

Toluene diisocyanate (TDI) pulmonary disease: Immunologic and inhalation challenge studies

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Clinical and serologic effects of TDI exposure were studied in 112 occupationally exposed plant workers. Sera were obtained before and after commencement of TDI production. All subjects were skin-tested with common inhalant allergens and a TDI-HSA conjugate. Total eosinophil counts, immunoglobulin quantitations, and specific antibody assays by PCA, P-K, and radioimmunoassay were performed. Clinically "sensitive" individuals were tested by provocative inhalation challenge with from 0.005 ppm to the threshold limit value of 0.02 ppm TDI. No TDI-induced immunologic changes were noted with the exception of 3 individuals who demonstrated small positive wheal-and-erythema reactions to TDI-HSA but not to HSA alone. Inhalation challenge with TDI vapor produced airways obstruction, as measured by FEF₍₂₅₋₇₅₎. These responses were of the immediate, delayed, and dual type, and were provoked in some cases with levels as low as 0.005 ppm TDI.

Toluene diisocyanate (TDI) is a simple molecule used in the production of polyurethane plastics. During the past 20 years there have been numerous reports of untoward pulmonary reactions often characterized by dyspnea, wheezing, and cough caused by TDI vapor.¹⁻¹⁰ Evidence suggests that these reactions, which clinically resemble the immediate, late, and dual bronchospastic reactions provoked by antigens such as *A. fumigatus*, avian allergens, and house dust, may be immunologic in nature.¹⁰ Currently there is no conclusive evidence that immunologic factors are involved in production of TDI-induced pulmonary symptoms. The present study represents the initial phase of a comprehensive study of workers exposed to TDI in the manufacturing process.

MATERIALS AND METHODS

Study population

The study population consisted of the work force at a TDI-producing plant which was part of a major chemical production complex. Initially, there were 167 participants, assigned to three groups on the basis of expected exposure to TDI: Group I—constant exposure (84), Group II—intermittent exposure (28), Group III—no exposure (55). A few participants resigned from the plant during the study and were replaced by new workers. When the work force expanded, every attempt was made to include all new personnel liable to TDI exposure.

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Operational procedure

In April-May, 1973, prior to commencement of TDI production, a visit was made to the plant to collect pre-exposure blood samples and to perform skin tests with common inhalant allergens and TDI-HSA (human serum albumin) conjugates. Further visits were made in November-December, 1973, following commencement of TDI production and in September-October, 1974, when blood samples were again collected and workers were skin-tested with TDI-HSA. Eosinophil counts were performed on all blood samples and serum was separated at the plant, frozen, and stored at -70° C until used to quantitate immunoglobulin levels and to perform passive cutaneous anaphylaxis (PCA) and P-K tests plus radioimmunoassay (RIA) for detection of TDI-specific antibodies.

Antigens and skin tests

TDI-serum albumin conjugate. TDI was conjugated to human serum albumin by the method of Scheel and associates.¹¹ In an ice bath, 100 ml of a 1% solution of the protein in 0.85% sodium chloride was adjusted to pH 8.5 with 1 N NaOH. While maintaining pH at 8.5 with 1 N NaOH, 100 ml of a 20 mg/100 ml solution of TDI in dioxane was added slowly over a 3-hr period. The mixture was left in the ice bath for 1 hr, transferred to a dialysis bag, and left in the fume hood overnight to allow the dioxane to evaporate. The conjugate was next dialyzed in the cold against 6 changes of phosphate-buffered saline (pH 7.4), lyophilized, and stored at -20° C until used. Conjugation was confirmed by ultraviolet spectroscopy at 245 m μ .

TDI-HSA SKIN TESTING. Subjects were first tested by standard prick tests with TDI albumin conjugate. When a negative prick test was obtained, 0.02 ml of a 5 mg/ml solution of the TDI-HSA conjugate in saline was injected intradermally and the injection site observed for 30 min. If a positive reaction was obtained with TDI-HSA, 0.02 ml of 5 mg/ml solution of HSA alone was injected intradermally.

Inhalant allergens for skin testing. A standard prick test for immediate wheal-and-erythema response was performed with 15 common inhalant allergens (Greer Laboratories, Lenoir, N. C.): *Fusarium solani*, *Helminthosporium* sp., *Hormodendrum hordei*, *Aspergillus* sp., Johnson grass, Bermuda grass, house dust, *Alternaria* sp., ragweed mix, pecan, oak, elm, plantain, and dog dander in 1:20 (w/v) concentration. Reactions were read at 30 min.

Eosinophil counts

Eosinophils were counted in a Fuchs-Rosenthal counting chamber by the method of Discombe¹² using a saponin-eosin stain. Levels were expressed as number of cells per cubic millimeter.

Immunoglobulin quantitation

Immunoglobulins G, A, M, and D were quantitated by single radial diffusion¹³ using Behring Diagnostics H-chain specific rabbit antihuman IgG, A, and M and Meloy H-chain specific goat antihuman IgD. IgE was quantitated by a double-antibody radioimmunoassay technique.¹⁴ Briefly, Sephadex G-25 superfine was activated with CNBr by the method of Axen and associates.¹⁵ To 200 mg of activated Sephadex, 0.1 ml of anti-IgE (Meloy, H-chain specific raised in rabbits) was added and treated as described in the original method. After final washing, the conjugate was stored in assay buffer at 4° C for immediate use or at -20° C for repeat analyses.

Anti-IgE was radioiodinated by a modification of the method of McConahey and Dixon,¹⁶ by reacting 0.06 ml of anti-IgE with 4 mCi of 125 I. To initiate the reaction, 20 μ g of chloramine T was allowed to react for 1 min at room temperature, then the reaction was stopped by addition of 100 μ g of sodium metabisulfite. The labeled antiserum was chromatographed on a 20×1.5 cm column of Sephadex G-150 to remove unconjugated iodine and the first eluant peak pooled for use.

For the RIA procedure, 100 μ g of a 1 mg/ml solution of Sephadex-anti-IgE in assay buffer was mixed with 50 μ g serum and incubated overnight at room temperature on a rotator

at 200 rpm. Conjugates were washed 3 times with assay buffer to remove unreacted serum, 100 μ l of radioiodinated anti-IgE was added, and the tubes were incubated overnight at room temperature on the rotator. After washing 3 times to remove unreacted labeled antibody, the complex was counted in a Beckman Biogamma counter for 10 minutes, and the results were used to obtain total serum IgE levels from a standard graph.

Radioimmunoassay for anti-TDI antibodies

Filter paper discs were activated by the method of Ceska and Lundkvist.¹⁷ To 500 mg of discs, 20 ml of a 20 mg/ml solution of TDI-HSA in borate buffer (pH 8.0) was added and incubated at room temperature for 6 hr on a rotator. Discs were washed 3 times in ethanolamine (1 M, pH 8.0) and left in ethanolamine at 4° C overnight. The following day discs were washed 3 times in assay buffer (500 ml 0.2 M tris buffer, pH 7.5; 500 ml 1.8% NaCl [w/v]; 10 ml 5% NaN₃; 5 ml Tween 20; 2 gm BSA).

For the test, 50 μ l serum was added to a tube containing a coupled disc, incubated overnight at room temperature on a rotator, and washed 3 times with 3 ml of physiologic saline per wash to remove unreacted serum. One hundred microliters of radiolabeled anti-IgE containing approximately 40,000 counts per minute (cpm) was added, the complex incubated overnight at room temperature on a rotator, washed 3 times in saline, and counted for 10 min on a Beckman Biogamma counter. A test:blank ratio of ≥ 2 was considered indicative of a positive result.

In vivo testing for homocytotropic and heterocytotropic anti-TDI antibodies

Sera were tested for heterocytotropic anti-TDI antibodies by the PCA technique in guinea pigs using the method of Ovary and associates,¹⁸ and for homocytotropic antibodies by the Prausnitz-Küstner technique described by Layton and associates.¹⁹ Briefly, for the PCA test, 0.1 ml of test serum was injected intradermally into the shaved back of 600 gm Hartley guinea pigs. Two hours after the intradermal injections, 1.0 ml of a 1 mg/ml TDI-HSA in 0.5% Evans blue dye solution in physiologic saline was injected intracardially and reaction sites were observed 4 hr for dye extravasation.

For the P-K test, 0.1 ml of test serum was injected intradermally into the shaved abdomen of Macaque monkeys. After 24 hr, 1.0 ml/kg of a 1 mg TDI-HSA per milliliter of 0.5% Evans blue dye solution in physiologic saline was injected intravenously and reaction sites were observed for 4 hr.

Lymphocyte transformation

TDI-HSA-induced lymphocyte transformation was determined in 5 "clinically sensitive" TDI workers at the time of provocative inhalation challenge by a previously described method.²⁰ Lymphocytes from 50 to 60 ml of heparinized blood were diluted to give 1×10^6 cells per tube and 0.1 ml of antigen as used by Avery and associates²¹ (500 μ g, 50 μ g, and 5 μ g TDI-HSA per milliliter) was added to appropriate tubes. Following incubation at 37° C in CO₂ atmosphere (72 hr for mitogen controls, 144 hr for tests), 1 μ Ci (2 Ci/mmol) of thymidine was added to each tube, and cells were incubated for 18 hr at 37° C in a CO₂ incubator prior to harvesting. A trichloroacetic acid precipitate was placed into scintillation cocktail containing toluene, 1,4 *bis* [2-(4-methyl-5 phenyloxazolyl)]-benzene, and 2,5-diphenyloxazole, and counted in a Beckman LS-230 liquid scintillation counter. Results were recorded as disintegrations per minute (dpm) per tube.

Provocative inhalation challenge

"Clinically sensitive" individuals were evaluated by TDI provocative inhalation challenge test. A worker was defined as "clinically sensitive" when he experienced clear-cut symptoms of airways obstruction each time he was in a TDI-containing area (i.e., wheezing, cough, and dyspnea). Detailed records of symptoms were recorded in each affected subject but will not be discussed in this report. The type of symptoms noted in these workers, together with the results of environmental monitoring, has been reported in preliminary form elsewhere. In ad-

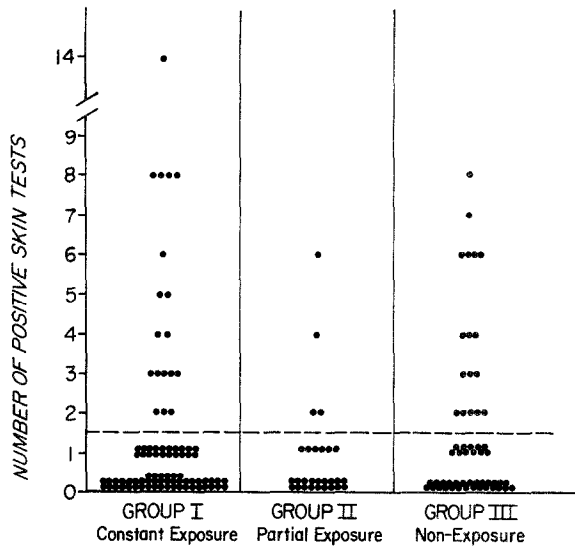


FIG. 1. Incidence of positive wheal-and-flare skin reactivity (prick test) of TDI-exposed and nonexposed workers to common inhalant allergens.

dition to cough, wheezing, and dyspnea, affected individuals at times presented with TDI-associated symptoms such as "chest tightness," substernal "chest constriction," "burning eyes," "phlegm production," vague "chest pains," rhinorrhea, generalized malaise, "dizziness," and "choking."²² The challenge procedure consisted of placing the individual for 15 min in a closed environment containing no TDI, or levels of TDI vapor from as low as 0.005 up to the threshold limit value (TLV) of 0.02 ppm (monitored by a model 700 TDI monitor [Universal Environmental Instruments, UK] and confirmed by the Marcali method). To obtain these levels, a large beaker containing TDI was placed in the room on a magnetic stirrer. Vapor emission was controlled by a glass plate over the beaker to give the appropriate TDI concentration. Pulmonary function studies were performed prior to and following exposure to TDI, at 15-min intervals during the first 2 hr, at 30-min intervals during the next hour, and hourly thereafter until 6 hr after challenge or, in the event of a positive response, until baseline, pre-exposure measurements were obtained. Further confirmatory lung function tests were performed at 24 hr after challenge to ensure that subjects had returned to pre-exposure baseline measurements. Pulmonary function tests, performed with a Stead-Wells spirometer, were forced vital capacity (FVC), forced expiratory volume in one second (FEV₁), and forced expiratory flow, 25% to 75% (FEF₂₅₋₇₅).

Immunologic testing during challenge consisted of intradermal skin testing with TDI-HSA, and collection of blood samples prior to challenge and at ½, 1, 2, 4, and 6 hr after exposure for total eosinophil counts and detection of TDI specific antibody.

RESULTS

Skin testing with common inhalant allergens

Fig. 1 shows the number of workers in each group who demonstrated positive wheal-and-erythema reactions on prick testing with common inhalant allergens. Since a negative history of allergy was supposedly a prerequisite for hiring of employees to work in the TDI area it was felt that history of atopy would be unreliable. If one accepts two or more clearly positive prick tests as being a likely indication of atopy, it can be seen that 21% of Group I, 14% of Group II, and

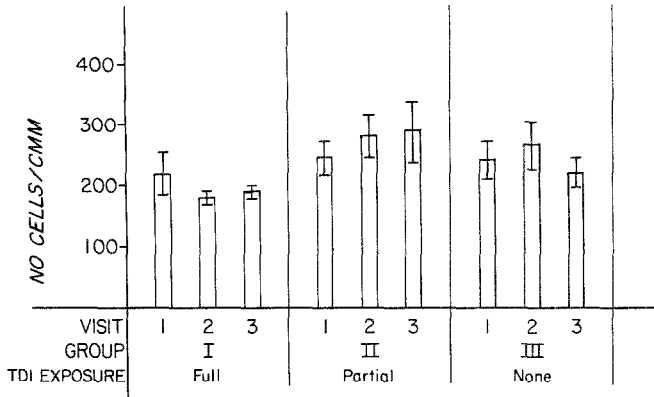


FIG. 2. Mean total eosinophil counts (\pm standard error) of TDI-exposed and nonexposed workers before (Visit 1) and at 6-month intervals (Visits 2 and 3) following commencement of TDI production.

31% of Group III subjects were atopic individuals. In Groups I, II, and III 14%, 7%, and 9%, respectively, had a past or present history of asthma or hay fever.

Skin testing with TDI-HSA antigen

On initial testing, positive immediate wheal-and-flare reactions to TDI-HSA conjugate were observed in 4 persons; however, testing with HSA alone gave similar reactions to those obtained with TDI-HSA conjugate in each case, indicating lack of TDI specificity. On the third plant visit, 3 subjects demonstrated positive wheal-and-erythema reactions to TDI-HSA but failed to react to HSA alone. In all 3 cases, the intradermal TDI-HSA test elicited definitely positive but small wheal-and-flare skin reactions between 7 and 10 mm edema.

Eosinophil counts

There were no marked differences in the mean total eosinophil counts between groups, and no significant change in counts was seen at the second and third visits following TDI exposure (Fig. 2).

Immunoglobulin quantitations

Results of immunoglobulin quantitations in the three subject groups are illustrated in Fig. 3. Pre-exposure samples showed a similar distribution with no significant intergroup differences in mean values. Postexposure samples showed a significant increase in IgG levels in all 3 groups indicating that this increase was not TDI-mediated. In the case of IgE levels, postexposure values were all increased, particularly in the nonexposed group, indicating that increased levels were not TDI-related but rather were possibly a reflection of seasonal variations.

PCA and P-K tests

PCA testing in guinea pigs and P-K testing in monkeys for TDI-specific antibody yielded uniformly negative results with all samples. The validity of these

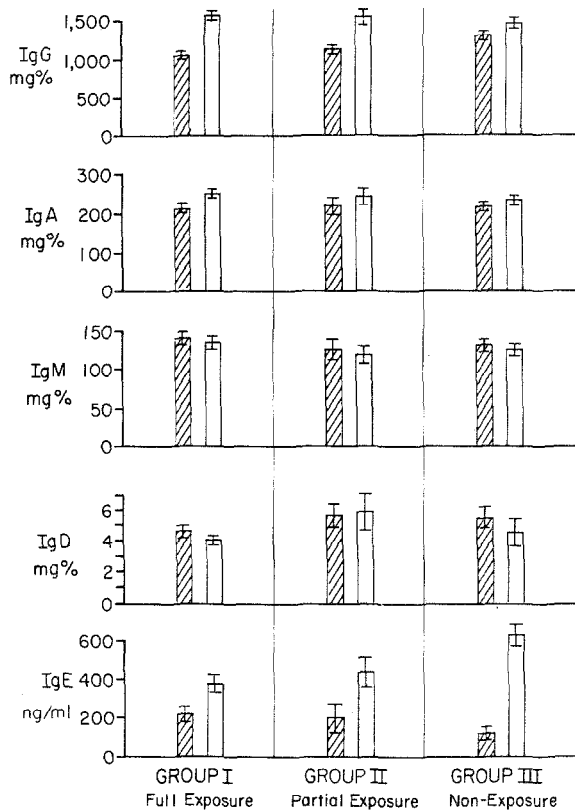


FIG. 3. Mean immunoglobulin levels (\pm standard error) of TDI-exposed and nonexposed workers before (cross-hatched) and at 6 months following (open bars) commencement of TDI production.

tests is not confirmed, however, due to inability to obtain known positive control human sera for these tests.

Radioimmunoassay for TDI-specific anti-IgE antibodies

RIA's for anti-TDI antibodies were also uniformly negative, yielding test: blank ratios of less than 2 (Fig. 4). To confirm that the test was valid, control sera from New Zealand white rabbits immunized with TDI-HSA in complete Freund's adjuvant (CFA) (1.5 ml of 3.3 mg TDI-HSA in 1 ml saline + 2 ml CFA) were used. For immunization 1.5 ml of the emulsified TDI-HSA in CFA was injected subcutaneously at weekly intervals for 12 wk. Animals were bled 1 mo following the last injection. A linear relationship was demonstrated with cpm when increasing amounts of serum were used. Absorption studies were performed with TDI-HSA and HSA alone (which had been processed by the conjugation procedure of Scheel and associates¹¹), to confirm specificity for TDI (Fig. 5). For absorption with HSA or TDI-HSA, 1 ml of serum was incubated at room temperature for 3 hr with 50 mg of either HSA or TDI-HSA. Following centrifugation to remove

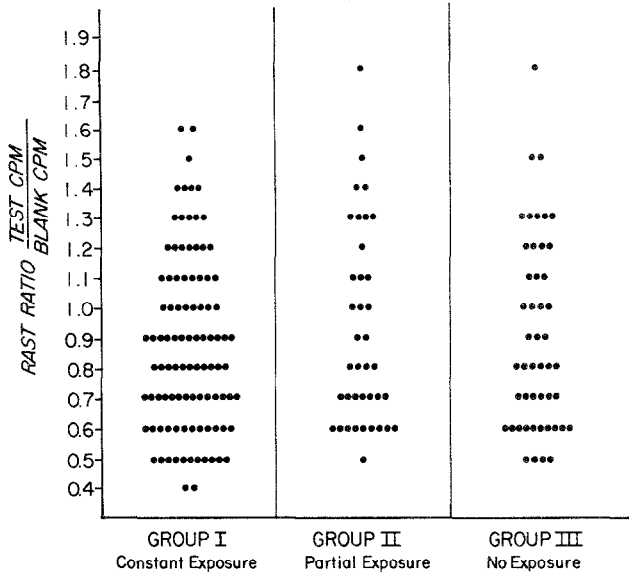


FIG. 4. RAST: serum test:blank ratios of TDI-exposed and nonexposed workers to TDI-HSA antigen 12 months after TDI exposure.

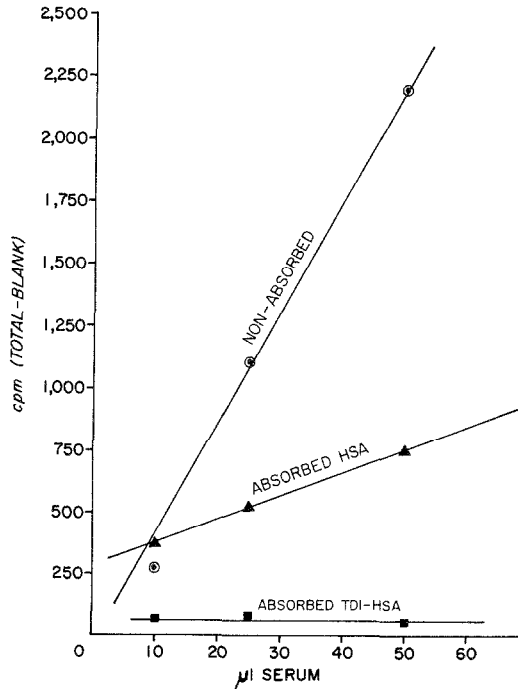


FIG. 5. Total cpm obtained with TDI-HSA-immunized rabbits serum: nonabsorbed, HSA absorbed, and TDI-HSA absorbed.

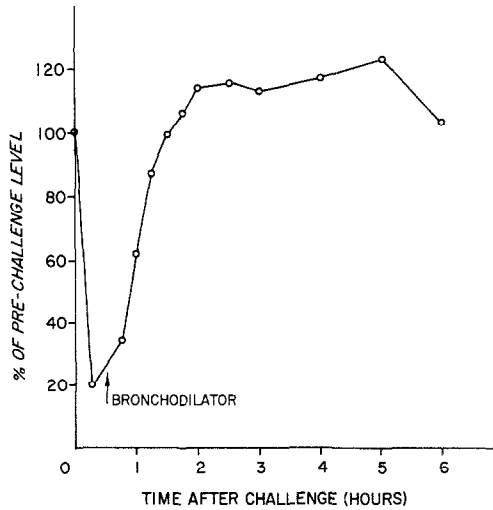


FIG. 6. Inhalation challenge: immediate response to 0.005 ppm TDI observed by FEF₍₂₅₋₇₅₎.

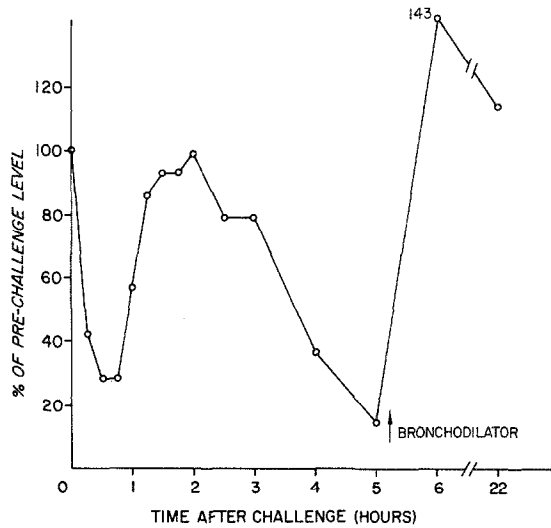


FIG. 7. Inhalation challenge: dual response to 0.01 ppm TDI observed by FEF₍₂₅₋₇₅₎.

precipitate, the supernatant was incubated overnight at 4° C with 50 mg of appropriate absorbent. After centrifugation, supernatant was incubated for 6 hr at room temperature with a further 50 mg of antigen, centrifuged, and the supernatant used in the RIA test.

Provocative inhalation challenge

Eleven of 14 workers who developed "clinical sensitivity" and three nonsensitive individuals were evaluated by provocative inhalation challenge. None of the control subjects exhibited demonstrable airways obstruction. Five showed a sig-

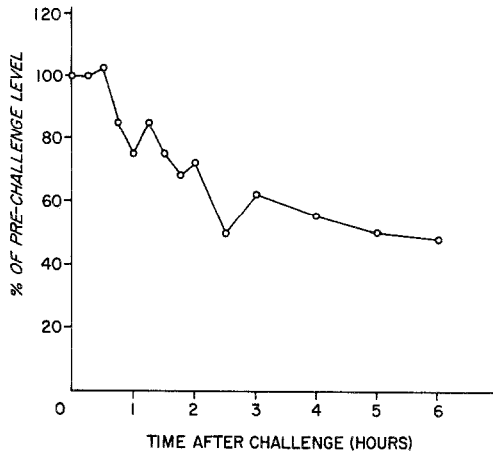


FIG. 8. Inhalation challenge: delayed response to 0.02 ppm TDI observed by $FEF_{(25-75)}$.

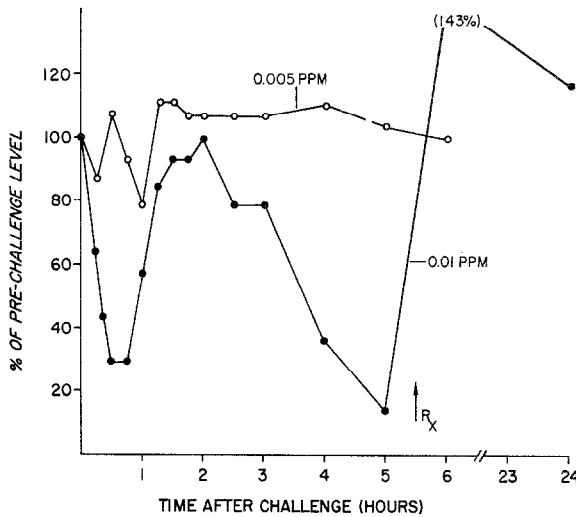


FIG. 9. Inhalation challenge: dosage effect to challenge with 0.005 ppm TDI (open circles) and 0.01 ppm TDI (closed circles) observed by $FEF_{(25-75)}$.

nificant decrease in FEF_{25-75} immediately following challenge as illustrated for Subject R. L. in Fig. 6. Of these 5, two showed dual immediate and late (5 to 6 hr) responses as illustrated for Subject P. B. in Fig. 7. Two other individuals developed a sustained decrease in FEF_{25-75} commencing within 1 hr after exposure and persisting for at least 6 hr, as illustrated for Subject N. L. in Fig. 8. These responses to challenge were shown to be dose-related as noted in Fig. 9, which shows the lack of response of an individual to TDI vapor in concentrations of 0.005 ppm but a dual response to 0.01 ppm as measured by FEF_{25-75} . Total eosinophil counts and immunoglobulin levels showed no change from baseline, pre-exposure levels. There was no pattern of atopy, hay fever, or asthma in the individ-

TABLE I. Lymphocyte transformation on 5 "clinically sensitive" individuals

	dpm at TDI-HSA concentration (mg/ml)*			dpm at HSA concentration (mg/ml)*			dpm with phytohemagglutinin (PHA) (dilution)*		Medium control
	0.5	0.05	0.005	0.5	0.05	0.005	1:10	1:100	
1	3,191	3,219	2,251	3,215	1,889	1,780	214,400	155,128	1,271
2	2,870	2,552	2,514	2,117	3,387	3,208	155,744	24,068	2,664
3	35,177	31,298	31,252	23,421	31,365	25,603	488,467	44,771	18,281
4	15,871	14,317	14,169	15,871	14,709	12,746	343,575	149,230	12,772
5	5,603	3,630	4,172	3,904	2,828	3,347	264,400	184,804	3,858

*Mean of 3 tubes.

uals showing "clinical sensitivity" or reacting to bronchial inhalation challenge.

Lymphocyte transformation

Results of attempted TDI-HSA-induced lymphocyte stimulation in 5 highly "clinically sensitive" TDI workers are illustrated in Table I. It is apparent that TDI-HSA failed to induce significant lymphocyte ³H-TdR incorporation in all individuals studied. These data fail to confirm the positive TDI-induced lymphocyte transformation tests obtained by Avery and associates.²¹ Although our coupling procedure was identical to that used by Avery and colleagues, we have been unable to determine the exact amount of TDI coupled to HSA and cannot, therefore, rule out differences in TDI concentration as a cause for these discrepancies.

DISCUSSION

Our results indicate that, in the 167 TDI plant workers studied to date, with the exception of 3 subjects who demonstrated positive wheal-and-erythema reactions to TDI-HSA, there are no detectable immunologic changes attributable to exposure to TDI vapor. Provocative challenge of some "clinically sensitive" subjects with TDI vapor for 15 min was, however, capable of initiating objective and subjective evidence of airways obstruction as evidenced by wheezing cough and decrease in FEF₂₅₋₇₅. The reactions resembled those seen when individuals sensitive to other respiratory allergens are challenged via the respiratory route; namely, immediate bronchoconstriction commencing within 15 min of exposure to TDI, a sustained late reaction, beginning within the first hour after exposure and increasing with time after challenge, and dual immediate and delayed reaction. In some cases these reactions could be elicited by as low a level as 0.005 ppm TDI and in others the pulmonary response to TDI vapor challenge also appeared to be dose-related. No correlations between atopic status or IgE levels and development of clinical sensitivity were demonstrable.

Our provocative challenge results confirm the observations of Pepys and associates,¹⁰ who were able to demonstrate immediate, delayed, and dual reactions using occupational type exposure to polyurethane-coated wire. Our challenge technique has the advantage that precise control of TDI levels, equal to or below the TLV, is possible. If our failure to detect TDI-specific antibody represents a valid observation, current data indicate a clear lack of relationship between positive dual bronchial challenge responses and either skin-sensitizing or precipitating antibody in TDI asthma.

Our ability to elicit positive wheal-and-erythema responses to TDI-HSA conjugates in several plant workers by direct skin test has not been previously reported. These reactions, although TDI-specific, were always small and unassociated with demonstrable TDI-specific antibody by P-K, PCA, or RIA. Their lack of correlation with clinical TDI "sensitivity" provides further evidence that TDI-induced bronchospasm is not likely IgE-mediated. Quantitation of IgE and other immunoglobulins in TDI workers before and after occupational exposure to date has also not been helpful in determining the pathogenesis of TDI-induced bronchospasm, since serum levels of three immunoglobulin classes (A, M, and D) were unchanged and postexposure IgG and IgE levels increased in the exposed and nonexposed groups. The slight rise in postexposure IgG levels is unexplained; the rise in IgE levels in all subjects might, however, be explained on the basis of known seasonal and postseasonal rises in IgE since the second and third plant visits were made during the Fall months either at the peak or immediately following the local ragweed pollination season.

The uniform lack of positive PCA and P-K tests in our current study is in keeping with previous findings of Avery²¹ and Bruchner⁵ and their associates.⁵ Other investigators have, however, reported a high incidence of positive PCA and P-K tests in exposed individuals^{23, 24} Our negative results cannot be validated since we were unable to obtain a P-K or PCA positive control serum from any of our clinically sensitive subjects or from other investigators. It is unlikely that our antigen was at fault, however, since TDI conjugation was confirmed by ultraviolet spectroscopy at 245 m μ as was the conjugate prepared by Scheel and associates,¹¹ Avery and colleagues,²¹ however, although using this same procedure, measured maximum absorbance of their conjugate at 255 m μ . The antigen was also capable of producing wheal-and-flare skin reactivity in several exposed workers, whereas HSA alone treated by the same conjugation procedure, did not, and it elicited positive RIA in TDI-HSA immunized rabbits which could be partially absorbed by HSA treated by the conjugation procedure and fully absorbed by TDI-HSA. A likely explanation for these discrepant results in P-K and PCA testing by various groups might be based on slight differences in antigen preparation or possibly in the degree of sensitivity of the study populations.

If our positive TDI inhalation challenge tests were indeed IgE-mediated, our current results might be explained by postulating that only small amounts of IgE antibody, locally produced in the respiratory tract in quantities insufficient to be demonstrated by radioimmunoassay or monkey P-K test, were involved. Against this hypothesis is the absence of wheal-and-flare skin reactivity in several highly "clinically sensitive" subjects who exhibited positive bronchial challenge tests.

Our studies to date are unable to explain the pathogenesis of TDI-induced pulmonary disease on an immunologic basis. Several other possibilities of TDI activity remain unexplored, however, including its effect on leukocyte histamine release (or other mediators of immediate hypersensitivity) and on intracellular cyclic AMP and GMP levels. Host factors such as abnormal airway response to mecholyl and histamine in TDI-"sensitive" workers might also be important determining factors in TDI-induced bronchospasms and are worthy of study.

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