

Biophysical determinants of toluene diisocyanate antigenicity associated with exposure and asthma

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Background: Toluene diisocyanate (TDI), a widely used aromatic diisocyanate with the potential to cause asthma, reacts with albumin in the airway fluid, which acts as a carrier protein for chemical presentation to the immune system. Structural elucidation of TDI-albumin conjugates is crucial to understanding the human immune response to TDI exposure. **Objective:** Investigate the dependence of TDI's antigenicity on the biophysics of exposure and its association with TDI asthma. **Methods:** Toluene diisocyanate-albumin conjugates were generated by exposing albumin to TDI in liquid or vapor phase (liquid or vapor TDI-albumin, respectively). Conjugates were characterized by native gel electrophoresis and matrix-assisted laser desorption/ionization-mass spectrometry, and used as antigens in ELISA assays for serum specific-IgE and IgG. **Results:** The physical phase of TDI (vapor vs liquid) affects the formation of TDI-albumin conjugates, with measurable differences in the amount of TDI per albumin molecule, migration in native gels, matrix-assisted laser desorption/ionization-mass spectrometry mass/charge spectra, and antigenicity. Vapor TDI-albumin conjugates were recognized by IgE from 44% of subjects with TDI asthma, whereas liquid TDI-albumin conjugates are recognized by IgE from only 17% of these patients. A significant ($P < .05$) association between TDI exposure and vapor TDI-albumin specific serum IgG was also observed. **Conclusion:** Biophysics of TDI exposure substantially affects formation of TDI-albumin conjugates recognized by the immune system in association with exposure and asthma. **Clinical implications:** The data suggest that serology may help identify TDI asthmatics and exposed workers if the appropriate form of TDI is used as the antigenic basis for analysis. (J Allergy Clin Immunol 2006;118:885-91.)

Key words: Occupational diseases, IgE, IgG, occupational exposures, asthma, MALDI-MS

Toluene diisocyanate (TDI), a highly reactive, widely used, low-molecular-weight chemical, may cause asthma in 5% to 15% of exposed workers.^{1,2} Considerable controversy exists regarding the pathogenesis of TDI-induced asthma, and the mechanisms by which the disease develops remain unclear.³⁻¹⁰ A leading hypothesis is that the chemical acts as a hapten and undergoes nucleophilic addition reactions (conjugates) *in vivo* with airway proteins.^{8,11} Strong evidence exists that albumin is the major carrier,^{12,13} and that reaction products between isocyanate and albumin include "neo-epitopes," a wide range of which may be possible depending upon the microenvironment.^{9,14} The structure of isocyanate-albumin conjugates that form *in vivo* in human beings, and which might elicit a pathogenic immune response, remains unknown and is critical to defining the primary events in the development of isocyanate asthma. In addition, biologically relevant forms of isocyanate-albumin conjugates may form the basis of much-needed diagnostic assays for isocyanate exposure and asthma.

We have recently reported that the biophysics of exposure has a strong influence on the formation of albumin conjugates with the aliphatic diisocyanate, hexamethylene diisocyanate (HDI), which has a high vapor pressure.¹⁵ Under mixed phase (vapor/liquid) conditions designed to mimic the airway microenvironment, vapor HDI exhibits limited and highly specific reactivity compared with liquid phase HDI, and appears to produce conjugates that better reflect those that occur *in vivo*. Among subjects with HDI asthma, the prevalence of serum IgE that recognized vapor HDI-albumin conjugates was greater than serum IgE that recognized liquid HDI albumin conjugates. In addition, the presence of vapor HDI-albumin specific IgG correlated much stronger with occupational exposure than the presence of IgG that recognized liquid HDI-albumin conjugates.

Aromatic isocyanates such as TDI and methane diphenyl diisocyanate differ from aliphatic isocyanates such as HDI in their hydrocarbon backbone, which influences their reactivity and may impose steric restrictions. The effect of exposure biophysics on the formation of albumin conjugates with aromatic isocyanates has not been well defined, and it remains unclear whether different

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TABLE I. Demographic data on the 4 study groups†

| | Subjects with TDI asthma (N = 66) | TDI-exposed control subjects (N = 167) | Subjects with allergic asthma (N = 64) | Unexposed control subjects (N = 113) |
|-----------------------|---|--|--|--|
| Age (y) | 42.8 ± 10.3 | 41.0 ± 8.4 | 31.0 ± 11.7 | 37.2 ± 11.2 |
| Sex (M/F) | 46/20 | 123/44 | 34/30 | 70/43 |
| Exposure duration (y) | 7.43 ± 4.3* | 11.53 ± 7.7* | NA | NA |

F, Female; M, male; NA, not applicable.

* $P < .05$, significant differences in exposure duration between subjects with TDI asthma and TDI-exposed control subjects.

†Values for age and exposure duration are presented as means ± SD.

Abbreviations used

HDI: Hexamethylene diisocyanate
MALDI-MS: Matrix-assisted laser desorption/
ionization-mass spectrometry
TDI: Toluene diisocyanate

isocyanates preferentially bind to different loci or amino acids on albumin, which might effect the immunogenicity of the resulting conjugate. The physical phase (vapor vs liquid) of the isocyanate used in preparing conjugates for immunologic testing is likely to be most critical for those isocyanates with high vapor pressures (eg, TDI and HDI), and where workers airways are exposed predominately to vapors, not liquid phases of the chemical. To date, the structure and antigenicity of TDI-albumin conjugates produced under vapor exposure conditions has not been investigated.

Another factor that may influence the antigenicity of TDI-albumin conjugates is the isomer ratio of 2,4/2,6-TDI. Although most industrial processes start with an 80/20 ratio of 2,4/2,6-TDI isomers, the mixture that reaches the airways may contain relatively higher levels of 2,6-TDI.¹⁶ The effect of the TDI isomer ratio on the formation of TDI-albumin conjugates and their antigenicity remains unclear.

In this study, biophysical analyses and experimental serology studies are undertaken to characterize and compare the antigenicity of an aromatic isocyanate (TDI) when conjugated to albumin by different exposure methods (vapor vs liquid). The influence of the TDI isomer ratio on antigenicity was also evaluated. The results are discussed in the context of TDI asthma pathogenesis and diagnosis.

METHODS

Human subjects

Blood was obtained from 66 patients with TDI-induced asthma, confirmed by positive responses to TDI bronchoprovocation tests, and 167 TDI-exposed asymptomatic workers age 21 to 61 years from the same working environments, including spray-painting and polishing departments of the furniture and musical instrument industries. Another 64 patients with atopic allergic asthma and no known TDI exposure and 113 unexposed healthy control subjects age 18 to 63 years were recruited from outpatients of Ajou University Hospital. The demographic data of the 4 study groups are summarized in

Table I. Atopy was determined by a positive skin test result to at least 1 common inhalant allergen, including house dust mite, tree and pollen mixtures, mugwort, ragweed pollens, and *Alternaria* (Bencard, Bredford, United Kingdom). Sera from subjects with TDI asthma and subjects with allergic asthma were collected at initial examination; all subjects stopped inhaled or oral steroids for 4 weeks before the study and underwent an interview, chest radiography, and skin prick test with common inhalant allergens. All subjects with asthma underwent lung function measurement and inhalation challenge with methacholine. TDI bronchial challenge test was performed according to the protocol described in previous studies.⁵ All subjects gave their informed consent, which was regulated by the Institutional Review Board of Ajou Medical Center, Suwon, Korea.

Preparation of liquid TDI-albumin conjugates

Liquid TDI-albumin conjugates were prepared as previously described, using a modification of the Scheel method.¹⁷ Low endotoxin human albumin (Sigma, St Louis, Mo) in PBS pH 7.2 at a concentration of 5 g/L (73 $\mu\text{mol/L}$) was mixed with liquid TDI (Sigma) dropwise until a final concentration of 1 g/L TDI (5.7 mmol/L) was achieved (roughly a 100-fold molar excess of TDI to albumin). The reaction was continued for 2 hours at room temperature, 0.2- μm filtered, dialyzed against PBS, and refiltered. Liquid TDI-albumin conjugates were prepared with an 80/20 and a 98/2 mixture of 2,4/2,6-TDI.

Preparation of vapor TDI-albumin conjugates

Vapor TDI-albumin conjugates were prepared by using our recently described isocyanate vapor phase exposure system.¹⁵ In brief, TDI vapor concentrations in the range of 0.14 to 1.4 mg/m^3 (~ 1 -10 $\mu\text{moles/m}^3$) were passively generated in a closed circuit system and monitored with an Autostep monitor (GMD, Pittsburgh, Pa). Low endotoxin human albumin in PBS pH 7.2 at a concentration of 5 g/L (73 $\mu\text{mol/L}$) was exposed in open 60-mm Petri dishes (Becton Dickinson, Franklin Lakes, NJ) overnight. The exposure unit was sterilized with 70% ethanol, and protein solutions were 0.2- μm filtered before and after exposure to ensure sterility. Vapor TDI-albumin conjugates were prepared with an 80/20 and a 98/2 mixture of 2,4/2,6-TDI.

Native gel analysis

Proteins were mixed with 10% glycerol in gel running buffer and then electrophoresed on 10% native polyacrylamide gels, pH 8.3, as previously described¹⁸ and stained with GelCode Blue from Pierce (Rockford, Ill).

Matrix-assisted laser desorption/ionization-mass spectrometry

Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) of acrylamide gel purified samples was performed by the Keck Center of the Yale University School of Medicine (<http://keck.med.yale.edu/prochem/maldi.htm>) as previously described.¹⁹ To

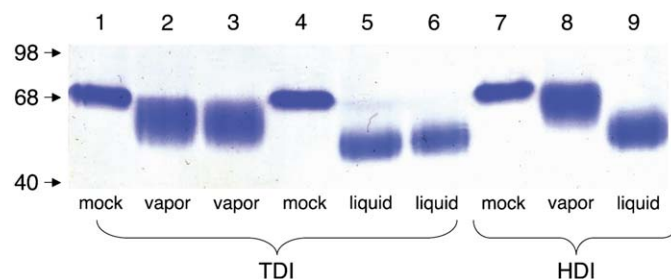


FIG 1. Native gel analysis of TDI-albumin conjugates prepared by vapor or liquid phase TDI exposure. Albumin exposed to air (mock), vapor, or liquid phase isocyanate was electrophoresed under native conditions. Lanes 2 and 5 used an 80/20 ratio of 2,4/2,6-TDI, whereas lanes 3 and 6 used a 98/2 ratio. For comparison, exposure with HDI is also shown as labeled.

generate the mass/charge spectra, a Fisons VG Tofspec SE instrument (Manchester, United Kingdom) was loaded with 500 fmol of trypsin digested sample along with 50 fmol bradykinin and 150 fmol of an adrenocorticotropic hormone peptide for internal calibration.

Specific IgG and IgE antibody to TDI-albumin conjugate by ELISA

Serum-specific IgE and IgG levels were detected by ELISA as described previously.⁵ In brief, TDI-albumin conjugate or mock conjugate was dissolved in normal saline and used to coat ELISA plates (Corning, New York, NY) with 1 μ g/well at 37°C for 2 hours followed by 4°C overnight. After washing with PBS-Tween 20, plates were blocked with 350 μ L blocking buffer (PBS containing 5% BSA and 0.05% Tween 20). Diluted serum (50 μ L) from patient or control subjects at a concentration of 1:50 for specific IgG and 1:2 for specific IgE in the preliminary experiments was incubated for 1 hour at 25°C in both TDI-albumin and mock albumin-coated wells and washed 4 times. Alkaline phosphatase-conjugated anti-IgG (1:2000, 100 μ L, Sigma) or biotinylated anti-IgE antibody (1:1000, Kirkegaard & Perry Laboratories, Gaithersburg, Md), then alkaline phosphatase-conjugated streptavidin (1:500, Sigma), were added into each well, incubated for 1 hour at room temperature, and washed, and 100 μ L substrate solution (Sigma) was added. After a 15-minute incubation at room temperature, absorbance values were read by using an ELISA reader at 405 nm and referenced to 450 nm. To quantify the antibody binding specific to TDI, the OD value of the mock conjugated albumin wells was subtracted from that of the TDI-albumin coated wells. Positive cutoff values (0.18 for IgG, 0.13 for IgE) were based on the mean + 2-fold SD of the OD values from tests with 80 unexposed healthy controls. Specificity for TDI-albumin in positive samples was confirmed by >50% reduction in OD in an inhibition ELISA with autologous TDI-albumin conjugate, and lack of inhibition (<10%) with control (mock) albumin.

Statistical analysis

The statistical analyses were performed by using SAS (SAS Institute, Cary, NC). Data not normally distributed were log-transformed. Cross-tab analysis was applied to compare the prevalence of specific IgG and IgE to 2 kinds of TDI-albumin conjugates. ANOVA test was applied to compare demographic data among the 4 study groups. *P* value of .05 or less was regarded as significant.

RESULTS

Demographics of the study population

Table I shows demographic data on the 66 patients with TDI-induced asthma and 3 control groups, 167 asymptomatic

TDI-exposed subjects, 64 patients with allergic asthma, and 113 unexposed healthy controls. There were no significant differences in mean age and sex proportion between the groups; however, exposure duration was significantly longer among asymptomatic exposed workers compared with subjects with TDI asthma.

Vapor TDI-albumin conjugates

Toluene diisocyanate-albumin conjugates were generated by exposing an albumin solution to TDI vapors in a closed circuit system, identical to that recently developed for HDI.¹⁵ Two mixtures of TDI isomers were used, one with an 80/20 and a second with a 98/2 ratio 2,4/2,6-TDI. Biophysical/biochemical changes in TDI vapor-exposed human albumin were evidenced by a change in migration during native gel electrophoresis (Fig 1). The migration of TDI vapor-exposed albumin differs from liquid exposed albumin and is likely related, at least in part, to the degree of albumin conjugation with TDI.¹⁵ Vapor TDI-albumin conjugates contain approximately 12 mol TDI/albumin molecule, whereas liquid TDI-albumin conjugates contain 40 mol TDI/albumin, on the basis of chemical substitution analysis and TDI's UV light absorbance at 245 nm (not shown).

To characterize better the TDI-albumin conjugates, we performed MALDI-MS on tryptic digests of albumin exposed to air (mock), vapor, and liquid TDI. TDI exposure reproducibly caused prominent changes the mass/charge spectra of albumin, the most notable being a new signal at 1323 observed in both vapor and liquid TDI exposed albumin (Fig 2). A new signal with a mass/charge ratio of 1137 was also observed in both vapor and liquid TDI-exposed albumin. The amounts of these novel components appeared to be relatively higher after liquid vs vapor exposure.

Qualitative differences in MALDI-MS mass/charge spectra that differentiate vapor from liquid TDI exposed albumin were also identified. New peaks in the mass/charge spectra at 1167 and 1203 were observed in vapor but not liquid TDI-exposed albumin, whereas new peaks at 709 and 1301 resulted exclusively after liquid TDI exposure. Additional changes unique to liquid TDI exposed albumin include an apparent loss of the 876 (amino acids 98-105, LCTVATLR), and unidentified 705 and 715 peaks (Fig 2).

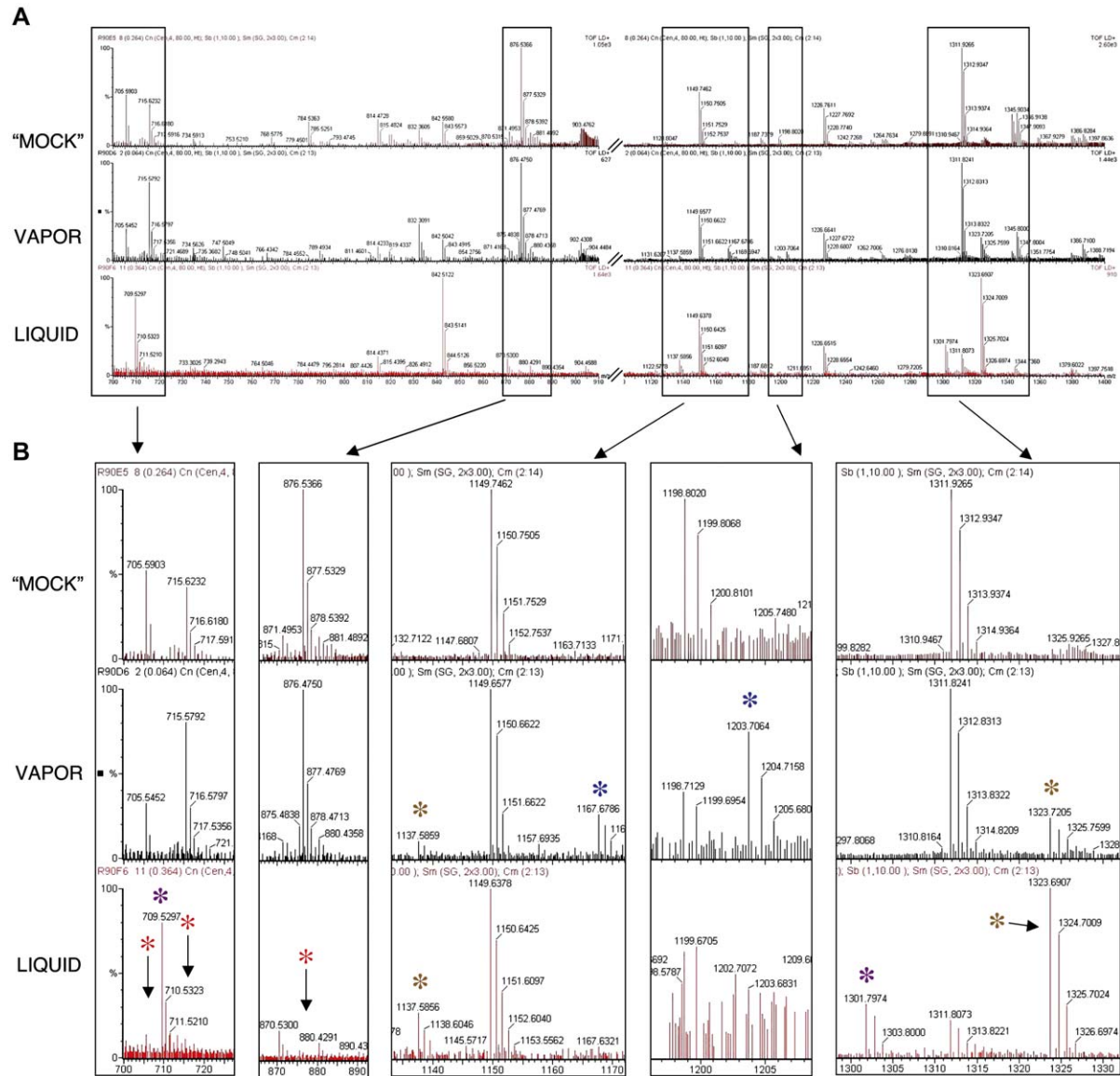


FIG 2. MALDI-MS analysis of TDI-albumin conjugates. **A**, The mass/charge spectra of mock, vapor, and liquid-exposed albumin are compared. **B**, Enlargement of regions that define consistent differences. *Blue*, Peaks unique to vapor exposed; *purple*, peaks unique to liquid exposed; *brown*, peaks found in vapor and liquid exposed; *red*, peaks missing from liquid exposed. Data are representative of 6 separate experiments.

TABLE II. Prevalence of serum specific IgE and IgG to vapor and liquid TDI-albumin conjugates among patients with TDI asthma (N = 66)†

| | Vapor | | Liquid | |
|--------------|------------|-----------|-----------|-----------|
| | 80/20 | 98/2 | 80/20 | 98/2 |
| Specific IgE | 29 (43.9)* | 9 (13.6) | 11 (16.7) | 6 (9.1) |
| Specific IgG | 20 (30.3)* | 12 (18.2) | 16 (24.2) | 13 (19.7) |

**P* < .001 compared with all other conjugates.

†Values shown represent absolute number of subjects with a positive ELISA test, whereas values in parentheses represent the percentage of the total (N = 66) tested, using TDI-albumin conjugates prepared with the isomeric ratio of TDI shown (ie, 80/20 or 98/2 isomer mixture of 2,4/2,6-TDI).

Serology assays using vapor TDI-albumin and liquid TDI-albumin conjugates

To compare the antigenicity of TDI-albumin conjugates prepared by different methods, we performed ELISA assays on the sera from 66 TDI asthmatics using either vapor-TDI albumin conjugates or liquid-TDI albumin conjugates as the antigen. As shown in Table II, significantly (*P* < .001) more subjects with TDI asthma had IgE that bound vapor TDI-albumin conjugates than liquid TDI-albumin conjugates. When TDI-albumin conjugates are prepared with an 80/20 mixture of 2,4/2,6-TDI, 44% of the TDI asthmatics had IgE that bound vapor TDI-albumin, whereas only 17% had IgE that bound liquid

TABLE III. Prevalence of serum specific IgE and IgG antibodies to vapor TDI-albumin conjugates in subjects with TDI asthma and control subjects†

| | Subjects with TDI asthma (N = 66) | TDI-exposed control subjects (N = 167) | Subjects with allergic asthma (N = 64) | Unexposed control subjects (N = 113) |
|--------------|---|--|--|--|
| Specific IgE | 29 (43.9)* | 7 (4.2) | 0 (0) | 1 (0.9) |
| Specific IgG | 20 (30.3)* | 16 (9.6)** | 1 (1.6) | 2 (1.8) |

* $P < .001$ comparing subjects with TDI asthma with any of the 3 control groups.

** $P < .05$ comparing asymptomatic TDI-exposed control subjects with the other 2 control groups.

†Values shown represent number of subjects with a positive ELISA test, whereas the values in parentheses are the percentage of subjects tested.

The vapor TDI-albumin conjugates used as test antigens were prepared with an 80/20 isomer mixture of 2,4/2,6-TDI.

TDI-albumin. In terms of serum IgG, vapor TDI-albumin conjugates were recognized by a slightly higher percentage of subjects than were liquid TDI-albumin conjugates, 30.3% vs 24.2%, but the differences were not as dramatic as those for IgE binding. Regardless of the method of conjugate production (vapor or liquid TDI exposure), the 80/20 isomeric mixture of 2,4/2,6-TDI yielded conjugates recognized by IgE and IgG from more subjects with TDI asthma than the conjugates made with the 98/2 mixture of TDI isomers. The presence of specific IgG or IgE was not associated with type of TDI asthmatic reaction (early, late, dual phase), a finding also reported by other investigators,²⁰⁻²³ which may suggest variable time-dependent mechanisms in response to exposure.

The prevalence of IgE, specific for vapor TDI-albumin conjugates, was determined in 3 control groups of human subjects: 167 asymptomatic exposed workers, 64 patients with allergic asthma, and 113 unexposed healthy individuals. For these studies, we focused on vapor TDI-albumin conjugates prepared with an 80/20 ratio of 2,4/2,6-TDI. As shown in Table III, serum IgE that specifically binds vapor TDI-albumin conjugates is present in a significantly ($P < .001$) higher proportion of subjects with TDI asthma than subjects in the other control groups (43.9% vs 4.2%, 0%, and 0.9%, as shown in Table III). There was no significant difference in the specific IgE levels to the vapor TDI-albumin conjugate between the 3 control groups ($P > .05$).

IgG specific for vapor TDI-albumin conjugates was also significantly more common among TDI asthmatics than among the 3 control groups ($P < .001$ for all), as shown in Table III. The prevalence of IgG that bound vapor TDI-albumin was more than 3 times greater among subjects with TDI asthma than asymptomatic TDI-exposed workers. However, the prevalence of vapor TDI-albumin specific IgG among asymptomatic TDI exposed workers (9.6%) was significantly ($P < .05$) increased compared with the other 2 control groups, which had prevalence rates of 1.6% and 1.8%, respectively. The potential significance of these data is further addressed in the discussion.

Lack of association between vapor TDI-albumin specific antibodies and clinical parameters

The prevalence of TDI-specific IgE and IgG in patients with TDI-induced asthma, as detected by using vapor

TDI-albumin conjugates, was not significantly ($P > .05$) associated with sex, atopy, and smoking status (data not shown), consistent with previous studies.³

DISCUSSION

We report for the first time the generation and characterization of TDI-albumin conjugates using vapor TDI exposure methods designed to better mimic the biophysics of exposure *in vivo* than conventional liquid phase methods used in studies to date. Albumin exposed to TDI vapors (vs liquid) undergoes limited conjugation that can be defined by MALDI-MS. Two changes in the mass/charge spectra (at 1167 and 1203) are unique to vapor TDI exposure, whereas 2 other changes (1323 and 1137) are found at reduced levels compared with liquid TDI exposed albumin. Other differences between vapor and liquid TDI-exposed albumin include migration in native gels, and consistent loss of MALDI-MS peaks (which correspond to known regions of albumin) in liquid-exposed samples. These biophysical differences between vapor and liquid TDI-exposed albumin conjugates are associated with their antigenicity as defined by serology. Most importantly, a substantially higher number of subjects with TDI asthma have serum IgE that recognize vapor TDI-albumin conjugates vs liquid TDI-albumin conjugates (44% vs 17%). In addition, vapor TDI-albumin specific IgG was significantly associated with TDI exposure. Together, the data provide important new insights into the antigenicity of TDI and its dependence on the biophysics of reactivity with albumin.

The reaction of aromatic isocyanates such as TDI, in vapor phase, with human albumin in liquid phase and the potential changes in antigenicity have not been well defined previously. However, we have recently demonstrated that the aliphatic isocyanate, HDI, with a similarly high vapor pressure as TDI, exhibits limited conjugation in vapor (vs liquid) phase,¹⁵ with specific amino acids of albumin, including His²⁴⁷ and Lys.⁴¹⁴ In the current study, we found the formation of TDI-albumin is also strongly influenced by the physical phase (vapor vs liquid) of isocyanate during exposure, and extend previous data by directly comparing vapor, mock, and liquid TDI exposed albumin. The specific sites of TDI conjugation to albumin remain unclear, as does the influence of isocyanate

concentration (which is linked to the physical phase) on the final reaction products.

The proportion of subjects with TDI asthma with a TDI-specific IgE response, detected by using vapor TDI-albumin conjugates as antigens, is substantially higher than that measured by using liquid TDI-albumin conjugates (44% vs 17%), and is also much higher than that reported in many other studies in the literature, including our own.^{5,10,20-27} In previous studies, the lack of TDI-specific IgE in subjects with TDI asthma has been interpreted to suggest that IgE is not an important mediator in TDI asthma, and further, that TDI asthma is not a manifestation of Type I hypersensitivity to the chemical.^{6,20,28} However, it is well recognized that this interpretation of serology studies to date is dependent on the TDI antigen used to screen for TDI-specific IgE, and it has been proposed that albumin might not be an appropriate carrier for TDI.²⁹ The current data suggest that TDI-albumin conjugates are associated with TDI asthma, but that their antigenicity is highly dependent on the conditions under which TDI exposure occurs. The vapor exposure conditions used in this report are likely closer to the conditions found in the microenvironment of the airways of workers in occupational settings than the conditions used for liquid phase exposure. Thus, vapor TDI-albumin conjugates may better mimic TDI *in vivo*, which could explain the higher proportion of subjects with TDI asthma with IgE that binds vapor TDI-albumin conjugates, compared with liquid conjugates used in this and previous studies.

The failure to detect TDI-specific IgE in 37 of 66 (56%) of our subjects with TDI asthma, despite the use of vapor TDI-albumin conjugates as test antigens, is notable and may be indicative of nonimmunologic or antigen-independent mechanisms of TDI asthma. However, several practical and theoretical issues that might also account for this finding cannot be definitively ruled out, including time since last exposure, isomeric ratio of 2,4/2,6-TDI used occupationally, lower TDI-conjugation levels, and the choice of carrier protein. Of these, the time since last exposure could be the biggest contributor to negative RAST tests in truly sensitized patients, given the short serum half-life (2 days) for IgE, as well as the nature of isocyanate asthma diagnosis, which often occurs after symptoms prevent the individual from performing exposure-related tasks. Unfortunately, reliable TDI exposure assessments for individuals in the current study were not available and can be highly variable in end-use settings such as spray coating operations.

Human IgG that bound vapor TDI-albumin conjugates was also present in a high proportion of TDI asthmatics (31%) as well as a lower percentage (9.6%) of asymptomatic exposed workers, but only 3 of 177 (1.7%) of individuals without known occupational TDI exposure. The prevalence of vapor TDI-albumin specific IgG among the subjects with TDI asthma in this study is similar to previous studies we have reported using liquid TDI-albumin conjugates, as well as analogous studies in HDI-exposed workers.^{15,24} The high prevalence of TDI

specific IgG among subjects with TDI asthma could reflect a specialized yet undetermined role in pathogenesis. However, the increased prevalence of TDI-specific IgG among exposed asymptomatic workers (vs unexposed individuals) suggests the equally likely possibility that TDI asthmatics had more TDI exposure than the asymptomatic groups, consistent with previous studies demonstrating a link between exposure and IgG responses.^{15,30,31} As mentioned, TDI workplace exposures can be highly variable, and limited information was available for individuals in the current study. Future studies combining exposure assessment with specific challenge and serology results should help clarify the association between TDI-specific IgG, exposure, and asthma.

The data also demonstrate the importance of the isomeric ratio of 2,4/2,6-TDI on the antigenicity of TDI-exposed albumin. Most industrial processes use an 80/20 mixture of 2,4/2,6-TDI, and thus, one might expect the 2,4 isomer to be present in the air at higher proportions occupationally. However, the reverse is actually true; airborne 2,6-TDI isomer concentrations are generally equal to or greater than those of 2,4-TDI, possibly because 2,4-TDI's higher reactivity makes it effectively less available to the vapor phase.¹⁶ In this study, we evaluated the effect of the TDI isomer ratio by comparing conjugates made with an 80/20 vs a 98/2 ratio of 2,4/2,6-TDI. The 80/20 (2,4/2,6-TDI) mixture yielded TDI-albumin conjugates that are recognized by IgE and IgG from more subjects with TDI asthma than TDI-albumin conjugates prepared with a 98/2 (2,4/2,6-TDI) mixture. It remains uncertain whether the differences we observed are a result of the absolute amount of 2,6-TDI used in generating the conjugates, or whether the precise 2,4/2,6-TDI ratio is critical to generating conjugates with appropriate antigenicity, an important consideration in industrial settings where other ratios of 2,4/2,6-TDI might also be used (eg, 65/35).

In summary, this study demonstrates that human albumin becomes conjugated with TDI when the chemical is in vapor phase, and that vapor TDI-albumin conjugates likely differ from TDI-albumin conjugates used in most immunologic studies to date, which traditionally have been prepared by using liquid phase TDI. The data highlight the dependence of TDI serology studies on the form of TDI used as the TDI antigen, and how this variable may influence the results of investigations on TDI asthma pathogenesis. The results provide strong circumstantial evidence for a Type I hypersensitivity-mediated mechanism of TDI asthma in a substantial proportion of patients (44%), and suggest that *in vitro* testing may be a useful adjunct to effective diagnosis and exposure assessment, if the appropriate TDI antigen is used. By analogy with other allergens, vapor TDI-albumin may also represent a target for immunotherapy.

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