Diverse Profiles of Immunoreactivity in Toluene Diisocyanate (TDI) Asthma

J. S. Gallagher, Ph.D.; C. S. Ted Tse, Pharm.D.; S. M. Brooks, M.D.; and I. L. Bernstein, M.D.

Possible immunoreactivity to chemically well-characterized mono- and diisocyanate protein conjugates was reevaluated in 15 workers with TDI asthma and 17 normal (nonexposed) volunteers. Lymphocytes of nine sensitive workers were incubated with TDI human serum albumin (HSA) conjugates. Leucocyte inhibitory factor (LIF) was produced. Leucocyte inhibitory factor was also induced by hexamethylene diisocyanate (HDI) protein conjugates in four of these workers who had no prior history of exposure to HDI. Disappearance of TDI- and HDI-induced LIF was noted in several sensitive workers who were removed from further TDI exposure. Three LIF-positive workers also demonstrated positive intracutaneous reactivity to TDI-HSA. One worker had a markedly positive RAST (25.5% binding) to a monofunctional (p-tolyl isocyanate) protein reagent. These studies suggest that isocyanates have the potential for eliciting heterogeneous immune responses in certain subpopulations of exposed workers. Continued contact with isocyanates may be necessary for maintenance of specific immunity. Possible cross reactivity between TDI and HDI may be determined by new antigenic sites created by isocyanate protein interactions.

Diisocyanate compounds are used extensively in industries which require polyurethane products.[1,2,3] Many of these compounds are volatile and may exert toxic effects on mucous membranes, especially conjunctivae and the respiratory tract.[4,5,6] Asthma-like reactions may also occur in workers after exposure to small amounts of various isocyanates. Since the natural history of these reactions appeared to be consistent with an adaptive immune response, early investigation of possible animal models sought to confirm this hypothesis.[7,8] Conflicting and inconclusive results were obtained in these preliminary experiments. Likewise, initial attempts to demonstrate humoral antibodies in exposed and sensitive workers were unsuccessful.[9,10,11] Since bifunctional diisocyanate protein conjugates were used as the test reagents in these studies, one proposed explanation for negative immunological results was the variability of hapten protein conjugates prepared in different laboratories.[12] Alternatively, a nihilistic view about the immunogenic potential of isocyanates was postulated by some investigators who reported that TDI-induced changes of airway reactivity might be due to direct toxic, pharmacologic and/or beta adrenergic blockage effects.[13,14,15]

More recently, the laboratory at the University of Cincinnati Medical Center developed a method of preparing stable diisocyanate protein conjugates.[16] These conjugates proved to be potent and reproducible immunogens for the production of reaginic antibodies in susceptible strains of mice.[12] Although circulating antibodies to bifunctional reagents were not observed in earlier clinical studies,[10,11] specific IgE responses to monofunctional p-tolyl isocyanate (PTI) conjugates were subsequently found in sensitive workers.[17] Additional evidence of isocyanate immunogenicity included the demonstration of $\lambda 1$ precipitating antibodies in guinea pigs immunized with PTI and HDI protein conjugates.[18,19]

If hypersensitivity is to be seriously considered as a pathogenic mechanism of isocyanate reactions, other possible immunologic mechanisms should be explored. Previous studies by Avery et al[20] had suggested that lymphocytes from exposed and sensitive workers would undergo transformation and proliferate after stimulation by TDI protein conjugates. This work had not been corroborated or extended, presumably because of the variability of reagents. Since the latter problem had been resolved,[16] it therefore seemed worthwhile to reinvestigate the possible role of cellular immunity in isocyanate reactions. The objectives of this investigation were to determine whether LIF, an in vitro correlate of cellular immunity, was present and to compare the results of this pro-

From the University of Cincinnati Medical Center, 231 Bethesda Ave., Cincinnati, OH 45267 (Dr. Gallagher, Assistant Professor, Experimental Medicine, Div. of Immunology), (Dr. Brooks, Head, Clinical Studies Div., and Professor of Medicine and Environmental Health), (Dr. Bernstein, Clinical Professor of Medicine and Environmental Health, Director of Allergy Laboratory and Allergy Training Program, Div. of Immunology), and the University of Chicago Hospital and Clinics, 950 E. 59th St., Chicago, IL 60637 (Dr. Tse). Dr. Tse was Research Associate, Div. of Immunology, University of Cincinnati Medical Center, at the time of this study.

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cedure with other available tests of immediate sensitivity in exposed and sensitive workers.

Materials and Methods

General Design of Clinical Studies. — Workers from a large manufacturing plant which requires extensive use of isocyanates in the production line are being studied on a long-term basis. By mutual agreement of all participants, the clinical investigation in this plant was divided into two separate phases: (1) study of currently symptomatic workers; (2) prospective evaluation of asymptomatic workers. This report deals exclusively with the Phase 1 study.

Patients. — Fifteen patients were included in the Phase 1 study. Eight were women and seven, men. Their ages ranged from 23 to 60 years. All patients had been exposed to TDI at work for at least three months and all had subsequently developed nasal symptoms, chest tightness, shortness of breath or frank evidence of wheezing. Before referral to the laboratory, it had been documented that all symptoms occurred during the active work week with symptoms improving appreciably or disappearing during two-day-off periods. In all cases symptoms had also cleared completely during longer vacation periods (two weeks or more).

Reagents. — Reagent grade TDI (Mondur TD-80; 80% 2,4-TDI; 20% 2,6-TDI) and HDI were supplied by the Mobay Chemical Corp., Pittsburgh, Pa. p-Tolyl monoisocyanate, 2-naphthol-3,6-disulfonic acid disodium salt, 2,4,6 trinitrobenzenesulfonic acid (TNBS) and hexamethylenediamine (HDA) were purchased from Eastman Organic Chemicals, Rochester, N.Y. p-Toluidine was obtained from the Aldrich Chemical Co., Milwaukee, Wis. Human serum albumin (HSA, 25% U.S.P., salt poor) was purchased from the Cutter Laboratories, Inc., Berkeley, Calif.

Preparation and Assay of Hapten-Protein Conjugates. — TDI and HDI were conjugated to 1% phosphate-buffered solutions of HSA by rapid stirring at room temperature and pH 7.4 for three different reaction times (5, 10 and 20 min) in order to adjust the degree of ligand substitution. Each reaction was interrupted by the addition of an equal volume of 2 M ammonium carbonate, centrifuged at 3000 g for 20 minutes and dialyzed extensively against 0.1 M ammonium carbonate. To avoid the possibility of non-protein contamination and to facilitate chemical analyses, various isocyanate protein conjugates were then precipitated with equal volumes of 20% tricholoracetic acid, centrifuged at 3000 g for 20 minutes, redissolved in 1 N sodium hydroxide and dialyzed extensively against distilled water. Aliquots of this solution were then stored at −20°C. HSA used as controls in all experiments was subjected to the same conditions of conjugation (including TCA precipitation and solubilization with 1 N NaOH) and analysis, except that TDI or HDI was not added. In previous and current animal experimental models, the antigenic properties of acid- and non-acid-treated conjugates were identical.[12]

Appropriate chemical assays were performed to determine the number of moles of isocyanate bound to protein under these preparatory conditions. Quantitative estimates of mono- and bisureido TDI derivatives, as well as total TDI bound to proteins, were obtained by a modified Guttman assay.[16] Monoureido linkage was assayed by stepwise monodiazotization, addition of chromophore (sodium 2 naphthol-3,6-disulphonate in 7.4 M NH_4OH) and spectrophotometric measurement at 500 nm. Total isocyanate binding was also determined spectrophotometrically at 500 nm after acid hydrolysis, bisdiazotization and addition of chromophore. The amount of isocyanate bound was measured from a standard calibration curve constructed from five different concentrations of p-toluidine. Total protein of all conjugated reagents was measured by a modified biuret assay.[21] Conjugates with minimal (TDI_2-HSA) ligand substitution were selected for the in vitro LIF tests.

HDI-protein conjugates were assayed for the total amount of HDA in the conjugates. Aliquots (1 ml) of HDI-protein conjugates were hydrolyzed for 144 hours with 6 N HCl at 110° in vacuum. The hydrolyzed samples were dissolved in 1 ml of 0.1 N aqueous NaOH and 5 µl of this solution was injected into a gas chromatographic system.[22] Specific conditions of gas chromatography were: column temperature, 200°C; injector temperature, 300°C; detector temperature, 320°C; carrier gas, nitrogen at column pressure of 29 psi; stainless steel column, 9.32 cm in diameter and 2 m in length, containing 25% Apiezon L plus 10% KOH coated on Chromosorb WHP (60/80 mesh). Triplicate samples were analyzed and their peak areas were measured. The concentrations of HDA produced from hydrolysis of various HDI-protein conjugates were determined by comparison with a standard calibration curve of HDA.

Monofunctional conjugates of PTI and HSA were prepared for use in the radioallergosorbent test (RAST). PTI 0.05 ml (76.5 mg) was added to 50 ml of a 1% phosphate-buffered saline solution of HSA under the same conditions described above for the bifunctional reagents. All conjugates were analyzed for degree of PTI substitution according to a previous report.[16] PTI_{13}-HSA adducts were used for RAST and RAST inhibition studies. These reagents were not available at the time skin and LIF test were performed.

Skin Tests. — Intracutaneous skin tests were performed with a 1:1000 (w/v) solution of TDI_{13}-HSA. Appropriate controls (solutions of unconjugated HSA, histamine [1:1000] and normal saline) were all tested at the same time. All intracutaneous tests were interpreted according to previously described criteria.[23] Only whealing responses to TDI_{13}-HSA occurring without reactions to HSA and saline were considered significant. All tests were performed with freshly diluted aliquots of previously stored reagents (at −20°C).

Measurement of Specific IgE. — Specific IgE was measured by RAST.[24] Cyanogen bromide-activated methylcellulose discs were reacted with the PTI_{13}-HSA conjugate at a concentration of 20 mg/ml. After stepwise incubation of antigen-coupled discs with patient's serum and ^{125}I anti-IgE, radioactivity bound to the paper discs was measured in a gamma counter. The amount of radioactivity bound by patient's serum was compared to similar measurements for individuals not exposed to isocyanates. Control tests were also performed with the carrier protein HSA alone. Total IgE was determined by the radioimmunosorbent test (RIST).*

*Phadebas, Pharmacia Laboratories, Piscataway, N.J.

Table 1. — Immunologic Tests in TDI-Sensitive Workers and Normal (Unexposed) Volunteers.

	Leucocyte Migration Inhibition (%)		Skin Test Grades*		Radioallergosorbent Test % Binding	
Workers	TDI-HSA*	HDI_6-HSA	TDI_{13}-HSA	HDI_6-HSA	TDI_{13}-HSA	PTI_{13}-HSA
1	43.0	—	—	—	1.5	2.6
2	53.1	—	—	—	1.8	1.1
3	89.6	—	—	—	1.8	1.4
4	98.5	—	—	—	2.3	2.4
5	23.3	28.9	—	—	—	—
6	63.0	—	—	—	—	—
7	99.0	99.9	—	—	—	—
8	35.3	81.8	3+	3+	1.4	1.1
9	37.0	42.5	2+	2+	2.4	25.5
10	19.1	3.3	2+‡	3+‡	2.1	1.1
11	15.4	8.1	0	0	1.6	1.1
12	14.8	—	—	—	—	—
13	6.2	0	—	—	1.2	1.4
14	0	10.6	1+	0	1.7	1.1
15	0	—	—	—	0.4	1.1
Controls						
1§	0	—	—	—	1.2	1.6
2 mean	2.2	3.7	0	0	1.8	1.4
to						
18¶ range	(1.2-10.3)	(1.9-15.5)			(0.7-2.8)	(0.1-1.7)

*0 — Same as control; 1+ — sum of crossed-diameter wheal twice the equivalent measurement of control plus erythema; 2+ — sum of crossed-diameter wheal three times the equivalent measurement of control plus erythema; 3+ — sum of crossed-diameter wheal four times the equivalent measurement of control plus erythema; 4+ — any wheal with pseudopodia

†TDI_2-HSA and TDI_{13}-HSA reagents used in Patients 1-4 and 6-15, respectively

‡Late reactions appearing four hours after application of tests

§Control worker whose last exposure was 7 years ago

¶Seventeen normal volunteers never exposed to isocyanates. Three of the volunteers consented to have direct skin tests

The RAST inhibition procedure was performed as previously described.[25] Briefly, varying concentrations of mono- and diisocyanate conjugates were incubated with a p-tolyl-specific serum with known high binding capacity for 2 hours at 37°C and the incubation mixtures were then assayed by the standard RAST technique. The amount of specific IgE binding (cpm) contained in the absorbed mixture compared to that in the unabsorbed allergic serum x 100 was defined as the percent of inhibition by respective absorbents.

LIF Tests. — Peripheral leucocytes were obtained by sedimentation of 25 ml of blood in a solution containing 250 U heparin/ml and 6% dextran.[26] After two separate washes with 0.83% ammonium chloride and Hank's balanced salt solution (HBSS), cells were suspended in TC199 containing 10% fetal calf serum. This suspension was aspirated into capillary tubes which were plugged, centrifuged at 2000 g, cut at cell fluid interfaces and mounted in tissue culture chambers filled with the suspending medium. To insure uniformity of cell density within capillary tubes, only those packed cells of similar length (1.5-3mm) were then incubated either with or without antigens at 37°C in a 5% CO_2 incubator for 18 to 24 hours.

The migration areas were measured planimetrically and the percent of inhibition of leucocyte migration was determined by the following formula:

Table 2. — Dose-Response Effects of Leucocyte Migration Inhibition Factor by TDI-HSA Conjugates.

		% Inhibition Test Dilution		
Workers	Conjugates	1-1	1-10	1-100
1	TDI_2-HSA*	7.8	TA	43.0
2	TDI_2-HSA*	TA	25.1	53.1
3	TDI_2-HSA*	89.6	38.2	TA
4	TDI_2-HSA*	—	30.9	98.5
5	TDI_{13}-HSA†	23.3	9.9	—
6	TDI_{13}-HSA†	63.0	35.7	—
7	TDI_{13}-HSA†	99.0	36.3	—

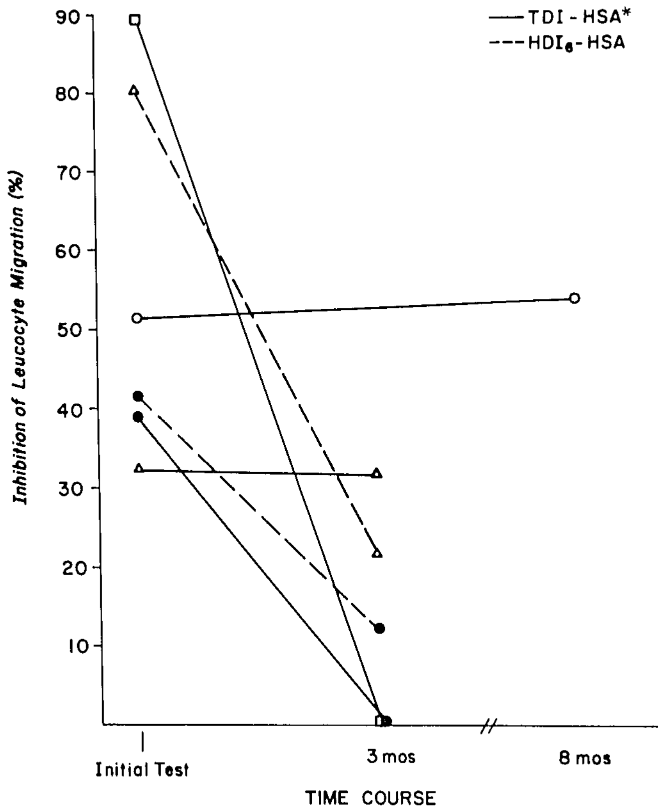
*Concentrated (undiluted) conjugate = 10 mg/ml

†Concentrated (undiluted) conjugate = 5 mg/ml

TA — Technical artifact prevented an accurate measurement at these dilutions

All experiments were conducted in triplicate and a mean percent inhibition was calculated. Inhibition percentages of 20% or more were considered significant. Each LIF experiment also included unconjugated HSA controls and leucocyte preparations from a normal volunteer without previous history of exposure. Occasionally, some degree of LIF inhibition was obtained with HSA control samples only. When this occurred, the non-specific inhibition of HSA-stimulated cultures was subtracted from the isocyanate conjugate-induced inhibition. This adjusted in-

$$\%\ \text{leucocyte migration inhibition} = \left\{ 1 - \frac{\text{migration area of cells in presence of antigen}}{\text{migration area of cells without antigen}} \right\} \times 100$$



*Patients 2 and 3 were tested with TDI_2-HSA; Patients 8 and 9 were tested with TDI_{13}-HSA.

Fig 1. — Serial studies of LIF (% inhibition) in 4 patients with TDI asthma. Patient 2 (○—○) was studied after continued exposure to TDI for eight months. Patients 3 (□—□), 8 (△—△) and 9 (●—●) were investigated three months after removal from further TDI exposure.

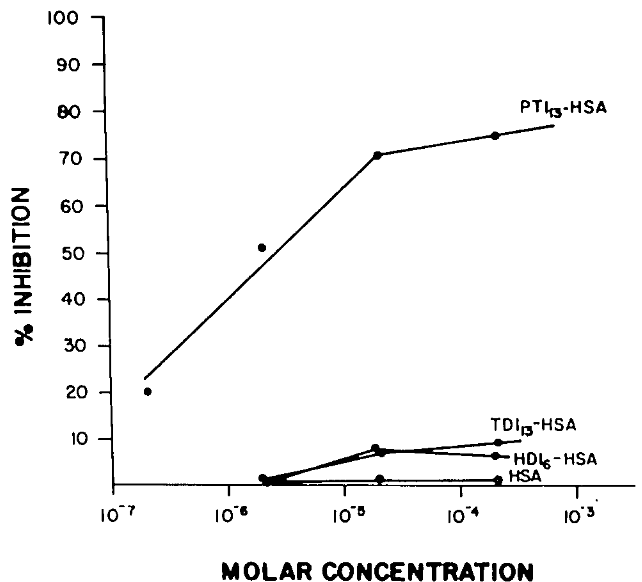


Fig 2. — RAST inhibition experiments of a serum (Patient 9) with 25.5% RAST binding to PTI_{13}-HSA coupled methylcellulose discs. Each of the above curves was plotted from dose-response RAST assays of serum obtained after absorption with each of the designated antigens.

hibition was considered significant only if it was 20% or more. In serial LIF experiments all antigen and control solutions were freshly diluted from aliquots of stored (−20°C) reagents

Results

Leucocyte migration tests were performed in 15 currently exposed and symptomatic patients, in one previously symptomatic worker who had not been exposed for seven years, and in 17 volunteer subjects with negative isocyanate exposure histories. These results are summarized in Table 1. The presence of LIF to TDI conjugates was demonstrated in nine symptomatic workers. Four of these subjects also exhibited LIF activity to the HDI conjugate. No evidence of LIF was observed in 17 nonexposed control subjects or in a previously symptomatic worker who had been removed from further exposure to TDI seven years prior to the test. Table 2 is a summary of dose-response assays which were measured in all but two LIF-positive cases (Patients 8 and 9). Symptomatic workers tested with minimally substituted TDI conjugates (Patients 1 through 4) tended to exhibit greater inhibition of leucocyte migration at dilute concentrations of test antigens, while the percent of inhibition of leucocyte migration was greater at more concentrated antigen solutions in those symptomatic subjects tested with more highly substituted TDI conjugates.

Four TDI-exposed and symptomatic workers (Patients 2, 3, 8 and 9, Table 1) were selected for a short-term prospective study of LIF activity. Patient 2 was tested again eight months after continued exposure to TDI, while repeat assays were conducted in Patients 3, 8 and 9 three months after they were removed from further TDI exposure.

Four of the 15 exposed, symptomatic workers and three normal volunteers without previous exposure to TDI gave informed consent for direct skin testing with the moderately substituted TDI protein conjugate (TDI_{13}-HSA). Equivalent concentrations of unconjugated HSA were also applied to control skin sites at the same time. Table 1 shows that two workers exhibited skin test reactivity. One other patient exhibited late onset reactions appearing four hours after application of the skin test. The LIF test was positive in both patients displaying immediate skin reactivity. Possible quenching of skin test reactivity was studied in one worker (Patient 8) three months after exposure to TDI was terminated. In this instance, skin test reactivity was completely abolished. Nonspecific direct skin test reactions to unconjugated HSA were not observed in either the workers or the volunteers. None of the unexposed volunteers exhibited skin reactivity to either the isocyanate reagents or the control solutions.

Table 1 also includes the results of RAST testing with both diisocyanate and monoisocyanate reagents in 11 of the 15 workers and in 18 control subjects. Similar to previous experience, RAST tests with the TDI bifunctional reagent were uniformly negative. However, one worker (Patient 9, Table 1) exhibited significant binding of specific IgE (25.5%; 14,591 cpm or 10 x the binding of a negative control serum) to insoluble matrices coupled with the monofunctional PTI conjugate. Fig 2 demonstrates that this reaction was significantly inhibited by monoisocya-

nate (PTI-HSA), but not by diisocyanate (TDI-HSA and HDI-HSA) conjugates or unconjugated HSA. Of further interest was the fact that this patient's serum IgE was well within normal limits (135 U/ml), a finding that has been observed consistently in other workers with specific IgE to industrial allergens.[27] The worker with p-tolyl-specific positive RAST also showed positive LIF and skin tests. Two of the other workers with positive skin tests did not show significant RAST binding.

Discussion

Although the immunopathogenesis of TDI asthma is currently a highly controversial subject, this immunologic reevaluation supports the view that isocyanates have the potential for sensitizing certain subpopulations of exposed workers. The immunologic responses encountered in this investigation were associated with both immediate and delayed hypersensitivity. Humoral immunoreactivity was demonstrated in some sensitive workers by direct skin testing and in one individual by the radioallergosorbent test. A possible role for cell-mediated mechanisms was also suggested by positive LIF results in the majority (9 of 15) of sensitive workers.

This clinical investigation confirmed the presence of specific IgE antibodies to the p-tolyl determinant, but not to the diisocyanate conjugate. However, only one sensitive worker in the current study and six others from a recently completed survey of 650 workers in a large TDI plant were found to have significant titers of p-tolyl-specific IgE. The low incidence of significant levels of specific IgE in these studies is at variance with several reports.[17,28] This discrepancy may be more apparent than real because of differing assumptions in interpreting the RAST test. For example, a positive test in the authors' laboratory is defined as 5% or greater binding (usually equivalent to residual radioactivity of at least 2000 cpm), whereas positive results in other laboratories were based upon counts as low as 600[17] and 350 cpm,[28] respectively.

One of the most noteworthy aspects of this investigation was the frequent occurrence of LIF in sensitive workers. This inhibitory lymphokine is generally regarded to be a component of cell-mediated immunity. Apart from several isolated case reports of hypersensitivity pneumonitis associated with isocyanate exposure[29,30] and a prior report of lymphocyte blastogenesis induced by TDI-protein conjugates,[20] little attention has been given to the possible role of cell-mediated hypersensitivity to isocyanates. The current LIF data suggest that delayed hypersensitivity responses may be evoked by isocyanates in subpopulations of sensitive workers. On the other hand, since it is also known that LIF might be activated by small amounts of circulating immune complexes,[31] it is possible that isocyanate-induced LIF could represent a heterogeneous type of immunologic adaptive response. An analogous situation was recently described in trimellitic anhydride (TMA) sensitivity, in which lymphocyte blastogenesis was abnormally high in a spectrum of clinical TMA syndromes, some of which were not associated with cell-mediated disease states.[32] In any case, the investigation of various in vitro correlates of delayed hypersensitivity deserves further attention in clinical evaluations of TDI reactions.

Simultaneous reactivity induced by TDI and HDI in the LIF test system was observed in several TDI-exposed, HDI-unexposed workers. A possible explanation for this occurrence is that new antigenic determinants (NAD) are created by interaction of either highly reactive aliphatic or aromatic ligands with carrier protein.[12] Presumably, HDI cross-reactivity in the LIF system could be accounted for by these NAD. Cross-responsiveness between several diisocyanate compounds has also been reported in bronchial challenge experiments,[34] but there was no substantive evidence of immunologic involvement. Although the current data provide indirect evidence that cross-immunity could have contributed to those reactions, it should be emphasized that they do not constitute direct experimental proof that TDI and HDI cross-immunity exists and further investigation along these lines is required.

New antigenic determinants may also explain the apparent paradoxical dose-response effects of minimally and maximally substituted adducts in this study. The minimally substituted conjugates may actually contain higher molar ratios of NAD and therefore LIF reactivity occurred at more dilute solutions of these antigens. On the other hand, relatively lower molar ratios of NAD may be present in the moderately substituted adducts because the dimer- and polymerization that occur during the preparation of these conjugates may actually mask a proportionately greater number of new antigenic determinants. Thus, the reactions to these conjugates might have been expected to occur only at greater test concentrations.

Disappearance of LIF reactivity in three patients three months after removal from isocyanate exposure suggests that continued contact with isocyanates may be necessary for maintenance of specific immunity. This interpretation appears to be supported by a negative LIF test in a clinically sensitive worker who had not been exposed to TDI for seven years and the persistent positive LIF test in a worker whose exposure to TDI was continued for eight months. These clinical observations are also consistent with a previous report wherein TDI-induced blastogenic responses declined after cessation of exposure.[20] The short-term nature of some of these LIF responses also illustrates the importance of the temporal factor in assessing immunoreactivity. Negative results of previous investigations could have been due to this variable, the significance of which had not yet been recognized when those experiments were performed.

Although direct skin testing was performed in a smaller number of sensitive workers, it was noteworthy that skin reactivity occurred in the majority of them. The test sites were not biopsied, but their evolution and clinical appearance were consistent with immediate hypersensitivity. After the immediate reactions subsided, no clinical evidence of delayed skin reactivity was observed in the same patients, all of whom exhibited positive LIF tests. How can this apparent dichotomy be explained? The exact biologic role of LIF is uncertain. In contrast to macrophage inhibitory factor, it has not been associated with the skin-reactive factor of delayed-type hypersensitivity reactions.[34] Recently, LIF has been identified as a hydrolytic enzyme regulated by cyclic GMP,[35] which also activates lymphocyte proliferation. Thus it is possible that

LIF could be produced simultaneously with mediators derived from carrier-specific T cells. These helper lymphokines could then trigger an anti-hapten antibody response by hapten-primed B cells.[36] Experimental results in mice suggest that a specific subclass of lymphocytes (Ly 1) is programmed to initiate both delayed-type hypersensitivity and helper function which stimulates B cells to produce antibody.[37] In rats there also is evidence that IgE may exert a regulatory role on the effector cells of delayed hypersensitivity.[38] Finally, in diseases associated with immediate hypersensitivity, it has been documented that there are circulating lymphocytes which produce mediators (mitogenic and MIF factors) in the absence of delayed-type skin reactions.[39]

Positive skin tests were obtained with TDI-HSA conjugates which failed to elicit positive RAST results. However, since all patients with positive direct skin tests to the bifunctional TDI adducts also exhibited positive LIF tests to the same reagents, it is possible that membrane stability (either of mast cells or lymphocytes) is a putative requirement for effective interaction of diisocyanate conjugates with their specific antibody-combining sites or receptors. This explanation would be consistent with the negative RAST inhibition results with diisocyanate conjugates, implying that the latter do not combine as readily with specific IgE molecules in free solution.

The immunologic response occurring after exposure to isocyanates is heterogeneous. This diversity may be advantageous in seeking ways of classifying the various clinical subpopulations of isocyanate reactions. In this particular study, for example, at least four clinical subpopulations of sensitive workers have been defined: (1) a small subpopulation with positive RAST tests to a monoisocyanate protein adduct; (2) a majority of sensitive workers with positive LIF responses to diisocyanate protein conjugates; (3) a small segment of workers with both positive LIF and direct skin test responses to diisocyanate protein conjugates; and (4) another group of workers showing no evidence of immunologic reactivity as measured by the tests used in this study.

In agreement with a recent report,[28] RAST was not determined to be an effective screening test for possible isocyanate immunoreactivity in a group of symptomatic TDI-exposed workers. On the othe hand, the results suggest that in vitro screening for mediators derived from sensitized lymphocytes may be an effective method of detecting reactivity in workers currently exposed to isocyanates. The case-finding potential of direct skin tests is less clear, but the preliminary results indicate that this diagnostic technique should be extended to future long-term studies. These data also establish that in vitro lymphokine or direct skin test screening will require the use of stable and well-characterized bifunctional isocyanate conjugates, while RAST tests are only positive using monofunctional isocyanate preparations.

None of the immunologic responses demonstrated in this study can as yet be implicated in the pathogenesis of TDI asthma. Indeed, immunoreactivity was conspicuously absent in six symptomatic workers of this investigation. It is hoped that the authors' Phase 2 prospective investigation will address these inconsistencies in a more direct manner by serial assessment of a large group of asymptomatic, exposed workers. In the meantime, however, it may be useful to conceptualize the pathophysiology of TDI asthma as a combination of factors which alter mucosal permeability and subsequently induce a state of hyperactive airways.[40]

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References

1. Simmons LF: Isocyanate resins, in *Modern Plastics Encyclopedia* **39**:216-218, 1961.

2. Foamed organics have bright future. *Chem Eng News* **39**:46-47, 1961.

3. Buist JM: Isocyanates in industry. *Proc R Soc Med* **63**:365-367, 1970.

4. Elkins HB, McCarl GW, Brugsch HG, and Fahy JP: Massachusetts experience with toluene diisocyanate. *Am Ind Hyg Assoc J* **23**:265-272, 1962.

5. Williamson KS: Studies of diisocyanate workers. *Trans Assoc Ind Med Off* **14**:81-88, 1964.

6. Bruckner HC, Avery SB, Stetson DM, and Dodson VN: Clinical and immunologic appraisal of workers exposed to diisocyanates. *Arch Environ Health* **16**:619-629, 1968.

7. Scheel LD, Killens R, and Josephson A: Immunological aspects of toluene diisocyanate (TDI) toxicity. *Am Ind Hyg Assoc J* **25**:179-184, 1964.

8. Steven MA and Palmer R: The effect of toluene diisocyanate on certain laboratory animals. *Proc R Soc Med* **63**:380-382, 1970.

9. Taylor G: Immune responses to toluene diisocyanate (TDI) exposure in man. *Proc R Soc Med* **63**:379-380, 1970.

10. Bernstein IL, Gallagher JS, Pesce AJ, et al: Immunologic studies of isocyanate-induced airways lability. *J Allergy Clin Immunol* **87**:212, 1976.

11. Butcher BT, Salvaggio JE, Weill H, and Ziskind MM: Toluene diisocyanate (TDI) pulmonary disease: Immunologic and inhalation challenge studies. *J Allergy Clin Immunol* **58**:89-100, 1976.

12. Tse CST, Chen SE, and Bernstein IL: Induction of murine reaginic antibodies by toluene diisocyanate. An animal model of immediate hypersensitivity reactions to isocyanates. *Am Rev Respir Dis* **120**:829-835, 1979.

13. Butcher BT, Salvaggio JE, O'Neil CE, et al: Toluene diisocyanate pulmonary disease: Immunopharmacologic and mecholyl challenge studies. *J Allergy Clin Immunol* **59**:223-227, 1977.

14. David RJ, Butcher BT, O'Neil CE, and Salvaggio JE: The in vitro effect of toluene diisocyanate on lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin and histamine: A possible mode of action. *J Allergy Clin Immunol* **60**:223-229, 1977.

15. Butcher BT, Karr RM, O'Neil CE, et al: Inhalation challenge and pharmacologic studies of toluene diisocyanate (TDI)-sensitive workers. *J Allergy Clin Immunol* **64**:146-152, 1979.

16. Tse CST and Pesce AJ: Chemical characterization of isocyanate-protein conjugates. *Toxicol Appl Pharmacol* **51**:39-46, 1979.

17. Karol MH, Ioset HH, and Alarie YC: Tolyl-specific IgE antibodies in workers with hypersensitivity to toluene diisocyanate. *Am Ind Hyg Assoc J* **39**:454-458, 1978.

18. Karol MH, Ioset HH, Riley EJ, and Alarie YC: Hapten-specific respiratory hypersensitivity in guinea pigs. *Am Ind Hyg Assoc J* **39**:546-556, 1978.

19. Karol MH, Hauth BA, and Alarie YC: Pulmonary hypersensitivity to hexyl isocyanate-ovalbumin aerosol in guinea pigs. *Toxicol Appl Pharmacol* **51**:73-80, 1979.

20. Avery SB, Stetson DM, Pan PM, and Mathews KP: Immunological investigation of individuals with toluene diisocyanate asthma. *Clin Exp Immunol* **4**:585-596, 1968.

21. Kabat BA and Mayer MM: Experimental Immunochemistry, 2nd Ed. Springfield, Ill.: Chas. C. Thomas, 1964, pp 559-560.

22. Sandridge RL: Laboratory Methods. New Martinsville, W.Va.: Mobay Chemical Corp., 1978.

23. Bernstein IL: Enzyme allergy in population exposed to long-term low level concentrations of household laundry products. *J Allergy Clin Immunol* **49**:219-237, 1972.

24. Wide L, Benich H, and Johansson SGO: Diagnosis of allergy by

an in vitro test for allergen antibodies. *Lancet* **2**:1105-1107, 1967.
25. Bernstein IL, Perera M, Gallagher J, et al: In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J Allergy Clin Immunol* **57**:141-152, 1976.
26. Soberg M and Bendixen G: Human lymphocyte migration as a parameter of hypersensitivity. *Acta Med Scand* **181**:247-256, 1967.
27. Maccia CA, Bernstein IL, Emmett EA, and Brooks SM: In vitro demonstration of specific IgE in phthalic anhydride hypersensitivity. *Am Rev Respir Dis* **113**:701-704, 1976.
28. Butcher BT, O'Neil CE, and Salvaggio JE: Radioallergosorbent test (RAST) with p-tolyl (mono) isocyanate on sera from toluene diisocyanate. *J Allergy Clin Immunol* **65**:168, 1980.
29. Charles J, Bernstein A, Jones B, et al: Hypersensitivity pneumonitis after exposure to isocyanates. *Thorax* **31**:127-136, 1976.
30. Fink JN and Schlueter DP: Bathtub refinisher's lung: An unusual response to toluene diisocyanate. *Am Rev Respir Dis* **118**:955-959, 1978.
31. Rocklin RE: Products of activated lymphocytes: Leucocyte inhibitory factor (LIF) distinct from migration inhibitory factor (MIF). *J Immunol* **112**:1461-1466, 1974.
32. Zeiss CR, Patterson R, Pruzansky JJ, et al: Trimellitic anhydride-induced airway syndromes: Clinical and immunologic studies. *J Allergy Clin Immunol* **60**:96-103, 1977.
33. O'Brien IM, Harries MG, Burge PS, and Pepys J: Toluene diisocyanate-induced asthma. I. Reactions to TDI, MDI, HDI, and histamine. *Clin Allergy* **9**:1-6, 1979.
34. Bennett B and Bloom BR: Reactions in vivo and in vitro produced by a soluble substance associated with delayed-type hypersensitivity. *Proc Natl Acad Sci USA* **59**:756-762, 1968.
35. Bendtzen K: Determination of the human lymphokine leukocyte migration inhibitory factor (LIF) by a sensitive radioenzymatic assay, inhibitory effect of cGMP on the esterolytic activity of highly purified LIF. *J Clin Lab Immunol* **2**:37-43, 1979.
36. Feldmann M and Basten A: Cell interactions in the immune response in vitro. III. Specific collaboration across a cell impermeable membrane. *J Exp Med* **136**:49-67, 1972.
37. Huber B, Devinsky O, Gershon RK, and Cantor H: Cell-mediated immunity: Delayed-type hypersensitivity and cytotoxic responses are mediated by different T-cell subclasses. *J Exp Med* **143**:1534-1539, 1976.
38. Pearson DJ and Taylor G: The influence of IgE-mediated reactions on the expression of delayed hypersensitivity in the rat. *Immunology* **33**:185-190, 1977.
39. Butcher BT, O'Neil CE, Reed MA, and Salvaggio JE: Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl isocyanate antigen. *J Allergy Clin Immunol* **66**:213-216, 1980.
40. Hogg JC, Pare PD, Boucher RC, and Michoud MC: The pathophysiology of asthma. *Can Med Assoc J* **121**:409-414, 1979.

Telling the Life Story

In a sense, each of us is a first novelist. Telling our life story is one of the luxuries of democracy, the equivalent of the identity papers and police dossier in an authoritarian state. Under American law, every citizen compiles his own dossier. He is guaranteed a life story and an equal opportunity to tell it.

Every listener is a literary critic, and how well you tell your life story will determine whether you become a best seller or an embittered victim of bad reviews. There is some debate about which is more authentic, your life or your story. One school of thought holds that the truth of your life lies in the tension, or discrepancy, between what actually happened and the way you tell it.

To the person who is contemplating you as a friend or lover, your life story is like the manual of instructions that comes with the purchase of an expensive appliance. The manufacturers of such appliances put a lot of careful thought and research into the composition of their manuals. And we can do no less.

— From "Reading and Writing: Life Story," by Anatole Broyard, in *The New York Times Book Review Section*, May 31, 1981.