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Subclinical Immunologic and Physiologic Responses in Hexamethylene Diisocyanate-Exposed Auto Body Shop Workers

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Background *Diisocyanates are potent sensitizing agents and currently the most commonly identified cause of occupational asthma in industrialized countries. However, diisocyanate asthma is difficult to diagnose and exposure and host risk factors are unclear. Auto body shops, one of the most common hexamethylene diisocyanate (HDI) exposure settings, are particularly difficult to study due to their small size and episodic exposures. Surveillance studies of such workers are limited.*

Objectives *We have initiated a cross-sectional field epidemiologic study, Survey of Painters and Repairers of Auto bodies by Yale (SPRAY), to characterize the effects of diisocyanate exposures on actively employed auto body shop workers.*

Methods and Results *We present here questionnaire, physiologic, immunologic, and exposure data on 75 subjects enrolled in the study. No overt cases of clinically apparent diisocyanate asthma were identified based on spirometry, methacholine challenge, peak flows, and symptoms. HDI-specific lymphocyte proliferation was present in 30% of HDI-exposed workers and HDI-specific IgG in 34% of HDI-exposed workers, but they were not associated. HDI-specific IgE was detected in two workers. HDI-specific lymphocyte proliferation, increased methacholine responsiveness, and symptoms of chest tightness and shortness of breath were more common in the most heavily HDI-exposed workers, the painters. More long-term follow-up of this cohort should clarify the significance of these HDI-specific immunologic responses, physiologic changes, and symptoms.*

Conclusions *These findings demonstrate the presence of HDI-specific immune responses in a large proportion of healthy HDI-exposed workers.* Am. J. Ind. Med. 39:587–597, 2001. *© 2001 Wiley-Liss, Inc.*

KEY WORDS: diisocyanates; asthma; hexamethylene diisocyanate; surveillance; lymphocyte proliferation

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Abbreviations: HDI, hexamethylene diisocyanate; BSA, bovine serum albumin; HSA, human serum albumin; PBMCs, peripheral blood mononuclear cells; SPRAY, Survey of Painters and Repairers of Auto bodies by Yale; IQR, interquartile range.

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INTRODUCTION

Diisocyanates, a group of highly reactive, low molecular weight compounds that are the obligate cross-linking agent for polyurethanes, are potent sensitizing agents and currently the most commonly identified cause of occupational asthma in industrialized countries [Vandenplas et al., 1993a; Raulf-Heimsoth and Baur, 1998]. Diisocyanate exposures frequently occur in small industries during end-user applications such as spray painting. Such industries have been difficult to investigate, in part due to their small size and sporadic exposures. One of the most common exposure settings is auto body shops where hexamethylene diisocyanate (HDI)-containing paints, primers, and coatings are used extensively, predominantly prepolymers of HDI such as HDI biuret. Studies investigating the immunologic and physiologic effects of HDI in such exposed populations have been limited. Both HDI monomer and prepolymers can induce occupational asthma [Vandenplas et al., 1993b]. A few small cross-sectional studies have shown a prevalence of diisocyanate asthma ranging from 0 to 33%, depending on the exposed population and criteria used to define diisocyanate asthma [Grammer et al., 1988; Welinder et al., 1988; Randolph et al., 1997] Most studies have relied on symptoms and spirometry, each of which has proven problematic for the clinical diagnosis of diisocyanate asthma. Immunologic assessment of HDI exposed workers has also been limited. HDI-specific IgE and IgG have been detected in selected patients with diisocyanate asthma or small populations of exposed workers, frequently without concurrent physiologic, symptom, and exposure data [Seguin et al., 1987; Grammer et al., 1988; Cartier et al., 1989; Baur et al., 1996; Tee et al., 1998].

How diisocyanates cause sensitization and asthma remains poorly defined, but available clinical and animal data implicate immune mediated mechanisms [Bernstein 1996, Maestrelli et al., 1997; Raulf-Heimsoth and Baur, 1998; Redlich et al., 1999]. The study of diisocyanate sensitization and asthma has been hampered by several factors. The innate reactivity of diisocyanates has hindered classical in vivo and in vitro approaches and made it unclear what the biologically relevant diisocyanate antigen is. Epidemiologic and clinical studies have suffered from the lack of well-defined exposure data and the lack of a simple diagnostic or screening test to identify those with sensitization or isocyanate asthma.

To address these problems we have initiated interrelated epidemiological and immunologic studies of auto body shop workers exposed to HDI, predominantly prepolymers of HDI. Our goals are to: characterize the physiologic and immune responses found in HDI-exposed workers; delineate diisocyanate exposure patterns in auto body shops; and identify host and exposure risk factors for diisocyanate sensitization and asthma. These studies should lead to a better understanding of the immune mechanisms by which diisocyanates cause sensitization and asthma and hopefully to an improved test or algorithm to screen for or diagnose diisocyanate asthma.

We present here questionnaire, physiologic, immunologic, and exposure data on 75 workers in the field epidemiologic study, a Survey of Painters and Repairers of Auto bodies by Yale (SPRAY). Surveillance of these HDI-exposed workers demonstrates a high prevalence of HDI-specific immunologic responses, although no cases of overt diisocyanate asthma were identified.

METHODS

Overall SPRAY Study Design

The epidemiological SPRAY study was designed as a cross-sectional survey of approximately 50 auto body shops and 300 workers with varying degrees of HDI exposure. Auto body shops and individual employees in the greater New Haven area were invited to participate. The investigative team spent a week at each shop obtaining extensive questionnaire, physiologic, and industrial hygiene data, as well as bloods for immunologic and other assays (see below). Questionnaires assessing general health, occupational history, and respiratory symptoms were administered to all participants. Information was also obtained about the auto body shop. The study protocol was approved by the Human Investigations Committee at Yale University and informed written consent obtained from each participant.

Questionnaires. An interviewer-administered questionnaire was obtained on each of the subjects. The subject questionnaire consisted of the IUATLD respiratory questionnaire for the diagnosis of asthma [Burney et al., 1989], part of the modified American Thoracic Society questionnaire [Ferris, 1978], with additional questions concerning the onset of respiratory symptoms and temporal relationship of symptoms to work, occupational history, respiratory protection, host factors, and medical history. Asthma-like symptoms included at least one of the following: cough; wheeze; chest tightness, and shortness of breath. The symptoms were classified as work-related if they got worse while at work or after leaving work, and improved away from work (weekend, vacation). Non-specific respiratory symptoms included wheezing, cough or shortness of breath occurring only with an upper respiratory tract infection or only in the early morning (i.e., smoker's cough).

The field industrial hygienist administered an additional questionnaire to the shop owner or manager regarding the auto body shop (including size, number of employees,

type of work, exposures, use of protective equipment, spray booth). The field industrial hygienist also completed a week-long work diary for each worker, recording all tasks performed during each half hour of the work day, such as spray painting, body repair work, or sanding.

Physiologic testing. Spirometry was performed on each participant at a given shop before and after work Monday through Thursday using a Nellcor Puritan Bennett spirometer (Mallinckrodt Inc., St Louis, MO), with subjects seated and wearing nose clips, according to the American Thoracic Society criteria [ATS, 1995]. A minimum of three acceptable forced expiratory maneuvers was obtained from each subject on each test occasion and the maximal FEV1 and FVC were used for analysis, according to American Thoracic Society standards for reproducibility. Cross shift and cross week changes in FEV1 were calculated. A 5% drop in FEV1 across shift or across the week was chosen to describe the population, a cut-off used in epidemiologic studies of exposed workers.

Peak expiratory flow rate (PEFR) measurements were performed using a mini-Wright peak flow meter for 1 week including one weekend and were recorded in a diary. Subjects were instructed to obtain PEFR measurements at home and at work every 4 h while awake and were reminded frequently to encourage maximal compliance. A minimum of 4 days of recordings was required for analysis, including at least one non-work day. Diurnal variation was calculated and visual interpretation made by a panel of two readers (CA Redlich, MR Cullen) to compare workday with non-work values.

Methacholine challenge testing was performed in the auto body shops on each individual to assess bronchial responsiveness on Monday morning before work and Thursday afternoon, near the end of the work week. The test was rescheduled at least 2 weeks later if the participant reported a recent upper respiratory tract infection. A modified Cockcroft methacholine challenge protocol was employed [Cockcroft et al., 1977; Sterk et al., 1993]. Normal saline for baseline was followed by increasing concentrations of methacholine which were nebulized for 2 min using a Devilbiss #644 nebulizer (Sunrise Medical Respiratory Products, Somerset, PA) with an output of 5 L/min. A forced expiratory maneuver was performed at 1 min after each concentration and the FEV1 recorded, providing the test was technically satisfactory. The following doses of methacholine were used: 1, 5, 10, and 25 mg/ml. The test was terminated when the FEV1 fell to 80% of the best post-saline level or the maximum concentration of methacholine was obtained. The PC_{20}, the concentration associated with a 20% drop in FEV1, was determined by linear interpolation or extrapolation. $PC_{20} < 25$ mg/ml, the highest dose of methacholine given, was chosen as the cutoff to describe airway responsiveness in the population.

Blood studies. Blood was drawn on the morning of the second or third day in the shop for several assays including total IgE, immunologic responses to HDI and genetic studies. Peripheral blood mononuclear cells (PBMCs) were isolated for immediate use in the proliferation assays (see below), as well as additional cytokine studies to better characterize the HDI-responsive cells and mediators. The remaining blood was processed within 2 h, and serum and whole blood aliquoted and frozen at $-70°C$ for the serologic assays (see below).

Antibody studies. Total IgE content of serum was determined with a radioimmunoassay kit (Hycor Biomedical, Inc., Garden Grove, CA). HDI-specific antibodies were determined using an enzyme-linked immunosorbent assay (ELISA) for IgG, and a radioallergosorbent test (RAST) for IgE, as previously described [Karol et al., 1995]. Human serum albumin (HSA) conjugates with hapten substitution ratios of 49:1 for HDI-monomer, or 37:1 for HDI-biuret were used as the antigens, as previously described [Karol and Hauth, 1982]. All antibody titres were confirmed by inhibition assays, as previously described [Karol et al., 1995].

Lymphocyte proliferation assay. PBMCs were isolated by Ficoll–Hypaque fractionation and plated in 96-well microtiter plates at a density of 1×10^5 cells/well in RPMI 1640 media supplemented with autologous patient plasma at a final concentration of 10% (v/v). Varying concentrations of HDI-monomer and HDI-biuret conjugated to HSA (HDI-HSA and HDI-biuret-HSA) and HSA alone were added in quadruplicate and cultures were incubated for 5 days. HDI-monomer and HDI-biuret HSA conjugates with hapten substitution ratios of 12:1 for HDI-monomer, or 37:1 for HDI-biuret were used as the antigens in the proliferation assays. Fifty to 500 ug/ml final concentrations of HDI-HSA and HDI-biuret-HSA were used based on pilot studies of HDI asthmatics and unexposed and atopic controls. Positive control stimuli included tetanus toxoid (TT) antigen (Massachusetts State Laboratories, Boston, MA) and the mitogen phytohemagglutinin A (Sigma, St. Louis, MO). Cultures were quantitatively assessed for proliferative responses based on ^{3}H-thymidine (Amersham, Piscataway, NJ) uptake. PBMCs were cultured for 5 days based on pilot proliferation time course studies that determined this time point to be optimal. During the final 18 h of culture, cells were pulsed with 1 mCi/well ^{3}H-thymidine and harvested using a semi-automated system (Tomtec, Hamden, CT). Uptake of ^{3}H-thymidine was quantitated using a ß-scintillation counter (Wallac, Turku, Finland). The means of quadruplicate cultures were determined and the results expressed as stimulation indices which were calculated based on (average cpm with test antigen—average cpm background)/(average cpm with control antigen—average

cpm background). A stimulation index of 2.0 was regarded as the cutoff criterion for a positive response, a cutoff used by other investigators [Zeiss et al., 1977; Frew et al., 1998].

HDI Exposure Assessment

Exposure information obtained from the shop questionnaire, the worker's individual questionnaire, and the daily work diaries is presented. Workers were assigned to one of the following three job categories based on the questionnaire: (1) office workers; (2) technicians (repairmen, detailmen, and helpers); and (3) painters. The number of unprotected short-term peak exposures, defined as exposures to spraying, mixing, and priming tasks, occurring daily and weekly, was calculated from the daily diaries. Extensive air, surface and skin sampling data were obtained and will be the subject of a subsequent report.

Statistical Analysis

Summary descriptive statistics were calculated for the different variables. Data that were not normally distributed were analyzed using non-parametric tests. Pearson or Spearman correlation coefficients were estimated to assess linear relationships between pairs of continuous variables, such as baseline FEV1, cross-shift and cross-week changes in FEV1, unprotected exposure episodes, and HDI-specific IgG. The frequencies of categorical variables were compared using Chi-squared tests (symptoms vs. atopy). Continuous physiologic variables were analyzed across categories using ANOVA (job category vs. age), Kruskal–Wallis tests (job category vs. unprotected exposure), Wilcoxon rank sum testing (HDI-specific IgG vs. atopy) or *t*-test (baseline FEV1 vs. PC_{20}), after appropriate transformations. The statistical analysis was performed using SAS (SAS Institute, Cary, NC).

RESULTS

Auto Body Shop Employees: Baseline Characteristics

Seventy-five auto body shop workers from 12 different shops were recruited and underwent questionnaires, week-long physiologic evaluation, blood studies, and worker diaries (Table I). This represented 93% of all workers at these shops; the remaining few were on vacation or refused to participate. Blood was obtained on 66 of these subjects. All but four (all office workers) were men; 88% were Caucasian, 8% Hispanic, and 4% African-American. Forty-five percent were never smokers and 28% current smokers. Four subjects (6%) reported a history of asthma diagnosed by a physician prior to work in auto body shops. One of these subjects reported that he still had asthma, but had no symptoms for the past 2 years, was on no-asthma medications, and his methacholine challenge test showed no airway hyper-responsiveness ($PC_{20} = 188$ mg/ml). No subjects reported currently being under treatment for asthma. Twenty-one percent of subjects had a history of allergies consistent with atopy. The median total years worked in the auto body industry was 15 years in two different shops.

Auto Body Shop Characteristics

The average number of employees per shop was seven (range 2–15), with an average of 1.9 painters, 3.8 shop workers or technicians, and 1.6 office workers. Technicians included repairmen, detailmen, and helpers. All of the shops had a negative pressure booth for painting. However, painting and priming also occurred on the shop floors,

TABLE I. Characteristics of Auto Body Workers (n = 75)

Age (years)	
Mean ± SD (range)	39.1 ±11.8 (18–67)
Gender (n, %)	
Male	71 (95%)
Female	4 (5%)
Race (n, %)	
Caucasian	66 (88%)
Hispanic	6 (8%)
African-American	3 (4%)
Education (n, %)	
Less than 12 years	10 (13%)
Graduated high school	43 (57%)
Technical/associate or some college	20 (27%)
College degree or more	2 (3%)
Smoking status (n, %)	
Never smoked	34 (45%)
Ex-smoker	20 (27%)
Current smoker	21 (28%)
History of asthma (n, %)	4 (6%)
History of atopy (n, %)	16 (21%)
Duration in auto body industry (years)	
Median (IQR[a]; range)	15 (15; 4 months–49 years)
Job category (n, %)	
Painter	18 (24%)
Technician[b]	39 (52%)
Office worker	18 (24%)
Number of shops worked at:	
Median (IQR[a]; range)	2 (2; 1–6)

[a]Interquartile range.
[b]Repairman, detailman, helper.

outside the booth. All of the shops used paints and/or primers which contain aliphatic diisocyanates, HDI and/or isophorone diisocyanate. The small, usually family owned and operated businesses had been in operation for an average of 32 years (range 4–70).

Exposure Assessment

Exposure was assessed based on shop and individual questionnaires and daily diaries. Based on job category 24% of the employees were painters, 52% technicians, and 24% office workers. The primary isocyanate containing product in use in the 12 different shops currently and over the past 10 years was HDI-oligomer, containing HDI-monomer and HDI-biuret, with some isophorone diisocyanate in use more recently. Current or past usage of aromatic diisocyanates including toluene diisocyanate and diphenylmethane diisocyanate was specifically asked about and not identified.

The number of daily and weekly short-term peak exposures to spraying, mixing and priming tasks, tasks demonstrated to be associated with highest airborne levels of HDI (data not shown), was calculated from the daily diaries. Fifty-three percent of workers had no exposures during the survey week, 23% had between 1 and 10 HDI peak exposures, and 24% had $\geq$10 peak exposures (maximum = 47). If only exposures without respiratory protection were counted, 58% had no unprotected exposures, 23% had between 1 and 5 unprotected peak HDI exposures, and 19% had $\geq$5 (maximum = 36). The number of such peak exposures was significantly associated with job category ($\chi^2 = 38.7$, $P < 0.001$ by Kruskal–Wallis). The median (IQR) number of peak exposures for office workers was 0 (0), for technicians 0 (3), and for painters 34 (16). Number of unprotected peak exposures was also associated with younger age (Spearman $r = -0.38$; $P = 0.002$) and fewer years in the industry (Spearman $r = -0.21$; $P = 0.097$). The relationships between exposure and the physiologic and immunologic endpoints are discussed below.

Symptoms

As shown in Table II approximately one half of the employees reported no respiratory symptoms, one-third had non-specific respiratory symptoms, 11% had work-related asthma-like symptoms, and 8% non-work-related asthma-like symptoms. The presence of symptoms was not associated with years of employment or smoking. Asthma-like symptoms, but not work-related asthma-like symptoms, were more common among those with atopy ($\chi^2 = 8.6$, $P = .035$). The relationships between symptoms, exposure, pulmonary function, and immunologic parameters are described below.

TABLE II. Respiratory Symptoms of Auto Body Shop Workers (n = 75)

	Number (%)
Respiratory symptoms:	
Wheezing or whistling	21 (28%)
Attacks of shortness of breath	17 (23%)
Chest tightness	15 (20%)
Cough attacks	26 (35%)
Work-related symptoms among those with symptoms:	
Symptoms get better on weekend/vacation	17 (50%)
Symptoms get worse returning to work	14 (40%)
Overall symptoms:	
No symptoms	36 (48%)
Non-specific respiratory symptoms	25 (33%)
Work-related asthma-like symptoms	8 (11%)
Non-work-related asthma-like symptoms	6 (8%)

Physiologic Measures: Spirometry, PEFRs, and Methacholine Challenge

The mean FEV1, FVC, and FEV1/FVC ratio were all within normal range, as expected (Table III). Three participants had percent predicted FEV1 or FEV1/FVC ratio slightly below the normal range. Mean change in FEV1 across shift (before and after work) and across week (Monday to Thursday) are shown in Table III: 28.6% of the participants had a cross-shift decline in FEV1 of >5% on any single day; 34.3% had a cross-week decline of >5%. These changes were not associated with atopy.

Compliance with PEFR measurements was excellent with 87% of the participants completing at least 4 of the 7 days of PEFR measurements (Table III). Two participants had PEFRs that were worse on workdays than on weekends and worse in the evening compared to the morning; two others had one or the other of these characteristics. However, there were too few ''positive'' recordings to assess relationships amongst the various endpoints.

The results of the methacholine challenge testing performed on Monday morning before work and Thursday afternoon are shown in Table III. The number of subjects with a $PC_{20} \leq 25$ on Monday was 5 (7.2%) and 10 (13.9%) on Thursday, with a total of 11 subjects (15.3%) on either day. Methacholine responsiveness, defined as $PC_{20} \leq 25$, was not associated with history of atopy, total IgE levels, or smoking status. Only one of the four subjects with a history of asthma had a $PC_{20} \leq 25$ on either day. Two of the subjects, neither of whom had a history of asthma or asthma symptoms, had a $PC_{20} \leq 8$, the cut-off frequently used clinically to diagnose asthma. One of these subjects was a 19-year-old technician with a HDI-specific lymphocyte stimulation index of 1.0, total IgE of 47.5, and no detectable HDI-specific IgE or IgG. The other was a 20-year-old

TABLE III. Physiology of Auto Body Shop Workers (n = 75)

	Mean ± SD
Baseline Measures	
Height (in.)	68.3 ± 3.3
Weight (p.)	181.6 ± 33.6
Baseline spirometry (liters)	
FEV1 (% predicted)	3.80 ± 0.73 (98.7 ± 14.4%)
# < 80% of predicted	3/67 (4.5%)
FVC (% predicted)	4.67 ± 0.83 (101.0 ± 13.3%)
# < 80% of predicted	3/67 (4.5%)
FEV1/FVC ratio (%)	81.1 ±5.4%
# < 75%	6/67 (9.0%)
Cross-shift changes in FEV1	
Cross-shift % change in FEV1 (n = 70)[a]	0.45 ± 3.5% (−9.6 to+8.9%)
>5% decrease in cross-shift FEV1 (any day)	20/70 (28.6%)
Cross-week % change in FEV1 (n = 67)	−2.7 ± 6.7% (−22 to+17%)
>5% decrease in cross-week FEV1	24/70 (34.3%)
Methacholine Challenge	
$PC_{20} \leq 25$ mg/ml	
Monday	5/69 (7.2%)
Thursday	10/72 (13.9%)
Either day	11/72 (15.3%)
Peak flow measurements	
Diurnal variation >20%	33/65 (50.8%)
PM worse than AM	3/65 (4.6%)
Work days worse than weekends	3/65 (4.6%)

[a]Each person's cross-shift changes were averaged and the mean of the means reported.

painter with a HDI-specific lymphocyte stimulation index of 1.3, total IgE of 4.9, an HDI-specific IgG titer of 1:8, and no HDI-specific IgE.

Relationships among the following physiologic parameters were assessed: baseline spirometry, cross-shift and cross-week changes in FEV1 and methacholine responsiveness. Lower baseline FEV1 (% predicted) was associated with a greater cross-shift decline in FEV1 ($r = -0.245$; $P = 0.046$), and cross-week decline in FEV1 ($r = -0.40$; $P = 0.001$). Lower baseline FEV1 was also associated with greater methacholine responsiveness ($PC_{20} \leq 25$, $P = 0.044$ by *t*-test). Those with a cross-shift drop in FEV1 $\geq$5% were more likely to also have a $\geq$5% fall in FEV1 over the week ($\chi^2 = 4.2$; $P = 0.041$). Methacholine responsiveness was not associated with cross-shift change in FEV1 or cross-week change in FEV1. The relationships between these parameters and symptoms, immunologic responses, and exposure are described below.

Immunologic Responses

The serum IgE values of the study subjects demonstrated a log-normal distribution with geometric mean serum IgE levels of 49 IU/ml (Table IV). The presence of HDI-specific and HDI biuret-specific IgE and IgG was determined in the subjects (Table IV). Only two subjects had positive diisocyanate-specific IgE: one had HDI-specific IgE and the second HDI-biuret specific IgE. These two subjects also had HDI-specific IgG (titers of 1:16 and 1:8), normal spirometry, no respiratory symptoms, but mildly increased airways hyperresponsiveness ($PC_{20} = 41$, $PC_{20} = 21$).

HDI-specific IgG antibodies were present in 34% of the subjects, although about half (59%) of these had low titers (<1:16). 7.7% of the subjects had HDI biuret-specific IgG antibodies (Table IV). The presence of HDI-specific IgG and HDI biuret-specific IgG were closely correlated (Spearman $r = 0.39$; $P = .002$). HDI-specific IgG did not correlate with total IgE levels or history of atopy.

Lymphoproliferative responses to HDI antigens in the auto body shop workers was evaluated by determining the proliferative responses of each subject's PBMCs to HDI-monomer-HSA conjugates in vitro (Table IV, Fig. 1). For comparison, in vitro proliferation responses to HDI-monomer-HSA are also shown for three known HDI asthmatics (diagnosis based on clinical evaluation and

TABLE IV. Immunological Studies on Auto Body Shop Workers

	Geometric mean ± GSD
Proliferation	
HDI-monomer	1.51 ±1.98
Proliferation index > 2	20/66 (30.3%)
Proliferation index > 3	12/66 (18.2%)
HDI-biuret	1.47 ± 1.99
Proliferation index > 2	13/53 (24.5%)
Proliferation index > 3	7/53 (13.2%)
Antibodies	
IgE total (IU/ml)	49.4 ± 4.8
> 50	30/65 (46.2%)
> 100	20/65 (30.8%)
> 200	12/65 (18.5%)
IgE-HDI	1 positive/65 (1.5%)
IgE-biuret	1 positive/65 (1.5%)
IgE-HDI	22 positive/65 (33.8%)
< 1:16	13/22 (59%)
≥ 1:16 to 1:80	6/22 (27%)
≥ 1:128	3/22 (14%)
IgG-biuret	5 positive/65 (7.7%)
< 1:16	0/5 (0.0%)
≥ 1:16 to 1:80	3/5 (60.0%)
≥ 1:128	2/5 (40.0%)

specific inhalation challenge or workplace PEFRs), seven unexposed nonatopic controls, and four unexposed atopic asthmatics (Fig. 1). Mean stimulation indices for HDI-monomer and HDI-biuret lymphocyte proliferation assay on the auto body shop workers are shown in Table IV. The HDI biuret-HSA and HDI monomer-HSA induced proliferative responses were strongly correlated (Spearman $r = 0.86$; $P = .0001$), suggesting that the monomer and biuret forms of HDI are antigenically similar, both capable of inducing HDI-specific lymphocyte responses and IgG. Twenty (30%) of the subjects had a stimulation index of >2 in response to HDI monomer-HSA, and 12 subjects (18%) had a stimulation index >3.0. There were no significant associations between proliferation index and either total IgE or HDI-specific IgG. Fifty-eight percent of subjects who had both proliferation and serology studies performed had either a positive HDI-IgG or HDI-specific proliferation of PBMCs. The two subjects with HDI-specific IgE had proliferation indices of 0.69 and 2.4 in response to HDI monomer-HSA.

Relationships Between Exposure, and Symptoms, Physiologic Parameters, and Immunologic Responses

The relationships among the various endpoints were evaluated, as well as the relationships between exposure and these endpoints. As shown in Table V, job category was associated with several parameters: symptoms of chest tightness and shortness of breath, cross-week change in FEV1, methacholine responsiveness, and HDI-specific proliferation and IgG. For example, 0% of office workers, 20.5% of technicians, and 38.9% of painters, the most heavily HDI-exposed workers, reported chest tightness. Also, 20.0% of office workers, 28.6% of technicians, and 43.8% of painters had HDI-specific proliferation indices >2. These differences, however, did not reach statistical significance with the current sample size. Greater number of peaks of unprotected HDI exposure per week was associated with greater cross-week decline in FEV1 (Spearman $r = -0.24$; $P = .067$), and shortness of breath ($P = .005$), but not with the other symptoms, or physiologic or immunologic parameters.

DISCUSSION

There are several notable findings in the data presented here on 75 HDI-exposed auto body shop workers. Small employers such as auto body shops are frequently the highest risk exposure environments and pose particular challenges to investigators due to their small size, geographic dispersion, non-uniform work structures and practices, sporadic exposures, potential unwillingness to participate in such studies, and difficulties in obtaining biologic specimens. We have demonstrated a successful multidisciplinary approach to studying the important and widespread auto body shop industry. A very high participation rate of individual shops and workers was obtained, with successful completion of extensive questionnaire data, physiologic testing including field methacholine challenge, immunologic blood tests, and exposure assessment.

The apparently low rate of overt asthma detected in the surveillance of these 75 auto body shop workers is of note. Only one of the subjects had a current diagnosis of asthma, and none were currently under treatment for asthma. Eleven (15.3%) had a $PC_{20} \leq 25$ mg and only two had a $PC_{20} < 8$ mg, the clinically used cut-off for a positive methacholine challenge test. There are several possible explanations for this low prevalence of asthma. First, the cross-sectional nature of the study design raises the possibility that the true prevalence is underestimated because the more responsive workers were not captured in the health survey as they had already left the industry (healthy worker effect). Records of past employees were not available for review, but our interviews suggest high turnover. Another possible explanation is the ''healthy shop'' effect, whereby owners of cleaner shops were more likely to participate, and exposures to the lower HDI levels in these cleaner shops are less likely to cause asthma

The immunologic findings, demonstrating a high prevalence of HDI-specific proliferative responses and HDI-

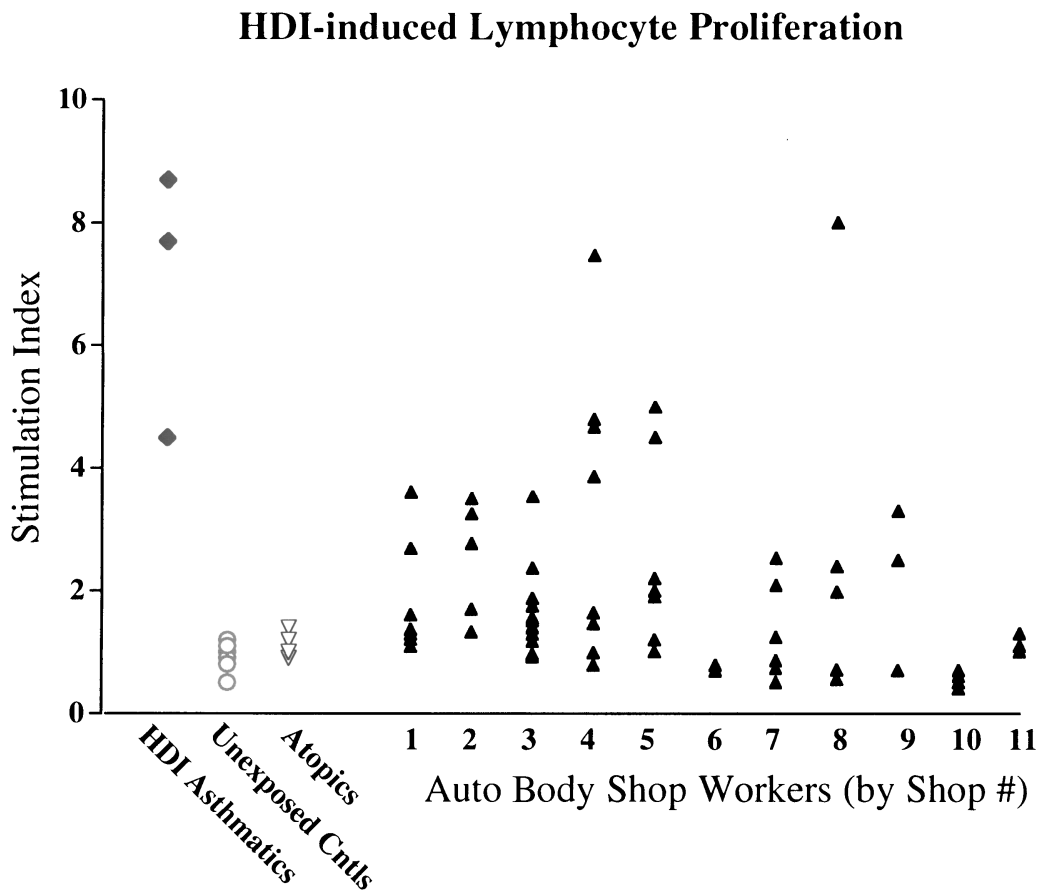

FIGURE 1. In vitro proliferation of PBMCs in response to HDI-HSA from 66 auto body shop workers (▲), 3 HDI asthmatics (◆), 6 unexposed controls (○), and 4 atopic asthmatics (▽).

specific IgG in this cohort of HDI-exposed healthy auto body shop workers, are intriguing and warrant further investigation. Fifty-eight percent of the auto body shop workers had either a positive HDI-IgG or HDI-specific proliferation of PBMCs. The HDI-specific proliferative responses are consistent with recent data suggesting that T lymphocytes play a key role in the pathogenesis of diisocyanate asthma [Bernstein et al., 1997; Raulf-Heimsoth and Baur, 1998; Redlich et al., 1999]. Whether such responses indicate some degree of immunologic sensitization to diisocyanates or predict future asthma is unclear. Our data on a limited number of specific challenge diisocyanate asthmatics have shown a proliferation index >3 in such subjects and a proliferation index <1.5 in unexposed controls and atopic asthmatics [Wisnewski et al., 1999a]. Given the high prevalence (30% of exposed workers) with an elevated proliferation index in the range of 2 or more, it appears unlikely that the majority of those subjects will go on to develop diisocyanate asthma, especially given the median years of employment in the industry (15 years). Further studies are in progress to determine which subgroups of T cells and T cell mediators are responding to the diisocyanate antigens in vitro, as well as which T cell populations become activated by HDI. Identification of certain cytokine profiles may identify those exposed subjects at greatest risk for asthma.

The low prevalence of positive HDI-specific IgE (3%) is not surprising. These findings are consistent with prior studies in the literature showing that generally less than 5–10% of diisocyanate exposed cohorts have detectable diisocyanate specific antibodies [Grammer et al., 1988; Welinder et al., 1988; Karol et al., 1994]. In contrast, HDI-specific IgG was quite prevalent, detected in 34% of the exposed workers, and was more common in the more heavily exposed workers (technicians and painters), but was not correlated with current exposure (unprotected peaks of exposure). These findings are difficult to compare to those in the literature as studies have used different assays to detect IgG and IgE levels and exposure data have been scanty. Limited studies have shown a higher prevalence of HDI-specific IgG than HDI-specific IgE in exposed populations, although the relationship to exposure has been variable [Grammer et al., 1990; Vandenplas et al., 1993a; Tee et al., 1998]. Our data suggest that HDI-specific IgG may reflect past HDI exposure, as has been shown with IgG specific to anhydrides and other low molecular weight asthmagens [Cullinan, 1998; Yokota et al., 1998). More long-term follow-up of this population is needed to determine the

TABLE V. Relationships between Exposure (Job Category) and Demographics, Symptoms, Physiology and Immunologic Parameters

	Exposure (job categroy)		
Parameter	**Office worker**	**Technician**	**Painter**
Mean ± SD or number (%)	**n = 18**	**n = 39**	**n = 18**
Demographics			
Age (years)	43.6 ± 10.5	39.7 ± 11.5	33.4 ± 11.8
Current smoker	5 (27.8%)	10 (25.6%)	6 (33.3%)
Gender (male/female)	14/4	39/0	18/0
History of asthma	0/17 (0%)	1/36 (2.7%)	3/17 (17.7%)
History of atopy	5/18 (27.8%)	6/39 (15.4%)	5/18 (27.8%)
Symptoms			
No symptoms	9 (50%)	21 (53.8%)	6 (33.3%)
Asthma-like symptoms	3 (16.7%)	5 (12.8%)	6 (33.3%)
Non-asthma-like symptoms	6 (33.3%)	13 (33.3%)	6 (33.3%)
Wheeze	5 (27%)	11 (28.0%)	5 (27.0%)
Chest tightness	0 (0%)	8 (20.5%)	7 (38.9%)
Shortness of breath	1 (5.6%)	10 (25.6%)	6 (33.3%)
Cough	6 (33.3%)	13 (33.3%)	7 (38.9%)
Physiology			
Baseline FEV1	3.44 ± 0.78	3.89 ± 0.63	3.95 ± 0.82
(% predicted)	(95.1 ±10.4%)	(102.7 ± 15.6%)	(94.7 ± 13.8%)
Cross-shift change in FEV1	0.3 ± 4.4%	1.0 ± 4.4%	1.6 ± 3.5%
Cross-week change in FEV1	−0.4% ± 4.5%	−2.5 ± 8.2%	−5.0 ± 4.2%
Methacholine challenge	1/17 (5.9%)	5/37 (13.5%)	4/18 (22.2%)
($PC_{20} \leq 25$ mg/ml)			
Immunologic Parameters			
Total IgE (IU/ml)			
Geometric Mean ± GSD	64.4 ± 4.8	50.4 ± 4.8	37.3 ± 5.2
HDI proliferation (index > 2)	3/15 (20.0%)	10/35 (28.6%)	7/16 (43.8%)
IgG-HDI (positive titre)	2/14 (14.2%)	14/35 (40%)	6/16 (37.5%)

significance of these different HDI-specific immunologic responses.

These immunologic findings should be interpreted in light of our limited understanding of the pathogenesis of diisocyanate asthma. Diisocyanates are extremely reactive compounds which likely bind to human proteins which are then presented to the immune system. The data shown here and most past immunologic studies have used HDI conjugated to albumin as the carrier protein, based on practical considerations. We are investigating the antigenicity of HDI conjugated to other human proteins, such as epithelial cell proteins, which may form more biologically relevant antigens and elicit different immune responses [Wisnewski et al., 1999b]. Use of such HDI antigens instead of HDI-albumin conjugates may in the future help elucidate the immunopathogenesis of isocyanate asthma, as well as develop greatly needed clinical markers of sensitization and early asthma.

The correlations between the different physiologic markers, symptoms, exposure and the immunologic responses studied to date are also of note. In a healthy population generally free of overt asthma, the different physiologic measures were not definitive in identifying possible subclinical disease. This is in contrast with findings in certain other industrial populations exposed to sensitizing agents and irritants, where exposures have usually been more constant, and standard physiologic parameters and/or symptoms have correlated with exposure [Huy et al., 1991; Greaves et al., 1997]. Some intriguing relationships, such as increased chest tightness, shortness of breath and methacholine responsiveness, were noted in the most heavily exposed workers, the painters. However, these findings did not reach statistical significance with the current sample size of 75 workers. Lack of stronger associations with exposure may be due to our limited assessment of exposure, thus far based on questionnaire and diary data, as well as our sample size.

As noted extensive quantitative exposure reconstruction is in progress. These findings also highlight the great need for a simple, widely available approach to screen for and diagnose diisocyanate asthma. Investigating the respiratory effects of intermittent sporadic exposures in end-use applications such as spray painting may require new diagnostic approaches, rather than standard physiology and questionnaires.

There are several limitations to the present findings. Foremost, as noted above, is the inherent limitation of a cross-sectional surveillance study of an exposed population. Although valuable data can be obtained, long-term follow-up of the population is required to appreciate the significance of the immunologic responses and other endpoints detected. Another problem is the low prevalence of overt asthma in this population, which may reflect the healthy worker effect commonly seen in cross-sectional studies. This limits our ability to determine which physiologic or immunologic endpoints best predict disease. Another limitation is that at this time we are unable to definitively classify HDI-exposed subjects by disease status since we have not yet performed HDI-specific challenges and do not know which of the 75 subjects may have subclinical HDI asthma. At best we can identify suspect cases, based on their symptoms, or physiologic or immunologic responses to diisocyanates. We are in the process of using our HDI exposure chamber to classify the HDI-exposed subjects into those with diisocyanate asthma and exposed controls. However, there are practical and technical limitations to performing HDI-specific challenges, which could misclassify cases. Another limitation is the exposure assessment based on the questionnaire data as noted above; it is likely that there is significant misclassification of exposure using only qualitative estimates.

Nonetheless, this initial cross-sectional investigation of auto body shop workers demonstrates a high prevalence of HDI-specific immune responses in a healthy cohort of HDI-exposed workers without overt asthma. In addition methacholine responsiveness, certain asthma symptoms, and HDI proliferation were more common in the workers most heavily exposed to HDI. Further evaluation of this cohort of HDI-exposed workers, including inclusion of a larger cross-sectional study population, specific HDI challenge, as well as longitudinal follow-up of those with ongoing exposures and those who leave the industry, will be needed in order to appreciate the significance of the immunologic, physiologic, and symptom findings. These data should improve our understanding of diisocyanate sensitization and asthma as well as identify optimal diagnostic and preventive modalities.

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