

Human γ/δ T-cell proliferation and IFN- γ production induced by hexamethylene diisocyanate

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Background: The human immune response to isocyanate, a leading cause of occupational asthma, remains incompletely characterized, including the cell types involved and the form of the chemical that acts as an antigen.

Objective: The purpose of this investigation was to characterize human T cells that respond to hexamethylene diisocyanate (HDI), an aliphatic isocyanate routinely used in the automobile body industry.

Methods: Human T-cell lines were generated and characterized from peripheral blood of HDI-exposed and HDI-unexposed subjects, using two different HDI antigens, HDI-conjugated albumin and HDI-exposed human airway epithelial cells (NCI-H292). Flow cytometry was used to characterize the phenotype of HDI-responsive T cells. ELISA and intracellular staining techniques were used to evaluate HDI-induced cytokine production. DNA sequence analysis of T-cell receptors was used to further define clonal populations of HDI-responsive T cells.

Results: HDI antigen preparations but not "mock exposed" control antigens lead to increased proliferation of specific cell types, CD3⁺CD4⁺CD8^{dim} and/or CD3⁺CD4⁺CD8⁻ cells, from HDI-exposed but not from HDI-unexposed subjects. These HDI-responsive T cells expressed unique oligoclonal γ/δ rather than α/β T-cell receptors, with characteristics suggestive of antigen-mediated selection and specificity. The HDI-stimulated γ/δ T cells were associated with T_H1-like cytokines and produce IFN- γ but not IL-5 or IL-13.

Conclusions: These data are the first to demonstrate that HDI can selectively stimulate γ/δ T cells with the potential to modulate the human immune response to exposure. (J Allergy Clin Immunol 2003;112:538-46.)

Key words: Hexamethylene diisocyanate, γ/δ T cell, interferon- γ , T-cell receptor, occupational asthma, CD8, albumin, airway epithelial cell

Abbreviations used

APC: Antigen-presenting cell
FITC: Fluorescein isothiocyanate
HDI: Hexamethylene diisocyanate
PBMC: Peripheral blood mononuclear cell
PE: Phycoerythrin
TDI: Toluene diisocyanate
TT: Tetanus toxoid

Hexamethylene diisocyanate (HDI) and its prepolymers are critical components of spray paints for automobile bodies and aircraft^{1,2} and belong to a class of commercially important highly reactive chemicals, isocyanates, which are immunogenic and a well-recognized cause of occupational asthma worldwide.^{3,4} An immune-based process is believed to mediate isocyanate asthma, based on clinical and pathological similarities with "atopic asthma" induced by common environmental allergens, which typically involves CD4⁺, T_H2-dominated cellular responses.^{5,6} However, certain characteristics of isocyanate-induced asthma may differ from atopic asthma, including (1) the lack of antigen-specific IgE in many patients,^{5,7-13} (2) associations of disease with neutrophils, IL-8 and IFN- γ (ie, T_H1-like responses),¹⁴⁻¹⁸ and (3) antigen-induced lymphocyte responses in vitro.^{1,15,18,19} Many questions remain unanswered about the cells that respond to isocyanates and the effects of exposure on the human immune system.

To better understand the immunogenicity of isocyanates and help elucidate the pathogenesis of isocyanate asthma, investigators have begun to characterize isocyanate-responsive human T cells. A priori, it has been hypothesized that isocyanates specifically activate CD4⁺/T_H2 type cells, given the central role of these cells in atopic asthma caused by environmental aeroallergens and immunochemical studies of endobronchial biopsy specimens from persons with isocyanate asthma.^{6,20} However, other clinical studies reported to date suggest that isocyanates may preferentially stimulate CD8⁺ T cells and/or a mixed T_H1/T_H2-type response.^{4,15,18,21,22} In the current study, we generated and characterized HDI-responsive T-cell lines from HDI-exposed automobile body shop workers. As the HDI antigen, we used

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HDI-conjugated human proteins (rather than HDI alone), based on the hypothesis that HDI “modified” self-proteins drive the human immune system, as well as the toxicity, lack of solubility in water, and rapid hydrolysis of the chemical on its own. We tested two different carrier proteins, albumin and epithelial cell proteins, based on prior studies demonstrating lymphocyte proliferative responses to HDI conjugated with these different carriers, and evidence that the carrier may be crucial to immune recognition.^{1,23,24} We found that HDI-albumin and HDI-exposed epithelial cells selectively stimulated proliferative expansion of clonal populations of T_H1-like γ/δ T cells from the peripheral blood of HDI-exposed workers but not unexposed control subjects. The ability of HDI to stimulate γ/δ T cells has not been previously recognized and may help explain some of the differences noted in the human immune response to HDI compared with other allergens.

METHODS

Human subjects

Twelve human subjects were recruited from the Study of Painters and Repairers of Autobodies at Yale (SPRAY) and from the Yale Occupational Medicine Clinic.¹ The study was approved by the Human Investigations Committee at Yale University, and written informed consent was obtained from each subject. Participants included 6 HDI-exposed automobile body shop workers with immunologic evidence of exposure (lymphoproliferative responses to HDI-albumin with stimulation indexes >3.0)¹ and 6 nonexposed subjects, including 2 atopic individuals with known sensitivity to cat and dust allergens. One hundred milliliters of heparinized blood was obtained from each participant and processed as described below.

Processing of peripheral blood

Peripheral blood was spun at 1000*g* for 10 minutes to separate plasma and cells. Plasma was sterile-filtered (0.2 μ m), aliquoted, and stored at -20°C . Mononuclear cells were purified on a ficoll-hypaque density gradient and used for culture or cryopreserved for later use as antigen-presenting cells (APCs). In all subjects, γ/δ T cells were $<10\%$ of the total peripheral blood mononuclear cells (PBMCs).

Generation of T-cell lines

PBMCs were plated at a density of 5×10^5 cells/mL in RPMI 1640 media (GibcoBRL; Grand Island, NY) supplemented with 10% autologous plasma. PBMCs were stimulated with HDI antigens or control stimuli for 7 days followed by weekly restimulation with antigen, plasma, IL-2 (R + D Systems; Minneapolis, Minn), and autologous mitomycin C-treated (Sigma, St Louis, Mo) PBMCs as APCs. HDI antigens were prepared as previously described.^{1,24} HDI-albumin was used at 500 $\mu\text{g/mL}$ and HDI-epithelial cell (NCI-H292) preparations were used at 30 $\mu\text{g/mL}$, based on prior studies.^{1,24} Negative control stimuli included media alone and “mock exposed” HDI antigens, that is, albumin and epithelial cells processed without HDI exposure. Positive controls, based on the a priori hypothesis that HDI would specifically stimulate CD4⁺, α/β T cells, included tetanus toxoid (TT) (Massachusetts Biological Laboratories; Jamaica Plain, Mass), concanavalin A (Sigma; St Louis, Mo), dust mite (Alk-Abello; Round Rock, Tex), and cat dander (Greer; Lenoir, NC) allergens at 10 $\mu\text{g/mL}$.

Phenotypic characterization of T-cell lines

T-cell lines were analyzed by 3-color flow cytometry analysis with combinations of the following monoclonal antibodies from Becton-Dickinson (San Jose, Calif): fluorescein isothiocyanate (FITC)-conjugated α -CD3; FITC and biotin-conjugated α - γ/δ ; phycoerythrin (PE)-conjugated α -CD4; PEcy5-conjugated α -CD8; PEcy5-conjugated α -CD3; PE-conjugated mouse IgG_{2b}; PE-conjugated rat IgG₁; PE-conjugated rat IgG_{2a}; PE-conjugated α -IFN- γ ; PE-conjugated α -IL-13; and PE-conjugated α -IL-5. The following reagents were also used from Pharmingen (San Diego, Calif): FITC and PE-conjugated α -V γ 9; PE-conjugated α - γ/δ ; and FITC and PE-conjugated α -V δ 2. Data were acquired on a FACScan and analyzed with Cell Quest software (Becton Dickinson).

Cytokine analysis

Culture supernatants of T-cell lines were analyzed for cytokine levels after 2 and 3 weeks of culture. IL-5, IL-13, and IFN- γ levels were quantified through the use of Opti EIA kits from Pharmingen, according to the manufacturer’s protocol. Statistical significance of differences in cytokine levels in supernatants of HDI-driven cell lines compared with control cultures was determined by using a paired Student *t* test. Intracellular cytokine staining was performed by 3-color flow cytometry on selected subjects. An aliquot of T-cell lines obtained during culture expansion (at week 2) was incubated with 2 $\mu\text{mol/L}$ monensin for 8 hours before flow cytometry, using CytoStain (Pharmingen) as directed.

Molecular analysis of TCR δ genes

RNA was extracted from Ficoll-hypaque-purified T-cell lines, with the use of RNeasy columns from Qiagen (Valencia, Calif). Two separate preparations of cDNA were generated by use of the Superscript kit from Gibco and the switch mechanism-activated reverse transcriptase system from Clontech (Palo Alto, Calif). PCR was performed with 2 μL of cDNA with Platinum Supermix (GibcoBRL) or Advantage 2 polymerase (Clontech). PCR conditions were $94^{\circ}\text{C}/58^{\circ}\text{C}/72^{\circ}\text{C}$ for 30 cycles, with the use of a Perkin-Elmer (Gaithersburg, Md) DNA Thermocycler and primer set 1 (forward-5’AGGAAGACCCAAGGTAACACAA 3’; reverse-5’CTTCACCAGACAAGCGACAT 3’) or primer set 2 (forward-5’ CACCATGCAGAGGATCTCCTCCCTC 3’; reverse-5’AGGAGTGTAGCTTCTCTCATGCCAGTCAGCC 3’). Controls for PCR contamination were negative in all experiments and included “mock” cDNA prepared without reverse transcriptase, water, and TOPO plasmid (Invitrogen). Sequence analysis of cloned, individual PCR products from 2 separate PCR reactions was performed by the Yale University DNA Sequencing Center (New Haven, Conn). Variable (V), diversity (D), and joining (J) region gene alignment was accomplished through the International ImMunoGeneTics Database (<http://imgt.cines.fr:8104/>).

RESULTS

Generation of isocyanate-responsive human T-cell lines

Human T-cell lines were generated from PBMCs of 6 automobile body shop workers with immunologic evidence of HDI exposure and 6 nonexposed control subjects. Table 1 summarizes the results of previous immunologic tests on these subjects¹ as well as the HDI-driven T-cell lines from the current study. T-cell lines were phenotyped starting at 2 weeks of culture, and supernatants were assayed for cytokine levels. The

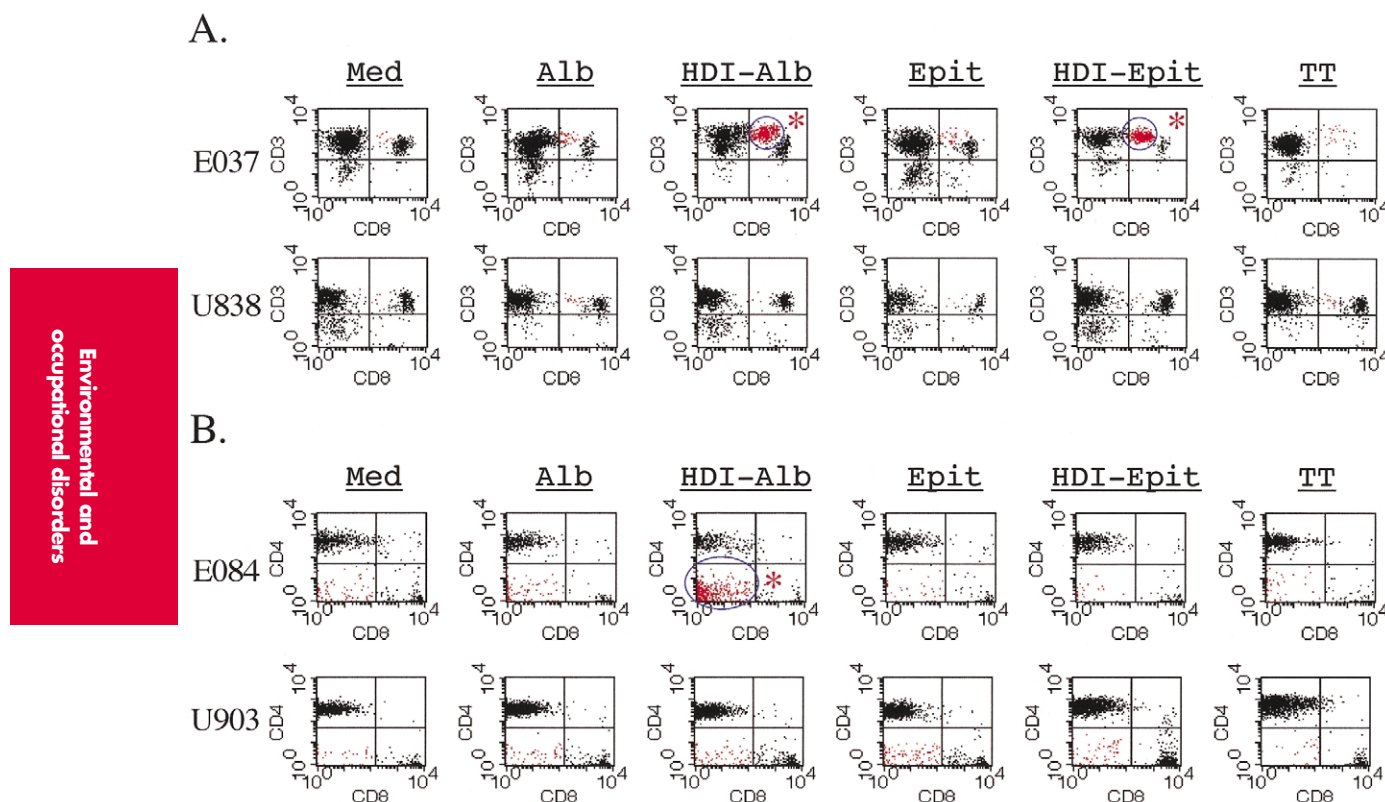


FIG 1. HDI induces proliferation of specific CD3⁺ cells in vitro. Human T-cell lines from 2 representative HDI-exposed (E037 and E078) and HDI-unexposed subjects (U903 and U838) were stimulated for 2 weeks with media (*Med*), albumin (*Alb*), HDI-conjugated albumin (*HDI-Alb*), epithelial cell antigen (*Epit*), HDI-exposed epithelial cell antigen (*HDI-Epit*), and tetanus toxoid (*TT*). Flow cytometry dot plots gated on live cells are shown for CD3 vs CD8 (**A**) and CD4 vs CD8, gated on CD3 (**B**). CD4-CD8^{dim} and CD4-CD8⁻ T cells selectively induced by HDI are circled.

HDI antigens tested included HDI-conjugated human albumin and HDI-exposed human airway epithelial cell preparations, based on previous studies. Control stimuli included tissue culture media, “mock exposed” human albumin, and airway epithelial cell preparations, TT, Concanavalin A, and high molecular-weight allergens (cat dander, dust mite).

Characterization of cell surface markers expressed by isocyanate-driven T cells

Although common allergens are known to specifically activate CD4⁺ T cells, it has previously been proposed that isocyanates may preferentially activate CD8⁺ T cells.^{21,22} Therefore, we initially assessed the expression of CD4 and CD8 on the surface of HDI-driven human T-cell lines. With the use of 3-color flow cytometry, we identified unique populations of CD3⁺ cells (CD4-CD8^{dim}, or CD4-CD8⁻) in HDI antigen-driven cultures from exposed workers but not unexposed control subjects (Fig 1).

On the basis of the profile of cell surface marker expression by the human T cells selectively induced by HDI, we hypothesized that they might be γ/δ T cells and performed direct staining for the γ/δ T-cell receptor. As shown in Fig 2 and by back-gating (not shown), we demonstrated that HDI antigens but not “mock exposed” control antigens, induced the expansion of γ/δ T cells from HDI-exposed workers but not unexposed control subjects. In 4 of the 6 HDI-exposed workers, 1 of the HDI antigens tested induced substantial γ/δ T-cell expansion, whereas in the other 2 HDI-exposed workers, both HDI-albumin and HDI-epithelial cell preparations effectively stimulated γ/δ T cells, which comprised up to 67% of the cultures (Table I). T cells expressing γ/δ receptors were not preferentially expanded in similar experiments with high-molecular-weight antigens (dust mite and cat dander) and PBMCs from the HDI exposed or unexposed control subjects with known sensitivity to these allergens (data not shown). TT, which was used as a positive control stimuli for CD4⁺ T cells and is also

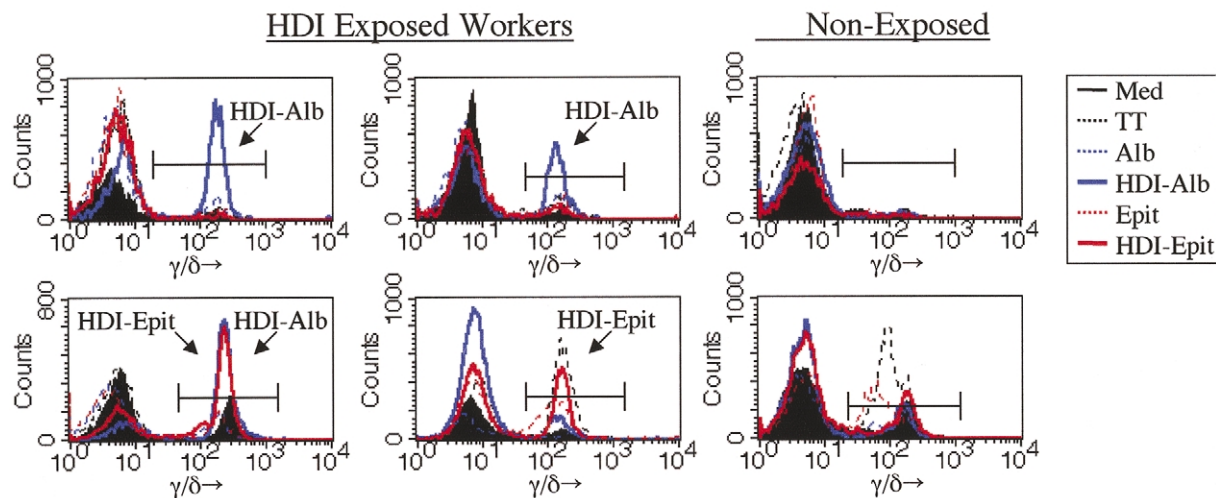


FIG 2. HDI stimulates proliferation of peripheral blood γ/δ T cells from exposed workers. PBMCs from HDI-exposed workers and nonexposed control subjects were stimulated with antigen for 2 to 3 weeks followed by flow cytometry staining for γ/δ T-cell receptor, as described in the Methods section. Stimuli included media (*Med*), albumin (*Alb*), HDI-conjugated albumin (*HDI-Alb*), epithelial cell antigen (*Epit*), HDI-exposed epithelial cell antigen (*HDI-Epit*), and tetanus toxoid (*TT*).

Environmental and
occupational disorders

TABLE I. Human study subject characteristics

ID no.	HDI-SI*	HDI-IgG	HDI-IgE	Mch**	Total IgE	HDI Challenge	HDI-Alb***% γ/δ	HDI-Epit***% γ/δ
HDI-exposed subjects								
E006	3.4	—	—	—	55	—	56	4
E013	3.1	1:128	—	+	19	ND	8	32
E037	3.9	1:64	—	—	86	—	63	65
E078	4.1	—	—	—	5	—	56	2
E084	3.4	—	—	—	49	—	48	3
E801	5.2	1:500	+	+	497	+	67	67
Unexposed control subjects								
U838	1.0	—	—	—	105	ND	1	2
U839	1.1	—	—	—	47	ND	4	3
U842	0.9	—	—	—	14	ND	3	4
U857	1.1	—	—	—	7	ND	4	3
U901	1.4	—	—	+	187	ND	6	4
U903	1.3	—	—	+	215	ND	8	9

ND, Not done.

*HDI-SI, Lymphocyte proliferation assay stimulation index induced by HDI-albumin in previous studies.¹

**Mch, Methacholine sensitivity, + indicates a PC₂₀ dose <8 mg/mL.

***Maximum percentage of γ/δ T cells present in T-cell lines driven with HDI-conjugated albumin or airway epithelial cells is reported.

known to stimulate to γ/δ T cells, was variably stimulatory to γ/δ T cells from both HDI-exposed and HDI-unexposed subjects (Fig 2).

Analysis of HDI-driven γ/δ T-cell antigen receptors

To further characterize isocyanate-driven γ/δ T cells, we analyzed their antigen receptors by using serologic and molecular techniques. We focused on 2 subjects,

E801 and E037, from whom γ/δ T cells were preferentially stimulated by both HDI-albumin and HDI-epithelial cell preparations. Each of the 4 HDI-driven γ/δ T-cell lines generated and tested from these 2 subjects predominately used receptors resulting from V γ 9/V δ 2 gene rearrangements, the most common in human peripheral blood (Fig 3).

To further define HDI-driven γ/δ T cells and distinguish them from γ/δ T cells driven by other stimuli, we

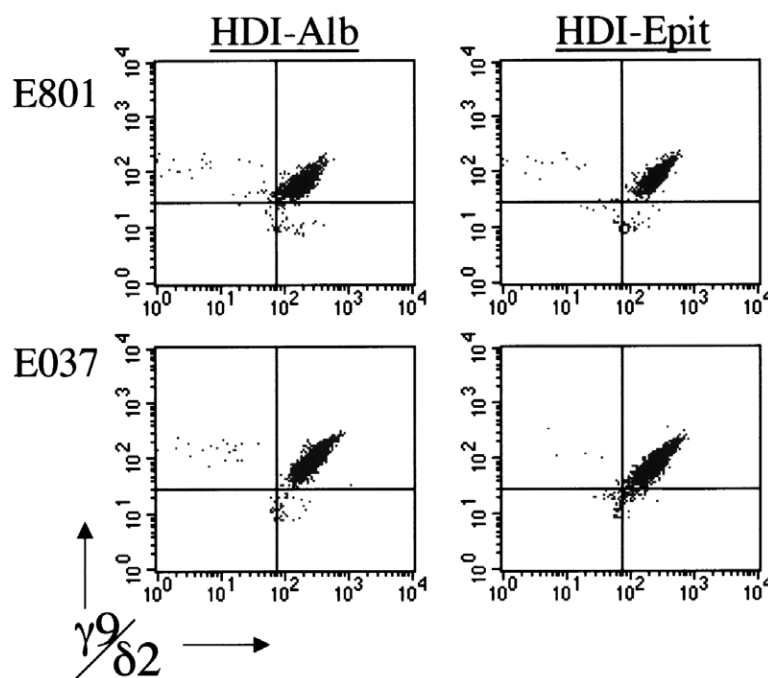


FIG 3. HDI-driven $\gamma\delta$ T cells use V γ 9/V δ 2 genes. T-cell lines from subject E801 and E037 driven with HDI-conjugated albumin (*HDI-Alb*) and HDI-exposed epithelial cell antigen (*HDI-Epit*) were 3-color-stained for $\gamma\delta$ TCR, V γ 9, and V δ 2. Flow cytometry dot plot analysis of V γ 9 vs V δ 2 cell surface expression by HDI-driven T-cell lines is gated on $\gamma\delta$ + T cells.

sequenced the δ -chain of the TCRs expressed by different cell lines. In subjects E801 and E037, we observed that HDI-driven $\gamma\delta$ T cells expressed oligo or monoclonal rearrangement of TCR δ genes, distinct from that induced by other stimuli (Table II). Furthermore, HDI antigens prepared with different carrier proteins (ie, albumin vs epithelial cell) were associated with different TCR δ genes from the same patient. The four predominate HDI-driven TCR δ genes from the 2 subjects tested used J1*01 junction genes and either D3*01 or D2*01 diversity genes. The specific P- and N-nucleotide additions/deletions (not shown; see GeneBank accession number in footnote of Table II) distinguish the HDI-responsive $\gamma\delta$ T cells from those previously defined molecularly and may be important determinants of HDI responsiveness. Together, the sequence and cell phenotype data support antigen receptor-mediated selection for the preferential expansion of specific $\gamma\delta$ T-cell clones in response to HDI.

Characterization of cytokine production by HDI-driven $\gamma\delta$ T cells

Given the central role of T_H2-type cytokines in atopic asthma and the potential for HDI to evoke airway inflam-

mation, we evaluated cytokine production by HDI-driven $\gamma\delta$ T cells. HDI-driven $\gamma\delta$ T cells were consistently associated with T_H1-like rather than T_H2-like cytokine profiles through analysis of cell culture supernatants. As shown in Fig 4, supernatants from HDI-driven T-cell lines derived from 3 different workers (E801, E078, E084), with high percentages of $\gamma\delta$ T cells, contained elevated IFN- γ levels (up to 20-fold increased) and unchanged or marginally decreased IL-5 and IL-13 levels compared with control cultures. HDI-driven $\gamma\delta$ T cells from these same 3 subjects were further shown to directly produce IFN- γ but not IL-5 or IL-13 by intracellular cytokine staining (Fig 5). Together, these cytokine studies demonstrate the T_H1-like properties of HDI-driven $\gamma\delta$ T cells from different subjects and thus their potential to modulate the effects of exposure.

DISCUSSION

This study demonstrates for the first time that HDI, an immunogenic, widely used industrial chemical, can selectively stimulate proliferation and cytokine production by human $\gamma\delta$ T cells and provides extensive characterization of these cells. HDI-driven human $\gamma\delta$ T cells were shown to lack cell surface CD4 expression, although many

TABLE II Predominate hypervariable region sequences of TCR δ from HDI-driven T-cell lines

Subject	Stimuli	V	CDR3 sequence	J	(#/total) ^A
E801	HDI-Alb	CACDT	VGGGYDPGRY	TDK	(12/14) ^B
		CAC	LLGNRN	TDK	(2/14)
	HDI-Epit	CACDT	VMLGDTHAY	TDK	(14/17) ^C
		CAC	TGGYTSSWDTRQMFFGTGDK	DK	(1/17)
		CAC	PMGDTRGGD	TDK	(1/17)
		CAC	PVLGDTRGGD	TDK	(1/17)
	TT	CACDT	VMLGDTHAY	TDK	(4/11)
		CAC	ESLSALGDPY	TDK	(3/11)
		CACDT	VGGGYDPGRY	TDK	(1/11)
		CAC	TLGSSDT	TDK	(1/11)
		CAC	TLG	TDK	(2/11)
	Non-Specific (IL-2)	CACDT	VMLGDTHAY	TDK	(1/5)
		CACDT	VGGGYDPGRY	TDK	(1/5)
		CAC	LLGNRN	TDK	(1/5)
		CAC	TGGYTSSWDTRQMFFGTG	DK	(1/5)
		CAC	NLGD	TDK	(1/5)
E037	HDI-Alb	CACD	RLGDPE	TDK	(10/10) ^D
	HDI-Epit	CACD	PLSPEY	TDK	(9/9) ^E
	Non-Specific (Con-A)	CACD	SILGDTRTR	K	(1/5)
		CAC	TVVLGDTRRGDG	DK	(1/5)
		CAC	SLTGGYA	DK	(1/5)
		CAC	VLLGATSLTP	DK	(1/5)
		CAC	TLGDTLQY	TDK	(1/5)

Environmental and occupational disorders

The deduced amino acid sequences of TCR δ chain hypervariable regions from T cell lines derived from 2 HDI-exposed subjects E801 and E037, stimulated with HDI-albumin (HDI-alb) and HDI-conjugated epithelial cell preparations (HDI-epit), tetanus toxoid (TT) or non-specific stimuli noted.
^A The number of individual PCR-derived clones/the total number of clones sequenced from each cell line is shown in the column to the right. The cDNA sequence for the HDI-driven TCR δs have been submitted to GeneBank under the accession numbers (AY312959)^B, (AY312958)^C, (AY312957)^D, (AY312956)^E.

expressed CD8, and were associated with T_H1-like cytokine profiles and produced IFN-γ but not IL-5 or IL-13. T-cell receptor analysis indicated that HDI induced clonal γδ T-cell expansion, suggestive of antigen-mediated selection. Together the data demonstrate the potential for HDI to stimulate human γδ T cells, a T-cell subset previously overlooked in considering the immunologic and toxicologic effects of occupational isocyanate exposure.

The present findings are consistent with the limited previous reports on T-cell responses to isocyanates, specifically their ability to stimulate IFN-γ secretion and non-CD4⁺ T cells.^{15,21,22} Maestrelli et al²² have previously shown that CD8⁺ T cells that secrete IFN-γ are preferentially expanded from airway biopsy specimens of 2 persons with toluene diisocyanate (TDI) asthma (in contrast to CD4⁺ T_H2-type cells from persons with

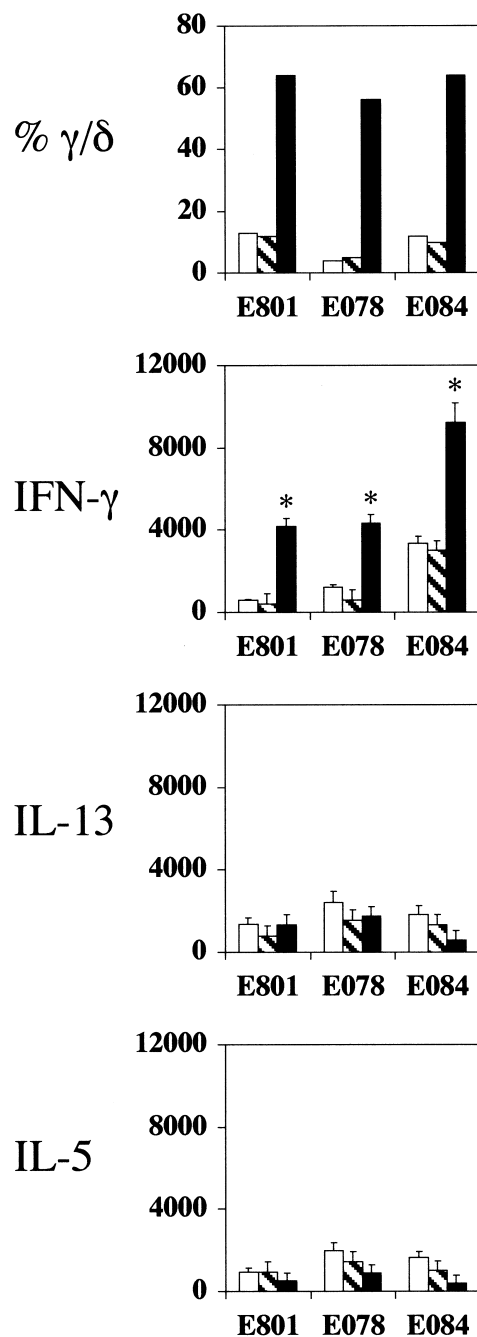


FIG 4. HDI-driven γ/δ T cells are associated with T_H1 -like cytokine profiles. Percentage of γ/δ T cells in HDI-albumin-stimulated cultures compared with control T cell lines is shown in top bar graph for 3 HDI-exposed workers. Lower 3 graphs show indicated cytokine levels (pg/mL) in culture supernatants at 2 weeks as detected by ELISA. In all 4 panels, stimuli were media (open bars), albumin (striped bars), and HDI-albumin (black bars). *Statistically significant difference ($P < .005$) compared with control cultures.

“atopic” asthma) but did not assess their TCR gene usage. Lee et al¹⁵ reported TDI-specific IFN- γ secretion by PBMCs from sensitized human subjects, and Herrick et al²⁵ documented similar findings by using lung-

derived lymphocytes in a murine model of HDI asthma. The current findings that HDI stimulates the proliferation of IFN- γ producing γ/δ T cells that can express CD8 may help explain, at least in part, some of the differences noted in the immune response to HDI compared with other allergens that cause asthma.^{4,6}

The results of the current study raise new questions on the human immune response to isocyanates as well as the biology of γ/δ T cells, which are poorly understood compared with α/β T cells. It remains uncertain if HDI-protein conjugates directly interact with γ/δ T-cell receptors or stimulate γ/δ T cells indirectly through “stress”-related or other HDI-induced molecules. If HDI-protein conjugates themselves are antigens, the role of antigen processing and classic MHC molecules is unclear, given that HDI-induced γ/δ T cells from some individuals lack both CD4 and CD8, which are coreceptors for MHC-presented peptides. If, on the other hand, isocyanates indirectly stimulate γ/δ T cells, possibly through stress-like or other secondary factors, what mechanisms govern this response? The answers to these questions should not only help elucidate the pathogenic immunologic cascade that can result from occupational isocyanate exposure but also provide further insights into the basic immunobiology of human γ/δ T cells. Although these issues are beyond the scope of this study, the current data provide clues that should be helpful to future investigations. The γ/δ TCRs from HDI antigen-driven human T-cell lines in this study are unique compared with control antigen-driven γ/δ T cells from the same subject and suggestive of clonal selection. Furthermore, the γ/δ TCRs from T-cell lines driven with different HDI antigens are molecularly distinct from each other as well as those previously reported to GenBank for other antigen-driven human γ/δ T cells. Studies with recombinant TCRs derived from HDI-driven γ/δ T-cell lines are underway in our laboratory and should provide important insights into both the antigenicity of HDI and the basic immunobiology of human γ/δ T cells.

In summary, the current data define the potential for HDI to stimulate proliferation and IFN- γ production by human γ/δ T cells from a limited number of HDI-exposed automobile body shop workers. Human γ/δ T cells have not previously been considered in the immune response to HDI but could modulate exposure-induced pathogenesis if they are similarly activated in vivo. The results lead to further questions such as the potential association of human γ/δ T cells with isocyanate-induced asthma and the ability of other isocyanates (methylene diphenyldiisocyanate, TDI) to stimulate human γ/δ T cells. Future studies should provide important information regarding the “antigen” specificity of HDI-responsive γ/δ T cells, their potential role in vivo in response to exposure, as well as the basic immunobiology of human γ/δ T cells.

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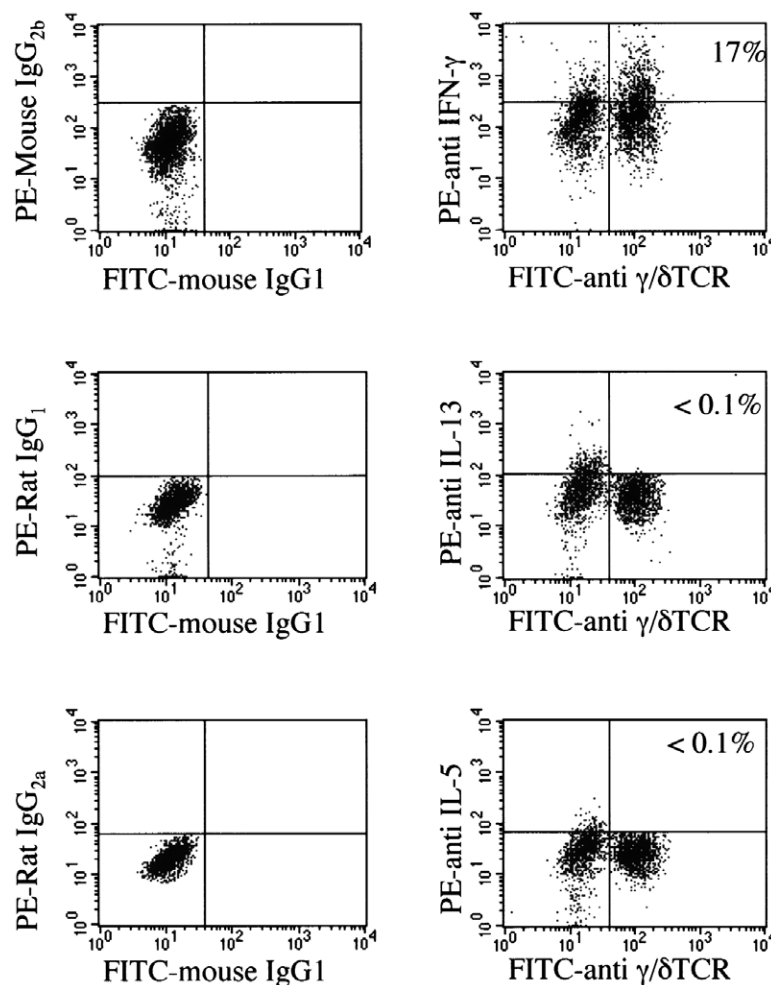


FIG 5. HDI-driven $\gamma\delta$ T cells produce IFN- γ . HDI-albumin-driven T-cell line from representative HDI-exposed worker (E801) were harvested after 2 weeks of in vitro stimulation and incubated with monensin 8 hours before intracellular cytokine analysis. Flow cytometry dot plots for $\gamma\delta$ TCR vs cytokines, IFN- γ , IL-13 and IL-5 (right), or control mAb staining (left) are gated on CD3⁺ cells.

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