. Traditionally, demonstration of bronchial sensitivity to occupational chemicals has relied on inhalation challenge by simulating the work place (O'Brien *et al.*, 1979; Pepys & Hutchcroft, 1975). Synthetic hapten-protein conjugates have not been used previously for bronchial provocation tests in man. By contrast, in a guinea-pig model of TDI asthma (Karol *et al.*, 1980), where animals were sensitized by inhalation of TDI vapour, bronchial provocations with TDI-GSA or *p*-TMI-GSA successfully elicited immediate respiratory reaction (Karol, 1981b). The negative results were possibly due to the fact that the bronchial provocation tests were performed 1 year after the patient's symptomatic occupational exposure. RAST titres at the time of challenge, although positive, were well below their values of 1 year earlier. Although the patient had retained a degree of skin sensitivity, respiratory sensitivity may have declined substantially during the year.

The patient's bronchial reactivity to methacholine had decreased toward normal 2 years after his last asthmatic reaction. This result is similar to that reported by Lam,

Wong and Yeung (1979), and suggests that the non-specific bronchial hyperreactivity is the consequence rather than the predisposing factor of his occupational asthma.

This study describes a patient with occupationally induced asthma in which two isocyanate-HSA antigens were each successful in detecting specific IgE antibodies and skin sensitivity. The immune response was considered to be a result of MDI exposure since the specificities of antibodies in the patient's serum were similar to those observed in animals deliberately immunized with MDI. Although the causal relationship between the immunologic response and the asthma could not be established, the value of using isocyanate-protein conjugate antigens to aid diagnosis of occupational asthma was apparent.

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