

Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocation test results

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Background: The role of specific IgG to toluene diisocyanate (TDI) in the pathogenesis of TDI-induced asthma still remains unclear.

Objective: We sought to evaluate the clinical significance of serum-specific IgG to TDI-human serum albumin (HSA) conjugate in subjects with TDI-induced asthma compared with specific IgE antibody.

Methods: One hundred three subjects were enrolled and divided into 4 groups according to specific bronchoprovocation test (BPT) results: 50 subjects with TDI-induced asthma with positive results on TDI BPT were defined as group 1, 13 symptomatic workers exposed to TDI with negative results on TDI BPT were defined as group 2, 20 unexposed patients with allergic asthma were defined as group 3, and 20 unexposed healthy control subjects were defined as group 4. Serum-specific IgG and IgE antibodies to TDI-HSA conjugate were detected by means of ELISA.

Results: The prevalence of specific IgG antibody to TDI-HSA conjugate was significantly higher in group 1 than in group 2 (46% vs 7.7%, $P = .01$) or group 3 (0%, $P < .01$). No significant difference was noted between group 2 and group 3 ($P > .05$). However, the prevalence of specific IgE antibody to TDI-HSA conjugate was not significantly different between group 1 and group 2 (14% vs 7.7%, $P > .05$) or group 2 and group 3 (7.7% vs 0%, $P > .05$). There was no significant difference in prevalence of specific IgE or specific IgG according to the type of asthmatic response during the TDI BPT ($P > .05$). Overall, statistically significant association was noted between the prevalence of specific IgE and IgG antibodies in 103 subjects ($P < .05$), but no difference was noted within group 1 subjects only ($P > .05$).

Conclusion: These findings demonstrate that the presence of serum-specific IgG is closely related to TDI BPT results, and it may contribute to the development of TDI-induced asthma. (*J Allergy Clin Immunol* 1999;104:847-51.)

Key words: Specific IgG, Specific IgE, toluene diisocyanate-human serum albumin conjugate, toluene diisocyanate bronchoprovocation test

Isocyanate chemicals are currently the most common causes of occupational asthma worldwide.¹ It has been reported among workers exposed to toluene diisocyanate (TDI), methylene diphenyldiisocyanate (MDI), and hexamethylene diisocyanate (HDI). TDI is the most common cause in Korea,² with a prevalence of 2.9% to 13% in exposed workers.^{3,4}

Although considerable controversy remains regarding the immunopathogenesis of TDI-induced asthma, several groups of investigators have identified serum-specific IgE antibody in sensitized workers.^{2,5-7} Although the role of specific IgE antibody has been investigated, the results thus far point to discrepancies or rather low associations between presence of specific IgE antibodies and the disease.⁶ There has been evidence to suggest that cell-mediated immunity is involved.^{8,9} With regard to the role of specific IgG, there has been one report suggesting a pathogenic role of specific IgG to MDI-human serum albumin (HSA) and HDI-HSA conjugates in exposed asthmatic workers.¹⁰ To the best of our knowledge, there is little data available on the role of specific IgG to TDI-HSA conjugate in TDI-induced asthma.

In this study we evaluated the clinical significance of serum-specific IgG antibody to TDI-HSA conjugate and its relationship with specific IgE in subjects with TDI-induced asthma compared with exposed and unexposed control subjects.

METHODS

Subjects

Fifty subjects with TDI-induced asthma were classified as group 1. Their bronchial sensitization was confirmed by positive responses to TDI bronchoprovocation tests (BPTs): 23 had early, 5 had late only, 9 had dual, and 13 had atypical asthmatic responses, including progressive, prolonged immediate, and square-shaped responses. Thirteen subjects who had worked in the same place with exposure to TDI and complained of lower respiratory symptoms but had negative responses on TDI BPT were categorized as group 2. There was no significant difference in demographic data, including age, sex, exposure duration, initial methacholine PC₂₀, and serum total IgE level (Table I). As control subjects, 20 subjects with allergic asthma who were sensitive to house dust mite allergen (group 3) and 20 unexposed healthy control subjects (group 4) were enrolled. Atopy

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Abbreviations used

BPT:	Bronchoprovocation test
HDI:	Hexamethylene diisocyanate
HSA:	Human serum albumin
MDI:	Methylene diphenyldiisocyanate
PBS-T	PBS-Tween 20
TDI:	Toluene diisocyanate

TABLE I. Comparison of demographic data between group 1 and group 2 subjects

	Group 1 (n = 50)	Group 2 (n = 13)	P value
Sex (M/F)	35/15	11/2	NS
Age (y)	39.1 ± 1.4	36.1 ± 3.6	NS
Atopy (presence/absence)	27/23	6/8	NS
Exposure duration to TDI (mo)	72.1 ± 9.7	56.0 ± 17.3	NS
Initial methacholine PC ₂₀ (mg/mL)	7.98 ± 1.8	12.2 ± 4.0	NS
Serum total IgE level (IU/mL)	389.6 ± 78.2	411.3 ± 120.9	NS

All values were presented as means ± SEM.

NS, Not significant.

was determined by a positive skin test response to at least one common inhalant allergen. Sera from all the subjects were collected before the treatments; all of them stopped using inhaled or oral steroids for 4 weeks before the study. They underwent an interview, chest radiography, skin prick tests with common allergens, lung function measurement, and inhalation challenge with both methacholine and TDI (Aldrich). All the subjects gave their informed consent, which was regulated by the Institutional Review Board of Ajou Medical Center, Suwon, Korea.

BPT test with methacholine and TDI

The methacholine bronchial challenge test was done according to the method described previously.⁹ Briefly, aerosols were generated by a DeVilbiss 646 nebulizer connected to a DeVilbiss dosimeter driven by compressed air (DeVilbiss Co). Five inhalations of normal saline at 5-minute intervals were taken, followed by a series of successively doubled doses of methacholine (0.075 to 25 mg/mL) until a 20% fall in FEV₁ was observed. FEV₁ was measured 5 minutes after the beginning of each inhalation, and the PC₂₀ level was determined by interpolation from the dose-response curve. The TDI bronchial challenge test was performed according to the previously described method.⁹ Briefly, the subjects were exposed to TDI (80:20 = 2,4 form; Aldrich) through tidal breathing in a small closed room for 5 to 15 minutes until asthmatic symptoms were induced. The concentration of TDI measured by TLD-1, a toxic gas detector with Cheakey (HDA Scientific), was 20 ppb. Pulmonary functions were measured with a spirometer (Multi-SPIRO SX/PC) immediately before and every hour after the exposure for 8 hours.

Preparation of TDI-HSA conjugates

2,4-TDI-HSA conjugates were prepared as described previously^{7,11} by using a modification of Tse and Pesce's method.¹² 2,4-TDI (2.4 g) was added to 90 mL of 1% HSA in PBS with constant stirring. Aliquots were taken at 5, 10, 20, 30, and 40 minutes after the reaction. Ten milliliters of 1% HSA solution was used as an uncon-

jugated control HSA. Ammonium carbonate (2 mol/L) was added to each aliquot to terminate reactions. All reactive samples were centrifuged at 3000g for 40 minutes at 4°C to remove unreactive TDI, extensively dialyzed for 3 days against 0.1 mol/L ammonium carbonate, precipitated with equal volume of 20% trichloroacetic acid, redissolved in 1 mol/L sodium hydroxide, and then dialyzed with 4 L of deionized water for 1 day. When the degrees of substitution were determined by a modified Gutmann assay,¹³ the molar ratio was 5. Protein content of a conjugated sample was determined by the Lowry method. All reagents for preparation of TDI-HSA conjugates were purchased from Sigma Chemical Co.

Specific IgG antibody to TDI-HSA conjugate as determined by ELISA

After 1 µg of TDI-HSA conjugate or unconjugated HSA was dissolved in 0.1 mol/L sodium bicarbonate buffer (pH 9.5), the mixture (pH 9.5) was coated in the ELISA plate individually (Costar Co) at 4°C, washed with PBS-Tween 20 (PBST), and blocked with 350 µL of blocking buffer (PBS containing 5% BSA and 0.1% Tween 20). Diluted serum (50 µL) from patients or control subjects at the optimal concentration (1:500) in the preliminary experiment was incubated overnight at 37°C in both TDI-HSA- and HSA-coated wells and washed 3 times. Peroxidase-conjugated anti-IgG antibody (50 µL, Vector Laboratory), diluted 1:5000 with 5% BSA-PBST, was added into each well and incubated for 1.5 hours at 37°C and washed with PBST, and then 100 µL of *o*-phenyl deamine was added. After 10 minutes' incubation at 37°C, reactions were stopped with 100 µL of 2N H₂SO₄ and read by using an ELISA reader (Molecular Devices Co) at 490 nm. The final absorbance value of each individual was decided after the subtraction of the HSA-coated value from that of the TDI-HSA-coated value. The positive cut-off value was determined as mean plus 2-fold SD of the absorbance values from group 4 subjects.

Specific IgE antibody as determined by ELISA

Diluted patient's serum or negative control serum (50 µL), which was determined to be the optimal concentration (1:10) in the preliminary experiment, was added to each well of ELISA plates (Costar Co) that had been coated with 1 µg/well TDI-HSA conjugate or control HSA dissolved in coating buffer (0.1 mol/L sodium bicarbonate buffer, pH 9.5) and blocked with 350 µL of blocking buffer (PBS containing 5% BSA and 0.1% Tween 20). After overnight incubation at 37°C, the plate was washed 3 times with PBST. Biotinylated anti-human IgE (50 µL, Sigma Co) diluted to 1:500 vol/vol with 5% BSA-PBST was incubated for 3 hours at 37°C and washed with PBST, and then streptavidin-peroxidase (Sigma Co), which was diluted to 1:2000 vol/vol with 5% BSA-PBST, was added to each well. After incubation for 1 hour at 37°C, the plate was washed, and 100 µL of 3,3',5,5' tetramethylbenzidine (Sigma Co) was added as the substrate solution. Reactions were stopped with the addition of 2.5N H₂SO₄. The OD of the solution was determined at 450 nm by using an ELISA reader (Molecular Devices Co). The final absorbance value of each individual was decided after subtraction of the control HSA-coated value from that of the TDI-HSA-coated value. The positive cut-off values were determined as mean plus 2-fold SD of the absorbance values from group 4 subjects.

Statistical analysis

The cross-tab analysis was applied by using SPSS version 7.0 software (Chicago) to compare the prevalence of both specific IgG and specific IgE antibodies between groups and to evaluate the association between 2 specific antibodies. A *P* value of .05 or less was regarded as significant.

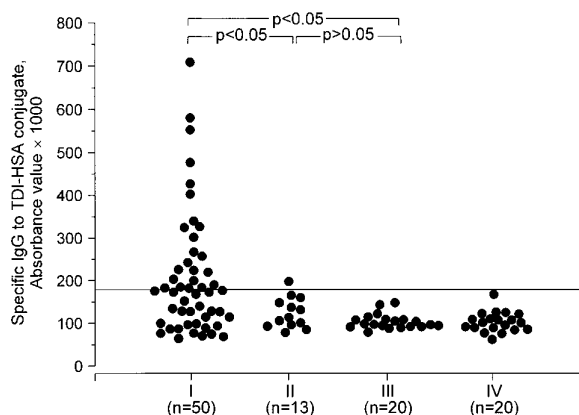


FIG 1. Comparison of specific IgG bindings to TDI-HSA conjugate as determined by using ELISA in 4 different groups: *group 1*, patients with TDI-induced asthma; *group 2*, exposed subjects with asthma who had negative results on TDI BPT; *group 3*, unexposed subjects with allergic asthma who are sensitive to house dust mite allergen; and *group 4*, unexposed healthy control subjects. Statistical significance was noted between group 1 and group 2 or group 3 by cross-tab analysis ($P < .05$), with no statistical significance between groups 2 and 3 ($P > .05$).

TABLE II. Test characteristics of TDI-HSA-specific IgE and IgG antibodies in identifying TDI-induced asthma confirmed by TDI BPT among exposed workers (group 1 and 2 subjects)

	Specific IgE antibody	Specific IgG antibody
Sensitivity (%)	14	46
Specificity (%)	92.3	92.3
Positive predictive value (%)	87.5	95.8
Negative predictive value (%)	21.8	30.8

RESULTS

Specific IgG antibody to TDI-HSA conjugate

Fig 1 demonstrates the prevalence of specific IgG antibody to TDI-HSA conjugate in 4 different groups. Twenty-three (46%) workers in group 1 and one (7.7%) worker in group 2 had high serum-specific IgG levels. Prevalence of specific IgG in group 1 was significantly higher than in group II ($P = .02$) or group 3 ($P < .001$). No significant difference was found between group 2 and group 3 ($P > .05$). Table II summarizes sensitivity, specificity, and positive and negative predictive values of specific IgG antibody for identifying TDI-induced asthma confirmed by TDI BPT in exposed subjects.

Specific IgE antibody to TDI-HSA conjugate

Fig 2 demonstrates the prevalence of specific IgE antibody to TDI-HSA conjugate in 4 different groups. Seven (14%) subjects in group 1 and one (7.7%) subject in group 2 had high specific IgE antibodies. There were no significant differences in the prevalence of specific IgE antibody between group 1 and group 2 or 3 ($P > .05$).

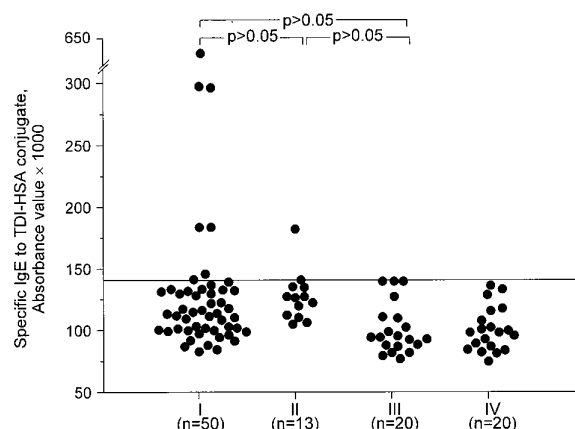


FIG 2. Comparison of specific IgE binding to TDI-HSA conjugate determined by using ELISA in 4 different groups: *group 1*, patients with TDI-induced asthma; *group 2*, exposed subjects with asthma who had negative results on TDI BPT; *group 3*, unexposed subjects with allergic asthma who are sensitive to house dust mite allergen; and *group 4*, unexposed healthy control subjects. No significant differences were noted between groups by cross-tab analysis ($P > .05$).

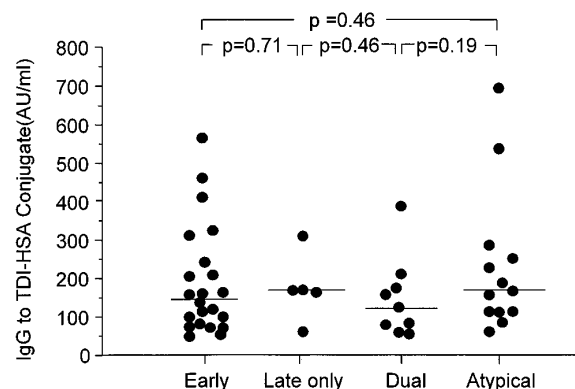


FIG 3. Comparison of specific IgG levels to TDI-HSA conjugate according to the type of asthmatic response during the TDI BPT. No significant differences were noted among the types of response ($P > .05$).

Table II summarizes sensitivity, specificity, and positive and negative predictive values of specific IgE antibody for identifying TDI-induced asthma. The correlation coefficient between total and specific IgE levels was 0.31 ($P = .04$, data not shown).

The influence of type of asthmatic response and association between specific IgE and IgG antibodies

There were no significant differences in the prevalence of specific IgG antibodies depending on the type of asthmatic response during TDI BPT ($P > .05$) in group 1 (Fig 3). There was no significant association between the prevalence of specific IgE and specific IgG antibodies in group 1 subjects ($P > .05$, data not shown).

DISCUSSION

Several groups of investigators have detected various prevalences of specific IgE antibody to TDI-HSA conjugate in the sera of workers with positive challenge results; the prevalence varied between 0% and 50% of the workers.^{1,2,5,6} In this study 7 (14%) of the subjects with TDI-induced asthma had high specific IgE antibody levels, whereas one (7.7%) in group 2 and none in the unexposed subjects or healthy control subjects had similarly high specific IgE antibody levels. These findings suggest that TDI exposure may induce IgE-mediated bronchoconstriction in some asthmatic patients and that there may be an asymptomatic sensitizer in exposed workers.

There has been evidence of cell-mediated immune response composed of eosinophils, mast cells, and T lymphocytes to induce airway inflammation in the bronchial mucosa of subjects with TDI-induced asthma.⁸ Furthermore, the production of IFN- γ was noted from T-cell lines stimulated by TDI-HSA conjugate from subjects with TDI-induced asthma.¹¹ As for the role of neutrophils, neutrophilia has been demonstrated in bronchoalveolar lavage fluid of isocyanate-induced asthma.¹⁴ Myeloperoxidase level, a neutrophil activation marker increased significantly after the TDI BPT, was accompanied by an increase of IL-8.⁹ These findings suggest that inflammatory cells, including T lymphocytes, eosinophils, mast cells, and neutrophils may contribute to the development of TDI-induced airway inflammation.

The specific IgG response to occupational antigens seems to be complex, eliciting variable responses according to different antigens. Forster et al¹⁵ reported that specific IgG to tetrachlorophthalic anhydride was detected in all asthmatic workers and some of the exposed asymptomatic workers. Moreover, the specific IgG4 antibody increased in 5 of 7 subjects with tetrachlorophthalic anhydride-induced asthma but was not detected in any of the asymptomatic individuals. Our previous study¹⁶ on reactive dye asthma revealed that the prevalence of specific IgG and IgG4 was significantly higher in symptomatic workers, but no association was noted according to exposure intensity or duration of exposure. However, several studies in other occupational settings have suggested that the presence of specific IgG may represent a response to high-dose exposure not related to the development of respiratory symptoms.^{17,18} In regard to specific IgG response in isocyanate-induced asthma, there has been a report suggesting a pathogenic role of specific IgG to HDI-HSA and MDI-HSA conjugates in the development of isocyanate-induced asthma,¹⁰ although other investigators have suggested that specific IgG to MDI may be an indicator of MDI exposure.¹⁹ Specific IgG antibodies to TDI-HSA and MDI-HSA conjugates are very closely related to hypersensitivity pneumonitis.²⁰

To the best of our knowledge, this is the first study to detect serum-specific IgG antibody to TDI-HSA conjugate in a significant number of subjects with TDI-

induced asthma compared with the exposed workers showing negative responses on TDI BPT. This study demonstrated that the prevalence of specific IgG to TDI-HSA conjugate was significantly higher in subjects with TDI-induced asthma than in those with negative responses on TDI BPT, as well as those of subjects with allergic asthma and unexposed healthy control subjects, whereas no significant differences were noted in the prevalence of specific IgE antibody. The sensitivity of specific IgG antibody to identify TDI-induced asthma was not high because the pathogenic mechanism of TDI-induced asthma is complicated. To the best of our knowledge, no single test has been found to be a useful marker for predicting TDI-induced asthma thus far. In conclusion, the presence of serum-specific IgG antibody to TDI-HSA conjugate is closely associated with TDI BPT results, and it may be used to monitor the condition before clinical diseases occur.

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