

Hypersensitivity pneumonitis (extrinsic allergic alveolitis) induced by isocyanates

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Background: Chemical-induced hypersensitivity pneumonitis has been so far rarely described. The purpose of this study was to find out whether hypersensitivity pneumonitis is a common disorder in isocyanate workers.

Methods: Company physicians' case histories of 1780 isocyanate workers were evaluated. In 16 subjects suspected of having isocyanate-induced hypersensitivity pneumonitis, chest x-ray films were made; levels of IgE and IgG antibodies to isocyanate-human serum albumin were estimated; conjugates and isocyanate challenge tests, bronchoalveolar lavage fluid analyses, and/or lung histologic investigations were performed.

Results: Each of the 14 study patients who had hypersensitivity pneumonitis had work-related dyspnea and fever occurring several hours after the start of work with isocyanates. Typical clinical findings were the reduction of lung diffusing capacity ($n = 10$), reticular or nodular lung patterns in the x-ray film ($n = 9$), and serum IgG antibodies specific to isocyanate-human serum albumin conjugates ($n = 10$). Restrictive ventilation patterns in the inhalation challenge tests ($n = 5$), lymphocytic and/or neutrophilic alveolitis seen in bronchoalveolar lavage fluid analyses ($n = 7$), and lymphohistiocytic patterns mostly associated with mild fibrosis in lung histology ($n = 5$) confirmed the diagnosis.

Conclusions: Occupational exposure to isocyanate vapors and aerosols induces typical hypersensitivity pneumonitis in at least 1% of the isocyanate workers with symptoms. Diphenylmethane diisocyanate was found to be the main cause of this disorder. (*J ALLERGY CLIN IMMUNOL* 1995;95:1004-10.)

Key words: Isocyanates, extrinsic allergic alveolitis, hypersensitivity pneumonitis, IgG antibodies, restrictive ventilation pattern, chest x-ray film, bronchoalveolar lavage fluid

Well-known substances causing hypersensitivity pneumonitis exist in *Thermoactinomyces*, *Aspergillus*, and several other microorganisms, and in different avian species. Corresponding disorders induced by chemicals have been rarely investigated.¹⁻⁴ Animal exposure to high concentrations of trimellitic anhydride fumes or dust was shown to cause the late respiratory systemic syndrome, which resembles hypersensitivity pneumonitis or the pulmonary disease-anemia syndrome.^{5,6} The situation in human beings is less clear, although some suspected cases have been reported.^{4,7-10} With regard to isocyanates, several case reports^{2,3,11-15} and a recently published article describing nine examined patients¹⁶ support the in-

Abbreviations used

BAL:	Bronchoalveolar lavage
DL _{co} :	Diffusing lung capacity for carbon monoxide
EAST:	Enzyme allergosorbent test
HDI:	Hexamethylene diisocyanate
HSA:	Human serum albumin
MDI:	Diphenylmethane diisocyanate
ppb:	Parts per billion
TDI:	Toluene diisocyanate

terpretation that these highly reactive and widely used reagents can also induce hypersensitivity pneumonitis. Summarizing the results from 14 patients of our clinic who underwent detailed clinical, immunologic, and partial bronchoscopic examinations, this study provides further evidence that isocyanates cause this chronic lung disease.

METHODS

Sixteen patients were initially recruited from a group of 1780 isocyanate workers. Two hundred ninety-four

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workers from this group comprised the whole staff of four car and chemical factories. The remaining subjects were involved in medical surveillance programs for isocyanate workers or were patients of our outpatient department. All subjects underwent physical examination by a company physician who also recorded detailed case histories. Furthermore, chest x-ray films and lung function testing (vital capacity, forced expiratory volume in 1 second) were routinely performed. According to case histories, 1095 probands reported workplace-related health problems, predominantly of the respiratory tract. Details of the study with special regard to bronchial asthma are published separately.¹⁷

The 16 subjects considered for this study comprised those who had repeatedly had dyspnea, fever, and malaise after latency periods of several hours after exposure to isocyanates. All but one had coughing and all but two reported excessive phlegm. Fourteen subjects agreed to take part in this study. Ten subjects had never smoked, and four were ex-smokers (patients 2, 6, 7, and 8; these ex-smokers had discontinued smoking 16, 20, 0.9, and 0.5 years before the study, respectively). According to case histories, none of them had had allergic rhinitis or bronchial asthma. However, routine skin prick testing with a panel of 20 common allergens revealed in one patient (No. 8, a former baker) an immediate-type response to an extract of wheat malt of approximately 50% of that obtained by 1% histamine.

The 14 workers underwent additional clinical investigations. Lung function tests were always performed and chest x-ray films were always made during working periods or within 1 month afterward. The lung function tests were conducted with a body plethysmograph (Masterlab-Transfer, Jaeger, Würzburg, Germany).

The results are expressed as a percentage of predicted normal values according to regression equations of Quanjer et al.¹⁸ and Cotes et al.¹⁹

We estimated IgE and IgG antibodies specific to human serum albumin (HSA)-bound toluene diisocyanate (TDI), diphenylmethan diisocyanate (MDI), and hexamethylene diisocyanate (HDI) in all patients by RAST²⁰ or enzyme allergosorbent test (EAST)²¹ and by a modified IgG-RAST or IgG-EAST,²² respectively. The EAST method differed from the RAST insofar as enzyme-linked instead of iodine 125-labeled secondary antibodies (β -galactosidase-conjugated anti-human IgE, alkaline phosphatase-conjugated anti-human IgG) with corresponding substrates were used. The isocyanate-HSA conjugates had been coupled to cyanogen bromide-activated paper disks.²⁰

Bronchoalveolar lavage (BAL) was performed as recommended by the Task Group of Bronchoalveolar Lavage of the European Society of Pneumology²³ with 100 to 140 ml of saline solution; recovery ranged from 30% to 60%. BAL fluid cells were analyzed by light microscopy (May-Grünwald-Giemsa staining) of cytocentrifuged preparations. Lymphocyte phenotyp-

ing, which was possible in only a few cases, was performed by flow cytometry with monoclonal antibodies for CD3, CD4, and CD8 (Becton Dickinson, Heidelberg, Germany).

If acceptable lung function values and informed consent were available, patients underwent inhalative MDI challenge tests in a chamber²² after occupational exposure was interrupted for at least one week. During the challenge test isocyanate air concentrations were continuously measured by MDA tape monitor (model 7005 or 7100). Usually we started with 5 parts per billion (ppb) of MDI for 15 minutes followed by 10 ppb for 30 minutes, and finally we applied 20 ppb for 5 minutes.

The final diagnosis of hypersensitivity pneumonitis was always based on compatible work-related complaints associated with at least two of the following parameters: reduced inspiratory vital capacity or diffusing lung capacity for carbon monoxide (DL_{CO}), interstitial lung shadowing, presence of isocyanate-specific IgG antibodies, positive response in the isocyanate challenge test (decreases in inspiratory vital capacity of at least 20%, in DL_{CO} of at least 15% or in arterial oxygen pressure of at least 7 mm Hg, increase in body temperature of at least 1° C, and malaise), lymphocytosis (decreased CD4/CD8 ratio) in BAL fluid in the chronic stage of the disease (characterized by persisting symptoms during ongoing exposure), or neutrophilic alveolitis in the acute stage.

RESULTS

Clinical investigations

Table I shows individual data on patients, occupational exposure to isocyanates, and symptoms. The subjects produced polyurethane foam, performed injection molding in foundries, sprayed paint, or worked in various other industrial branches. The preponderance of MDI exposure was noticeable (nine cases). Three paint sprayers had contact with an HDI biuret, and three handled TDI and other isocyanates. Workers' mean exposure period to isocyanates was 6.0 years (range, 0.5 to 20 years).

Each worker reported recurrent episodes of fever (38° to 40° C; in two cases temperature was not measured) and dyspnea, usually 2 to 5 hours after exposure. Two subjects had weight loss (≥ 4 kg), and one had work-related rhinitis.

Chest x-ray films showed a preponderance of reticular lung patterns (Table II); nodular opacities, patchy infiltrates, and alveolar patterns were also seen (Fig. 1). Five probands had a normal findings on chest x-ray film.

The vital capacity was reduced in seven cases, and DL_{CO} was abnormal in 10 subjects. None of the patients had a typically obstructive ventilation

TABLE I. Work-related exposure and symptoms

Patient No.	Age (yr)/sex	Occupation	Exposure/duration (yr)	Symptoms/duration (yr)
1	54/M	Foam production	MDI/3	F,D/1.5
2	53/M	Paint spraying	HDI/20	F,D,W/0.5
3	46/M	Foam production	MDI/5	F,D/1.0
4	42/M	Plastic welding	TDA, TIPHP/0.5	F,D/0.2
5	48/F	Adhesive application	MDI (TDI)/0.5	F,D/0.4
6	46/M	Injection molding*	MDI/12	F,D/2.0
7	53/M	Injection molding*	MDI/1.2	F,D,W/0.9
8	25/M	Paint spraying	HDI/0.5	F,D/0.1
9	59/M	Paint spraying	HDI (TDI)/13	F,D/2.0
10	15/M	Foam production	MDI/1	F,D/0.2
11	28/M	Adhesive application	MDI/1	F,D/0.3
12	38/M	Foam production	MDI/1	(F),D/0.1
13	54/M	Injection molding*	MDI/16	F,D,R/0.5
14	53/M	Foam production	MDI/9	(F),D/5.0

D, Dyspnea; F, fever; (F), fever not substantiated; R, rhinitis; TIPHP, tris(4-isocyanatophenyl)-thiophosphate; W, loss of body weight; parentheses indicate minor exposure.

*Use of silica sand for so-called cold box system in steel or aluminum foundries.

TABLE II. Chest x-ray film findings and lung function parameters

Patient No.	Chest x-ray film findings	Lung function				Isocyanate-specific IgG (kU/L)†
		IVC (% of normal)	FEV ₁ (% of normal)	Airway resistance (% of normal*)	DL _{CO}	
1	Normal	85	70	0.1	81	6.21
2	Reticular, patchy	85	80	0.1	54‡	15.21
3	Reticular, patchy	82‡	77	0.2	68‡	4.00
4	Reticulonodular	61‡	60‡	0.2	46‡	2.85
5	Reticulonodular	99	81	0.2	50‡	3.17
6	Normal	99	77	0.2	74‡	5.74
7	Reticular, patchy	83‡	69	0.3	49‡	4.04
8	Reticular	70‡	82	0.2	73‡	1.36§
9	Reticular, patchy	68‡	72	0.1	60‡	1.46§
10	Alveolar	93	92	ND	97	2.92
11	Nodular	89	88	0.1	63‡	2.53
12	Normal	80‡	65‡	0.1	68‡	7.90
13	Alveolar	108	93	0.2	82	1.00§
14	Normal	80‡	81	0.3	112	1.30§

FEV₁, Forced expiratory volume in 1 second; ND, not done.

*Normal range, <0.3 kPa · second · L⁻¹.

†Values of greater than 2 kU/L are regarded as positive because 25 unexposed control subjects had mean values +2 SD of 1.86 to 1.98 kU/L. IgG values were always obtained with the HSA conjugate of the diisocyanate mainly or exclusively used in the workplace (see Table I). Tris(4-isocyanatophenyl)-thiophosphate could not be investigated. Subjects 5 and 9 had 1.21 and 1.53 kU/L for TDI-HSA, respectively.

‡Less than regression equations minus 1.64 residual SD.

§At least 1 year after cessation of exposure; in all other cases time lapse since last exposure was less than 1 month.

pattern. Forced expiratory volume in 1 second as a percentage of vital capacity was slightly reduced in two probands, however.

Ten of the 14 workers had significant levels of

IgG antibodies specific to isocyanate-HSA conjugates (HSA conjugates corresponding to isocyanates used in the workplace were always tested). IgE antibodies were not detectable (<0.35 kU/L),

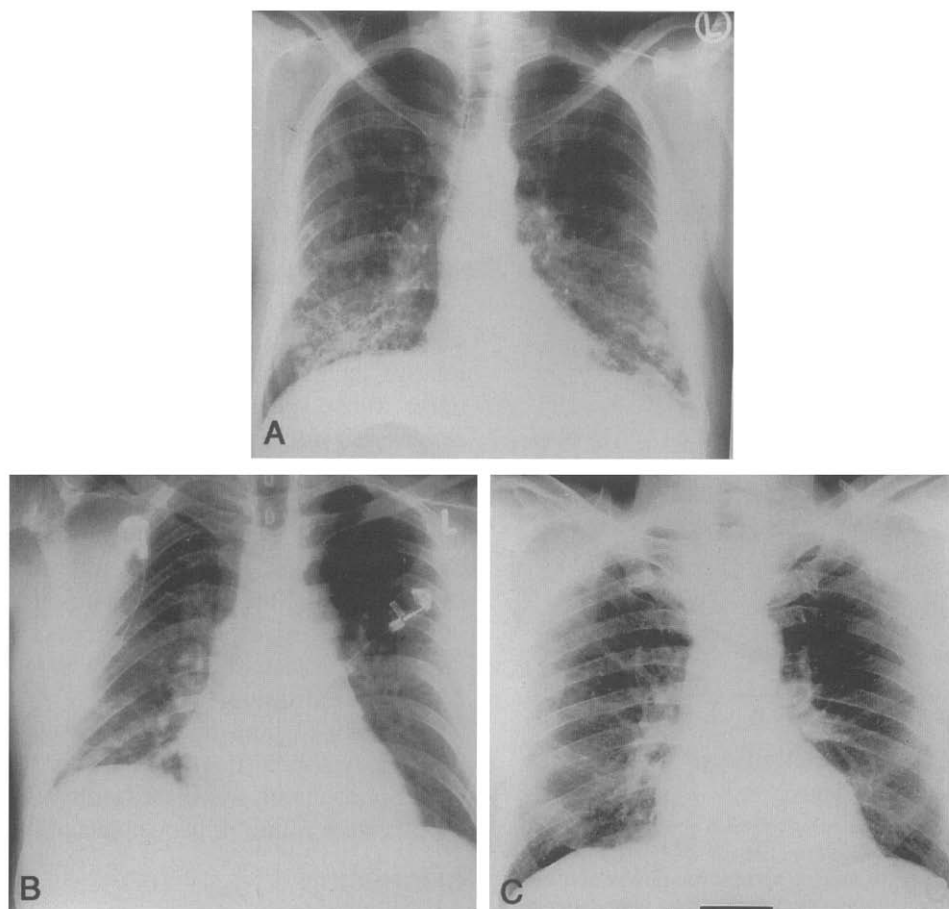


FIG. 1. Chest x-ray films of patients 2 (A), 4 (B), and 9 (C).

TABLE III. Inhalation MDI challenge tests

	Patient 1		Patient 6		Patient 7		Patient 13		Patient 14	
	Before	After (hr*)	Before	After (hr*)	Before	After (hr*)	Before	After (hr*)	Before	After (hr*)
Airway resistance (kPa/L/sec)	0.13	0.53† 0.32 (7)	0.29	0.38 (6.5)	ND	ND	0.17	0.30 (6)	0.31	0.45 (6)
FEV ₁ (L)	2.15	1.10† 1.55 (7)	2.69	1.50 (5.5)	2.75	1.75 (8)	4.04	3.40 (4-24)	2.88	2.28 (6)
IVC (L)	3.75	2.85 (7)	3.30	2.45 (5.5)	3.08	2.06 (8)	4.38	3.69 (6)	3.74	3.01 (6)
DL _{co} (mmol/min/kPa)	8.2	5.8 (7)	ND	ND	ND	ND	11.4	9.5 (4)	10.4	9.56 (5)
Pao ₂ (mm Hg)	74.0	58.0 (7)	87.0	76.0 (6)	ND	ND	80.0	62.0 (6)	79.0	65.0 (4)
Temperature (° C)	37.3	39.0 (7.5)	36.3	38.3 (5.5)	37.1	38.6 (8)	35.2	39.7 (8)	36.7	38.5 (7)
Blood leukocytes (×10 ³ cells/mm ³)	4.4	13.5 (6)	5.7	17.2 (6.5)	7.2	14.9 (6)	6.0	13.0 (8)	7.5	9.9 (6)

FEV₁, Forced expiratory volume in 1 second; IVC, inspiratory vital capacity; ND, not done.

MDI concentrations used for inhalative challenge were 5 ppb for 15 minutes, 10 ppb for 30 minutes except in case 14 (3 ppb for 25 minutes, 10 ppb for 5 minutes), 20 ppb for 5 minutes (patients 6 and 13), 10 ppb for 30 minutes, and 20 ppb for 25 minutes (patients 1 and 7).

*Hour in which maximum change occurred.

†Immediately after challenge.

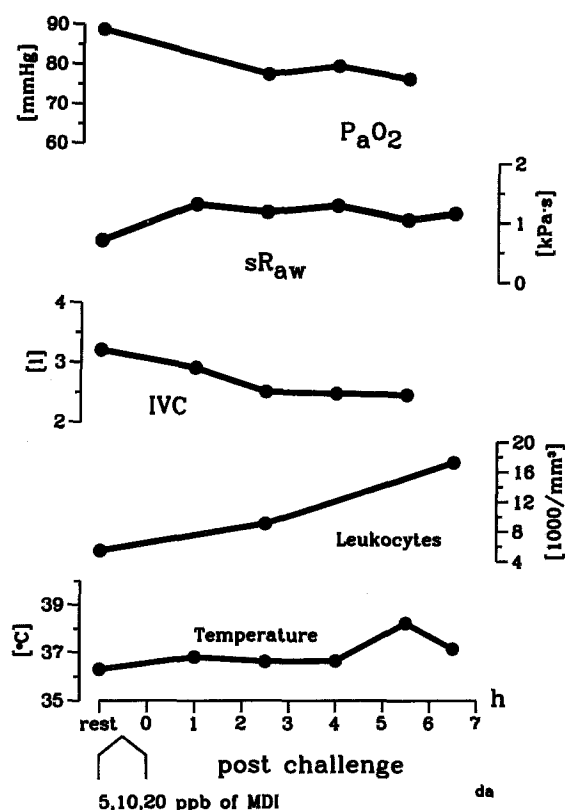


FIG. 2. Patient 6. Results of inhalation challenge test with isocyanate MDI performed in chamber.¹⁷ Restrictive ventilation pattern and systemic symptoms developed several hours after challenge. IVC, Inspiratory vital capacity; FEV₁, forced expiratory volume in 1 second; sR_{aw}, specific airway resistance; PaO₂, arterial pressure of oxygen.

an outcome different from results obtained in 14% of patients with isocyanate asthma.¹⁷

Isocyanate challenge tests

After patients granted informed consent, inhalative challenges with the isocyanate MDI were performed in five subjects during asymptomatic periods, to confirm the diagnosis of isocyanate alveolitis.

Results are shown in Table III, and a detailed example is given in Fig. 2. Parameters under rest conditions did not significantly differ from those obtained in a control measurement on the day before the test (variation of lung function values was always <8%). After a latency period of 2 to 8 hours, systemic symptoms such as fever, malaise, and leukocytosis associated with a restrictive ventilation pattern developed in each of the five subjects. Impairment of DL_{CO} and/or hypoxemia could be substantiated in two and four cases, respectively. In addition, one obstructive ventilation pattern was observed. It is interesting that

patient 13 responded in a manner typical of allergic alveolitis despite his negative IgG antibody finding.

BAL fluid analyses and lung biopsies

Bronchoscopy showed in most cases reddening and swelling of bronchial mucosa. This finding corresponds to that in patients with farmer's lung or pigeon breeder's disease.

In BAL fluid analysis five of six investigated subjects demonstrated an absolute and four also a relative increase in lymphocytes. In the two patients whose specimens could be subtyped, CD8 cells were borderline and strongly elevated, respectively. Three patients examined 5 to 6 hours after challenge showed strongly elevated polymorphonuclear granulocyte counts. The examinations of one patient (No. 12) and of four others in the chronic stage of the disease (continuing symptoms during persisting exposure) exhibited lymphocytic alveolitis associated mostly with a moderate increase in neutrophils.

Lung biopsy, performed in five subjects, always revealed lymphohistiocytic infiltrates. Granulomatous lesions mainly associated with mild interstitial fibrosis were found in two subjects (Table IV).

DISCUSSION

Hypersensitivity pneumonitis is caused by the inhalation of different organic substances and mediated by cytotoxic mechanisms and various cellular and humoral immune responses. Although the individual pathogenetic role of these responses is not clear, it can be assumed that diagnostically used parameters such as specific IgG antibodies (which may lead to the formation of immune complexes and the activation of the complement cascade) and antigen-specific lymphocytic reactions are also operative in this chronic interstitial lung disease.

Symptoms and clinical findings of the 14 cases described here are in line with the generally accepted pathogenesis and diagnostic criteria of hypersensitivity pneumonitis. Strong clues for this interpretation are lymphocytic alveolitis, increased neutrophils in BAL fluid during the acute stage, histology of lung specimens, impaired lung function parameters, presence of isocyanate-HSA-specific IgG antibodies, and exposure-related pulmonary and systemic symptoms.

Isocyanate-HSA conjugates, which were shown to comprise new antigenic determinants,²¹ may not be optimal antigens for quantifying specific antibodies in sensitized persons. However, several

TABLE IV. Results of bronchoscopy, transbronchial biopsies, and BAL analysis performed in seven isocyanate workers with allergic alveolitis

Patient No.*	Bronchoscopy		BAL analysis†					
	Macroscopy	Lung histology	Total cells ($\times 10^6/L$)	Macro-phages (%)	Neutro-phils (%)	Eosino-phils (%)	Lympho-cytes (%)	CD4/CD8 ratio
6	Bronchitis	ND	27.0	29	62	1	8	ND
12	Bronchitis	ND	7.8	73	16	1	9	ND
13	Bronchitis	ND	6.4	66	24	4	7	0.5
7	Normal	Mixed cellular pneumonitis, discrete interstitial fibrosis	73.6	58	1	1	40	ND
8	Bronchitis	Lymphohistiocytic infiltration	51.0	55	2	2	41	ND
10	Normal	Lymphohistiocytic infiltration	ND	ND	ND	ND	ND	<0.9
11	Bronchitis	Lymphohistiocytic infiltration, discrete fibrosis	43.2	54	0	3	43	ND
12	Bronchitis	Interstitial pneumonitis, discrete fibrosis	6.0	35	5	1	59	0.4

ND, Not done.

*Examinations were performed 5 to 6 hours after MDI challenge in patients 6, 12, and 13, and during chronic stage in patients 7, 8, 10, 11, and 12.

†Normal values: Total cells, $<13 \times 10^6$ cells; macrophages, $>84\%$; neutrophils, $<3\%$; eosinophils, $<0.5\%$; lymphocytes, $<13\%$; CD4/CD8 ratio, 1.1 to 3.5.

trials with a variety of carrier proteins and peptides have not yielded more suitable antigens.^{3, 20} In any case, the sensitivity of antibody detection in isocyanate workers is low, which may explain the typical response observed in the MDI challenge test of an antibody-negative subject.

Isocyanate-HSA-specific IgG antibodies were also found in healthy workers.¹⁷ Thus their presence is per se not a reliable indicator of isocyanate-induced allergic alveolitis.

The DL_{CO} was the most sensitive lung function parameter and was decreased in 10 of the 14 subjects on the day of investigation.

Absence or only discrete signs of lung fibrosis in our patients may be due to the relatively short disease duration and/or to the low exposure in comparison with patients with farmer's lung disease or pigeon breeder's disease.

The challenge test performed in five workers resulted in restrictive ventilation patterns, hypoxemia, malaise, fever, increased leukocyte counts in blood, and an elevated neutrophil count in BAL fluid. These findings together with the time lapse of several hours are typical of hypersensitivity pneumonitis.²⁴

Duration of isocyanate exposure, time of onset of symptoms, and lag period until diagnosis varied considerably. An association between these data on the one hand and symptoms, chest x-ray film findings, lung function parameters, and levels of

isocyanate-HSA-specific IgG antibodies on the other hand could not be found. However, the preponderance of MDI as the causative substance is striking. Because no worker was exclusively exposed to TDI, it remains unclear whether this compound was the cause of the observed disorders. These results differ from exposure data obtained in our total group of 1780 isocyanate workers; here we found comparable numbers of subjects exposed to MDI, TDI, HDI, or their derivatives during the last 15 years, and in none of the 82 challenge tests with TDI or HDI atmospheres was hypersensitivity pneumonitis observed.¹⁷

Corresponding to the experiences with patients with farmer's lung, humidifier lung, or bird breeder's disease, the strong preponderance in the isocyanate alveolitis group of subjects who had never smoked (9/13) is notable. Two subjects stopped smoking more than 15 years ago, and two other probands quit at the beginning of isocyanate exposure or of workplace-related symptoms, respectively. This suggests that never-smoking and ex-smoking status is associated with an increased risk of isocyanate alveolitis.

Our results indicate that MDI and in a few cases HDI induce typical hypersensitivity pneumonitis. Because we did not find any other convincing case in clinical studies of 1780 isocyanate workers, this disease seems to be rare. In contrast to our find-

ings, Vandenplas et al.¹⁶ reported a prevalence of allergic alveolitis of 4.7% in a factory where MDI-based binding resin was used to manufacture wood chip boards. The reason is unclear, but special working conditions might be important.

We conclude that symptoms such as fever, malaise, and dyspnea recurring several hours after working in at-risk environments such as foam production, injection molding, and paint spraying sites should initiate specific screening and surveillance programs so that definite diagnoses of exposed workers can be obtained and appropriate preventive measures can be introduced.

REFERENCES

1. Malo JL, Pineau L, Cartier A. Reference values of the provocative concentrations of methacholine that cause 6% and 20% changes in forced expiratory volume in one second in a normal population. *Am Rev Respir Dis* 1983;128:8-11.
2. Walker CL, Grammer LC, Shaughnessy MA, Duffy M, Stoltzfuss VD, Patterson R. Diphenylmethane diisocyanate hypersensitivity pneumonitis: a serologic evaluation. *J Occup Med* 1989;31:315-9.
3. Wass U, Belin L. An in vitro method for predicting sensitizing properties of inhaled chemicals. *Scand J Work Environ Health* 1990;16:208-24.
4. Zeiss CR, Wolkonsky P, Chacon R, et al. Syndromes in workers exposed to trimellitic anhydride: a longitudinal clinical and immunologic study. *Ann Intern Med* 1983;98:8-12.
5. Biagini RE, Bernstein DI, Gallagher IS, et al. Immune response of cynomolgus monkeys to phthalic anhydride. *J ALLERGY CLIN IMMUNOL* 1988;82:25-9.
6. Zeiss CR, Levitz D, Leach CL, et al. A model of immunologic lung injury induced by trimellitic anhydride inhalation: antibody response. *J ALLERGY CLIN IMMUNOL* 1987;79:59-63.
7. Czappon AB, Kaplan V, Speich R, Baur X. Acute autoimmune response in a case of pyromellitic acid dianhydride-induced hemorrhagic alveolitis. *Allergy* 1994;49:337-41.
8. Gerhardsson L, Grammer LC, Shaughnessy MA, Patterson R. IgG subclass antibody against trimellitic anhydride in workers with and without immunologic lung diseases. *J Occup Med* 1992;34:989-92.
9. Grammer LC, Shaughnessy MA, Henderson J, et al. A clinical and immunologic study of workers with trimellitic-anhydride-induced immunologic lung disease after transfer to low exposure jobs. *Am Rev Respir Dis* 1993;148:54-7.
10. Patterson R, Addington W, Banner AS, et al. Antihapten antibodies in worker exposed to trimellitic anhydride fumes: a potential immunopathogenetic mechanism for trimellitic anhydride pulmonary disease-anemia syndrome. *Am Rev Respir Dis* 1979;120:1259-67.
11. Bascom R, Kennedy TP, Levitz D, Zeiss CR. Specific bronchoalveolar lavage IgG antibody in hypersensitivity pneumonitis from diphenylmethane diisocyanate. *Am Rev Respir Dis* 1985;131:463-5.
12. Charles J, Bernstein A, Jones B, et al. Hypersensitivity pneumonitis after exposure to isocyanates. *Thorax* 1976;31:127-36.
13. Fink JN, Schlueter DP. Bathtub refinisher's lung: an unusual response to toluene diisocyanate. *Am Rev Respir Dis* 1978;118:955-9.
14. Selden 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;15:234-7.
15. Yoshizawa Y, Ohtsuka M, Noguchi K, Uchida Y, Suko M, Hasegawa S. Hypersensitivity pneumonitis induced by toluene diisocyanate: sequelae of continuous exposure. *Ann Intern Med* 1989;110:31-4.
16. Vandenplas O, Malo JL, Dugas M, et al. Hypersensitivity pneumonitis-like reaction among workers exposed to diphenylmethane diisocyanate (MDI). *Am Rev Respir Dis* 1993;147:338-46.
17. Baur X, Ammon J, Czappon AB, et al. Respiratory hazards of isocyanates. *Int Arch Occup Environ Health* 1994;66:141-52.
18. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. In: Standardized lung function testing: official statement of the European Respiratory Society. *Eur Respir J* 1993;16:5-40.
19. Cotes JE, Chinn DJ, Quanjer PhH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity): official statement of the European Respiratory Society. *Eur Respir J* 1993;16:41-52.
20. Dewair MA, Baur X. Studies on antigens useful for detection of IgE antibodies in isocyanate-sensitized workers. *J Clin Chem Clin Biochem* 1982;20:337-40.
21. Baur X. Immunologic cross-reactivity between different albumin-bound isocyanates. *J ALLERGY CLIN IMMUNOL* 1983;71:197-205.
22. Baur X, Dewair M, Roemmelt H. Acute airway obstruction followed by hypersensitivity pneumonitis in an isocyanate (MDI) worker. *J Occup Med* 1984;26:285-7.
23. Klech H, Pohl W. Technical recommendations and guidelines for bronchoalveolar lavage (BAL): report of the European Society of Pneumology Task Group on BAL. *Eur Respir J* 1989;2:561-85.
24. Hendrick DJ, Marshall R, Faux JA, Krall JM. Positive alveolar responses to antigen inhalation provocation tests: their validity and recognition. *Thorax* 1980;35:415-27.