

T-cell receptor V β gene segment expression in diisocyanate-induced occupational asthma

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Background: Diisocyanates are the most common cause of occupational asthma induced by low-molecular-weight chemicals. The disease appears to be immunologically mediated but is independent of IgE antibody synthesis. An underlying genetic susceptibility is suggested by the fact that the disease only develops in approximately 5% to 10% of exposed workers.

Objective: The study was designed to determine whether disease susceptibility is influenced by HLA and T-cell receptor V β gene segment usage.

Methods: T-cell receptor V β gene repertoires were quantitated by using primer pairs specific for V β gene segments in conjunction with a common C β region primer. One group of workers with diisocyanate-induced occupational asthma produced diisocyanate-specific IgG and IgE antibodies, whereas the other group did not produce specific antibodies. Occupational asthma was previously confirmed by either workplace challenge or laboratory specific diisocyanate bronchoprovocation. Control groups consisted of diisocyanate-exposed workers who were free of symptoms, patients with nonoccupational asthma, and unexposed subjects who were free of symptoms.

Results: Lymphocytes from workers with diisocyanate-induced occupational asthma had significantly decreased V β 1 and V β 5 gene segment expression before in vitro exposure to diisocyanates, compared with control groups. Percent V β 1 and V β 5 gene segment expression was selectively increased when peripheral blood mononuclear cells were stimulated in vitro with diisocyanate-conjugated proteins. Low-resolution HLA class II phenotyping revealed no significant differences in the distribution of HLA-DR or HLA-DQ alleles between diisocyanate-induced occupational asthma and control groups.

Conclusions: These findings are consistent with a hypothesis that antigen-specific T-cell subpopulations may be sequestered in the lungs of workers with diisocyanate-induced occupational asthma and clonally expand after further exposure to diisocyanates. (*J Allergy Clin Immunol* 1997;99:245-50.)

Key words: Diisocyanates, occupational asthma, cell-mediated, T-cell receptor, V β gene segments, HLA phenotyping

Diisocyanates are the most common cause of occupational asthma induced by low-molecular-weight chemicals.¹ The underlying mechanisms of diisocyanate-induced occupational asthma (DOA) remain unclear. However, in vitro investigations support a role for cell-mediated immune responses.^{2,3} Evidence for T-cell involvement in DOA was first reported by Avery et al.,² who demonstrated lymphoblast transformation and lymphocyte proliferation in workers with DOA. Gallagher et al.³ confirmed this observation by demonstrating the presence of leukocyte inhibitory

Abbreviations used

DOA:	Diisocyanate-induced occupational asthma
HDI:	Hexamethylene diisocyanate
HSA:	Human serum albumin
MDI:	Diphenylmethane diisocyanate
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
PHA:	Phytohemagglutinin
SSP:	Sequence-specific primer
TCR:	T-cell receptor
TDI:	Toluene diisocyanate
V β :	Variable β gene segment

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factor activity, a cytokine derived from lymphocytes, in the majority of their subjects with DOA. Histopathologic studies of workers with DOA have reported increased circulating peripheral and bronchoalveolar lavage CD8⁺ cells in addition to eosinophils.⁴ Both activated T cells and eosinophils have also been found in bronchial biopsy specimens of workers with DOA.⁵

Dearman et al.⁶ demonstrated that different chemical allergens promote divergent immune responses in a

BALB/c murine model. They found that mice exposed to diphenylmethane diisocyanate (MDI) had increased numbers of T_{H2} -like cells, whereas exposure to dinitrochlorobenzene, a common skin-sensitizing agent, resulted in increased numbers of T_{H1} -like cells.⁶ These results indicate that chemical haptens may regulate specific T-cell clone recognition and activation.

Renz et al.⁷ demonstrated that ovalbumin-reactive T-cell hybridomas selectively express T-cell receptors (TCRs) with increased V β 8 and V β 2 gene segments in mice. T cells with increased V β 8 gene segment expression augmented IgE production in sensitized mice, whereas V β 2 T cells inhibited IgE production.⁷ Transfer of V β 8 T cells from ragweed-sensitized mice to naive mice resulted in augmentation of the IgE response and increased airway responsiveness in the naive recipients.⁷ These results demonstrate that antigen-driven T-cell subpopulations expressing selective TCR V β repertoires are important in regulating immune responses.

Precedence for studying TCR V β repertoires in chronic respiratory diseases has been previously established for pulmonary sarcoidosis and asthma.⁸⁻¹⁰ No discernible differences in peripheral blood or bronchoalveolar lavage baseline V β repertoires could be detected in patients with pulmonary sarcoidosis compared with control subjects. However, IL-2-treated bronchoalveolar lavage lymphocytes from patients with sarcoidosis produced selective expansion of T-cell subpopulations with specific V β repertoires compared with control subjects.^{8,9} Davey et al.¹⁰ reported heterogeneous TCR V β gene expression in a single pair of monozygotic twins discordant for asthma. The twin proband with asthma had persistent expression of the CD8+ V β 8 gene segment compared with the unaffected twin over a 2-year period. Whether this difference in CD8+ V β expression was associated with clinical disease is still unknown.

Because of the emerging evidence supporting T-cell involvement in DOA, we analyzed the TCR V β repertoires of peripheral blood mononuclear cells (PBMCs), obtained from workers with DOA, before and after in vitro diisocyanate exposure.

METHODS

Experimental design

Subject population. The diagnosis of DOA was based on history of diisocyanate exposure in the workplace, clinical symptoms that decreased away from work, and peak flow variability at work compared with away from work. The diagnosis of DOA was confirmed by either a workplace or laboratory challenge with diisocyanates. Workers with DOA were further characterized by whether they produced diisocyanate-specific IgE and/or IgG antibodies by using a standardized ELISA method described by Sarlo et al.¹¹ An increased level of specific IgE and IgG antibodies to diisocyanates conjugated to human serum albumin (HSA) (MDI-HSA, toluene diisocyanate [TDI]-HSA, or hexamethylene diisocyanate [HDI]-HSA) has been defined as an optical density greater than 3 standard deviations above the mean of 10 unexposed control subjects who have isotype-specific antibodies below the limit of detection.

The group of workers with DOA consisted of five workers who produced specific diisocyanate IgE and/or IgG antibodies and five workers without specific IgE and/or IgG antibody production. Of the workers with DOA, one had workplace exposure to TDI, three to MDI, three to HDI, two to MDI and TDI, and one to HDI and MDI. Three control groups consisted of five normal individuals without prior exposure to diisocyanates in the workplace, five patients with asthma without prior exposure to diisocyanates, and five individuals exposed to MDI in the workplace for at least 1 year. The diisocyanate-exposed control subjects without symptoms were evaluated in a similar fashion to workers with DOA. They all had no history of asthma, normal lung function, and no peak expiratory flow rate variability at or away from work. Study protocols were reviewed and approved by the University of Cincinnati Human Subject Institutional Review Board, and informed consent was obtained from each subject.

Isolation and culture of PBMCs. Peripheral venous blood (90 to 120 ml) was collected and mixed with 10 ml of 0.2 mol/L (pH = 7.2) ethylenediaminetetraacetic acid. PBMCs were purified by density-gradient centrifugation (LeucoPREP; Becton-Dickinson Laboratories and Co., Lincoln Park, N.J.), yielding approximately 1 to 2×10^6 PBMCs/ml. PBMCs were washed to deplete platelets, and a differential cell count was obtained by using an automated laser counter (Cell Dyn 3500SL, Abbott Laboratories). Cells were resuspended at a concentration of 10^6 cells/ml in RPMI-1640 medium (Gibco, Grand Island, N.Y.) containing 10% fetal bovine serum (Hyland City), 4 mmol/L glutamine, 100 U/ml penicillin, 100 U/ml streptomycin (Gibco), 10 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid, 2 mmol/L pyruvate (Sigma Chemical Co., St. Louis, Mo.). Cell cultures were maintained for 7 to 10 days at 37°C, 5% CO₂ in complete medium, medium containing a 100 μ g/ml concentration of phytohemagglutinin (PHA), or medium containing a 10 μ g/ml concentration of the diisocyanate-HSA antigen (HDI-HSA, MDI-HSA, or TDI-HSA).^{12,13} Recombinant IL-2, 20 μ g/ml (Sigma), was added at the beginning of culture and on day 3 to maintain viability of T cells for several days.^{14,15} Total cell numbers and viability with trypan blue exclusion were assessed after 7 days.

PBMC proliferation. The method used for PBMC proliferation has been well described elsewhere.¹² PBMCs isolated from eight of the workers with DOA, four of the exposed workers without symptoms, and four of the normal subjects were counted and adjusted to a concentration of 1×10^6 cells/ml in complete RPMI-10 medium, and 100 μ l of the cell suspension was added in triplicate to each well of a 96-well plate (1×10^5 cells/well). Duplicate plates for each subject were harvested at 3 and 7 days. Assays were performed in groups of three to four subjects on different days. For each plate, RPMI-10 media, HSA, and PHA (Sigma Chemical Co.) were added to each well in triplicate at a final concentration of 10, 1, and 0.1 μ l/100 μ l as negative and positive controls, respectively. HDI-HSA, TDI-HSA, or MDI-HSA was added to each well in triplicate at final concentrations ranging from 1×10^{-8} to 1×10^3 μ g/ml. The plates were incubated (37°C, 5% CO₂) for 3 days to detect maximal mitogen stimulation and for 7 days to detect maximal antigen stimulation.³ On day 3 or 7, 1 μ Ci of tritiated methylthymidine (Amersham Corp., Arlington Heights, Ill.), diluted in 10 μ l of RPMI-10, was added to each well and incubated for 24 hours (37°C, 5% CO₂). Cells were harvested by using a Skatron multiwell harvester (SKATRION, Sterling, Va.) by filling and aspirating each row with RPMI ten times to ensure complete cell transfer onto filter paper and removal of unincorporated

thymidine. Each filter paper was placed in a 20 ml scintillation vial and allowed to dry overnight. Scintillation fluid was added, and samples were counted as counts per minute in a Beckman LS 9000 microprocessor beta scintillation counter, which estimates the amount of DNA synthesis and cell proliferation in an entire cell population.

TCR V β analysis. To determine TCR V β gene segment expression before and after *in vitro* diisocyanate stimulation, fresh PBMCs and PBMCs incubated with diisocyanate conjugated to protein for 7 days were analyzed by reverse-transcriptase polymerase chain reaction (PCR). TCR V β analysis was performed on each subject with the PBMCs that were exposed to their specific workplace diisocyanate compound. In those situations in which a worker was exposed to more than one diisocyanate compound, PBMCs incubated with the diisocyanate compound to which the worker was most recently exposed in the workplace were used. Total RNA was extracted from 10⁷ PBMCs by the guanidine thiocyanate-phenol-chloroform extraction method, and the first-strand complementary DNA was synthesized with oligo-dT priming.^{16,17} Enzymatic gene amplification of cDNA was performed by using methods described by Choi et al.¹⁸ TCR primers specific for V β 1-20 were used in conjunction with a common C β region primer (5'-ACACCAGTGTGCCCTTTTGGGTG).¹⁹ Coamplification of a C α gene segment from the α chain constant region was included as an internal control with the primer pair (forward primer 5'-ATCATAAATTCGGGTAGGATCC and reverse primer 5'-GAACCTGACCCTGCCGTGTACC).¹⁹ Reaction products were maintained within the linear dose-response relationship between initial amounts of cDNA and quantity of amplified DNA. Equivalent concentrations of C α primers and V β primers resulted in competition for reagents and preferential amplification of C α (because of a greater amount of constant α gene segment relative to each variable β gene family). Titration experiments followed by hybridization of PCR products with both C β and C α internal probes indicated that results were optimized when V β primers were added at a 40-fold greater concentration than C α primers.¹⁹ The reaction mixture consisted of a total volume of 50 μ l containing equivalent amounts of cDNA, 1.5 mmol/L MgCl₂, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 0.02% gelatin, 5 pmol of each V β /V α primer + 0.012 pmol of C α primers, all four deoxyribonucleoside triphosphates (0.2 mmol/L each), and 2 units of Taq polymerase (Perkin-Elmer Cetus, Emeryville, Calif.). Thirty cycles of amplification were performed (1 minute at 94° C, 1 minute at 55° C, and 2 minutes at 72° C) with a thermocycler (Perkin-Elmer Cetus).

The C α region primer concentrations were decreased to one-fortieth the concentration of the V β /C β primers to minimize preferential amplification of the C α template relative to the V β template. Lack of interference by the C α template was confirmed by separating the products by size, transferring them onto a nylon membrane, and hybridizing them for 18 hours at 37° C with internal C β - and C α -specific probes.¹⁹ Once probe specificity was demonstrated, Southern blotting was performed with transfer of PCR products to nylon membranes by using a Minifold II slot blot apparatus (Schleicher & Schuell, Keene, N.H.), and the blots were hybridized sequentially with three phosphorous 32 γ adenosine triphosphate-labeled internal oligonucleotide probes specific for C β 1 (5'-CTCGGGTGGGAACAC-GTTTTC), C β 2 (5'-CTCGGGTGGGAACACCTTGTTTC), and C α (5'-TTTAGAGTCTCTCAGCTGGTA).^{19,20} Blots were washed at a final stringency of 0.1 mol/L sodium

chloride/sodium citrate solution/1% sodium dodecylsulfate at 60° C and processed for autoradiography.²⁰ Washed Southern blots were exposed to x-ray film, and the amount of amplified DNA was measured by densitometric analysis with a desktop image scanner (Microtek) and scan analysis software (NIH Image) for determination of bound radioactivity.²¹ Dilution analysis showed that the yield of amplified product, as measured by densitometry, was proportional to the initial dilution of cDNA in reaction mixtures. Hence, the technique was judged to be semiquantitative.¹⁹ To make comparisons between individuals and changes resulting from antigen or mitogen stimulation of T cells, the relative amount of each V β gene family usage was expressed as a percentage of the total 100% V β repertoire for each subject studied.¹⁹ This value was calculated by dividing the amount of bound radioactivity in each V β reaction by the total amount of bound radioactivity incorporated in all V β reactions after normalizing for amounts of C α products (V β /C α internal control).¹⁹ When possible, TCR V β analysis was performed with the same initial messenger RNA samples. The mitogen, PHA, was used to control for the effect of *in vitro* culture conditions on the stimulated TCR repertoires. The TCR repertoires of the PHA-stimulated PBMCs from workers with DOA and control subjects remained unchanged from baseline peripheral TCR repertoires, a finding consistent with previous published investigations.¹⁸

Class II HLA antigen phenotyping. HLA-DR and HLA-DQ serologic typing was performed by a microlymphocytotoxicity assay and confirmed by PCR amplification with sequence-specific primers (SSPs).²²⁻²⁴ Genomic DNA was extracted by conventional methods from PBMCs, then amplified by using PCR and SSP primers for DRB1 and DQB1 alleles (SSP Primers AB; Novum, Huddinge, Sweden).²⁴ HLA-DR typing was conducted by PCR amplification with SSPs by using 19 PCR reactions per individual, 17 for assigning DR1-DR18 and two for the DR52 and DR53. Patients who were positive for DR4 were subtyped by using DRB1*04 primers.²⁴ For complete DQB1 PCR-SSP typing, the first set of DQB1 primers of 14 PCRs was performed per DNA sample. The second set of DQB1 primers included eight additional primer mixes for a total of 22 reactions per DNA sample. The DNA was amplified by 30 three-temperature cycles that included denaturation at 94° C for 20 seconds, annealing at 65° C for 50 seconds, and extension at 72° C for 20 seconds.²⁴ These PCR cycling parameters were the same for DR "low-resolution" PCR-SSP typing and subtyping. Presence or absence of amplified PCR products was determined by ethidium bromide-stained agarose gel electrophoresis. The gels were examined under UV illumination and photographed.

Statistical analysis

PBMC proliferative responses of workers with DOA were analyzed in comparison with those of control subjects by using Fisher's exact test, which takes into account small numbers of subjects. A *p* value of less than 0.05 was considered significant.

The average V β gene usage of the 20 specific primers used in this investigation correlated well with previously published frequencies.¹⁹ All subjects displayed a normal distribution of percent V β usage for each V β family according to frequency distribution analysis and *z*-histogram curves.¹⁹ This permitted the use of parametric methods for data analysis. Analysis of variance was used to compare the mean differences of V β usage between two or more balanced groups. The unpaired *t* test was used to compare mean differences of V β usage between two

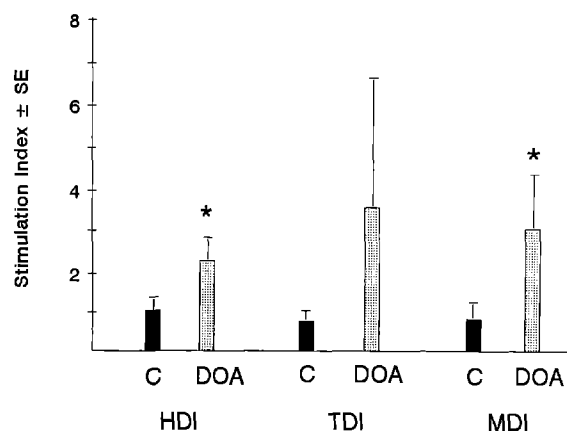


FIG. 1. Proliferative responses of PBMCs obtained from workers with DOA ($n = 8$) compared with control subjects (C) ($n = 8$). PBMCs from control subjects (normal and exposed, free of symptoms) and workers with DOA (DOA) were stimulated with 10 $\mu\text{g/ml}$ HDI-HSA, TDI-HSA, or MDI-HSA for 7 days. Significant p values of less than 0.05 were obtained for workers with DOA compared with control subjects in response to HDI-HSA and MDI-HSA but not to TDI-HSA. Results are expressed as "stimulation index," defined as the average sample counts per minute (cpm)/average control medium cpm. The control medium was RPMI-10. The stimulation index was calculated by dividing the cpm value for PBMCs incubated with each of the diisocyanate-HSA-conjugated compounds by the cpm value for the RPMI-10 cell cultures.

groups of different individuals, and the paired t test was used to compare before and after measurements of V β usage in the same group of individuals. Means were considered significantly different when a p value was less than 0.05.

Sharing of common HLA alleles among workers with DOA and control groups was analyzed by using Fisher's exact test. Common distribution of HLA alleles among these groups was expressed as an "uncorrected" p value because of the small number of subjects analyzed.²⁵

RESULTS

PBMC proliferation

The mean proliferative response ranged between 100 and 300 cpm for the HSA control and 50,000 and 100,000 cpm for mitogen (PHA). The maximal proliferative response was observed on day 4 for mitogen and on day 7 for antigen (HDI-HSA, TDI-HSA, or MDI-HSA). The mean proliferative response of PBMCs obtained from workers with DOA ($n = 8$) stimulated with HDI-HSA and MDI-HSA was significantly greater than the mean proliferative response of PBMCs obtained from diisocyanate-exposed and unexposed control subjects without symptoms ($n = 8$) stimulated with HDI-HSA and MDI-HSA (Fig. 1). The proliferative response of workers with DOA to TDI-HSA was variable compared with that of control subjects, thereby abrogating a statistically significant result. Workers with DOA who produced diisocyanate-specific antibodies manifested similar proliferative responses compared with workers with DOA who did not produce antibodies. In two cases the PBMC proliferative response was greater for a

different diisocyanate compound than that to which the worker had been originally exposed in the workplace. There was no statistically significant difference in PBMC proliferation between diisocyanate-exposed control subjects without symptoms and unexposed control subjects.

Analysis of TCR V β expression

Frequency distribution analysis demonstrated that the percent usage of individual V β families by this subject population was normally distributed across the general population. Baseline (before diisocyanate stimulation) expression of TCRs expressing V β 1 and V β 5 was significantly lower among all workers with DOA ($n = 10$) as compared with all control subjects ($n = 10$) ($p < 0.05$) (Table I). When either subgroup of workers with DOA, with or without specific antibody production ($n = 5$ for each group), was compared with each control subgroup, baseline expression of V β 1 and V β 5 gene segments was either significantly decreased or approached statistical significance. There was no consistent rise in any one specific V β gene segment among workers with DOA, which compensated for the decreased baseline expression of V β 1 and V β 5.

Significant increases in T-cell subpopulations expressing V β 1 and V β 5 gene segments were observed in all workers with DOA after diisocyanate stimulation compared with prestimulation (baseline) analysis ($p < 0.05$) (Table I). Moreover, the percentage of T cells expressing these V β gene repertoires was increased to comparable levels expressed by control subjects after stimulation (Table I). Stimulation of T cells with mitogen or HSA did not result in increased expression of V β 1 or V β 5 gene segments in either workers with DOA or control subjects. This observation is similar to that reported by other investigators who found that stimulation of T cells with mitogen did not significantly change the percentages of T cells bearing specific V β gene segments from that seen in the starting population.¹⁸

Comparison of TCR V β gene segment usage by diisocyanate-exposed subjects without DOA ($n = 4$) with that by workers with DOA ($n = 10$) and unexposed subjects ($n = 6$) revealed that antigen exposure alone was not responsible for identifiable differences in these baseline TCR V β repertoires (Table I).

HLA phenotyping

Sharing of HLA-DR and HLA-DQ Class II antigens among workers with DOA was not demonstrated (Table II).

DISCUSSION

Previous investigations of DOA have attempted to identify specific biomarkers that could be used to detect workers at risk for developing occupational asthma. Diisocyanate-specific IgG antibodies have been previously reported to represent a marker of exposure to diisocyanates, but neither IgG nor IgE antibodies have correlated well with disease activity.²⁶ Similarly, use of PBMC proliferation as a biomarker for identifying work-

TABLE I. Percent V β 1 and V β 5 expression before and after diisocyanate in vitro exposure in control subjects and workers with DOA

Subject group	Preexposure %V β 1	Postexposure %V β 1	Preexposure %V β 5	Postexposure %V β 5
Normal control subjects (<i>n</i> = 3)	5.77 $\pm$ 0.09	5.53 $\pm$ 0.35	6.20 $\pm$ 0.49	6.67 $\pm$ 0.64
Control subjects with occupational asthma (<i>n</i> = 3)	5.18 $\pm$ 0.36	4.43 $\pm$ 0.99	6.83 $\pm$ 0.44	4.97 $\pm$ 0.74
Diisocyanate-exposed normal control subjects (<i>n</i> = 4)	5.63 $\pm$ 0.63	5.80 $\pm$ 0.29	7.75 $\pm$ 1.08	6.65 $\pm$ 0.57
Total control subjects (<i>n</i> = 10)	5.36 $\pm$ 0.32	5.31 $\pm$ 0.35	7.01 $\pm$ 0.48	6.15 $\pm$ 0.42
DOA antibody producers (<i>n</i> = 5)	4.66 $\pm$ 0.22	6.08 $\pm$ 0.68	5.42 $\pm$ 0.24	6.52 $\pm$ 0.10
DOA nonantibody producers (<i>n</i> = 5)	4.18 $\pm$ 0.31	5.18 $\pm$ 0.45	5.84 $\pm$ 0.46	6.94 $\pm$ 0.46
Total workers with DOA (<i>n</i> = 10)	4.42 $\pm$ 0.20*	5.63 $\pm$ 0.41	5.63 $\pm$ 0.25*	6.73 $\pm$ 0.23

Values are expressed as means $\pm$ SEM.

* Prediisocyanate stimulation of peripheral blood T-cell subpopulations bearing TCRs expressing V β 1 and V β 5 gene segments is significantly greater for all control subjects (*n* = 10) compared with all subjects with DOA (*n* = 10) when Student's *t* test is used (*p* < 0.05). After diisocyanate stimulation, T-cell subpopulations bearing TCRs expressing V β 1 and V β 5 gene segments are approximately the same for control subjects and subjects with DOA.

TABLE II. Number of control subjects compared with workers with DOA expressing specific Class II HLA-DQ and HLA-DR phenotypes

HLA	DQ						DR									
	1	2	3	4	6	7	1	3	4	7	8	11	13	15	52	53
Control subjects (<i>n</i> = 15)*	1	6	6	2	7	7	1	1	5	7	2	5	4	4	9	10
DOA (<i>n</i> = 10)	3	3	3	1	4	0	1	3	3	1	1	0	4	3	7	3

* Control population was expanded to 15 subjects for HLA phenotyping.

ers at risk for DOA appears to be nonspecific for disease activity.^{2,3} In our experience, PBMC proliferation was more pronounced among workers with DOA compared with control subjects, but, in general, the magnitude of proliferation was too variable among workers with DOA to be considered useful as a reliable biomarker for disease activity (Fig. 1). Recently, our laboratory has reported that histamine-releasing factor, produced by subpopulations of PBMCs, may also be a potential biomarker for workers who have DOA.²⁷ The mean histamine-releasing factor response to diisocyanate-conjugated proteins was significantly greater in subjects with DOA compared with exposed subjects who were free of symptoms.²⁷ Neither PBMC proliferation nor histamine release correlated with the presence of diisocyanate-specific IgG or IgE antibodies in workers with DOA.²⁷

HLA Class II gene products have been investigated as possible genetic markers for predicting whether workers are at risk for developing DOA. Bignon et al.²⁸ studied a population of 28 workers with DOA and observed that the HLA class II allele DQB1*0501 conferred protection from disease in healthy diisocyanate-exposed workers, whereas the allele DQB1*503 was associated with an increased susceptibility to DOA. From this study it was concluded that specific HLA alleles may serve as important biomarkers for identifying workers at risk for developing DOA.²⁸ Low-resolution HLA-DR and HLA-DQ phenotyping of our workers with DOA did not reveal sharing of common Class II antigens. The absence of

HLA antigen sharing among our workers compared with those findings of other investigators may reflect the small size of our subject population, the phenotyping methods used, and/or geographic differences between North American and European indigenous populations.²⁸ Larger populations of workers with DOA need to be HLA phenotyped before associations between disease susceptibility and dominant HLA alleles can be confirmed.

This investigation of TCR V β repertoires in a population of workers with DOA revealed prestimulatory (baseline) decreases in T-cell subpopulations expressing V β 1 and V β 5 gene segments. These baseline differences were not present among normal, asymptomatic diisocyanate-exposed or asymptomatic unexposed control groups. Interestingly, after stimulation with diisocyanate-conjugated protein, these V β gene segments increased to the same levels expressed by control subjects (Table I). Poststimulatory changes in V β repertoires did not appear to correlate with whether PBMCs from a worker with DOA proliferated or produced specific IgE and IgG antibodies in response to diisocyanates. These findings are impressive if one considers that statistical significance was achieved between subgroups with such small numbers of subjects.

These results indicate that further investigation of disease-specific T-cell subpopulations in the immunopathogenesis of DOA would be worthwhile. Furthermore, they are consistent with a hypothesis that specific

T-cell subpopulations in DOA may be sequestered in the lungs of these workers and selectively expand on repetitive exposure to diisocyanates. Whether a selective V β gene repertoire is a useful biomarker or a possible risk factor for DOA also requires further investigation.

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