
Increased levels of IgG to cytokeratin 19 in sera of patients with toluene diisocyanate-induced asthma

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Background: Inhaled isocyanate binds with cytokeratin (CK) of the epithelial cells, which could induce immune responses.

Objective: To elucidate the possible existence of an isocyanate-induced, asthma-associated autoantigen from the bronchial epithelial cells, which may be associated with toluene diisocyanate (TDI)-induced asthma development.

Methods: We cultured bronchial epithelial cells with incubation of TDI-human serum albumin (HSA) conjugate. Gene expression profiles of cultured epithelial cells were analyzed using a microarray technique. CK19 protein expression within the epithelial cells was confirmed by IgG immunoblot using monoclonal antibody to CK19. Serum IgG to CK19 and specific IgG and IgE antibodies to TDI-HSA conjugate were detected by enzyme-linked immunosorbent assay in 68 TDI asthma patients (group 1) and compared with 40 allergic asthma patients (group 2) and 80 unexposed healthy controls (group 3).

Results: After TDI exposure, increased expression of *CK19* and *CK14* genes from the culture bronchial epithelial cells was noted using microarray analysis. IgG immunoblot analysis confirmed increased expression of CK19 after the TDI exposure. The levels of serum IgG to CK19 were significantly higher in the TDI asthma group than in groups 2 and 3 ($P=.008$). The prevalence of IgG to CK19 was significantly higher in group 1 (38.2%) than group 2 (22.5%) or group 3 (1.3%) ($P=.008$). Significant associations were noted between IgG to CK19 and specific IgG to TDI-HSA conjugate and transglutaminase ($P=.02$) but not with specific IgE to TDI-HSA conjugate.

Conclusion: We suggest that TDI exposure can augment CK19 expression from the bronchial epithelial cell, which may involve immune responses as an autoantigen to induce airway inflammation in TDI-induced asthma.

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INTRODUCTION

The pathogenic mechanisms of toluene diisocyanate (TDI)-induced asthma are complicated, because several immunological and nonimmunological mechanisms are involved.¹ Recent investigations suggest that isocyanate, especially hexamethylene diisocyanate, binds to airway epithelial cell proteins and leads to airway inflammation, which is composed of cytokine and chemokine productions, and cellular recruitment, which is mediated by lung epithelial cell protein that presents the chemicals.² A previous study demonstrated that specific IgG to TDI-human serum albumin (HSA) conjugate was apparent in 46% of TDI asthma patients and was closely associated with TDI bronchoprovocation test results,³ suggesting a possible involvement of IgG in the pathogenic mechanism of TDI asthma. However, no further evidence

exists on how these IgG antibodies contribute to TDI asthma development.

The possible involvement of an autoimmune mechanism in the pathogenesis of asthma has been proposed by previous studies that have demonstrated the presence of circulating autoantibodies in bronchial mucosa tissue in patients with asthma, especially in patients with nonallergic asthma.^{4,5} After considering the results of a long-term follow-up study of TDI-induced asthma patients that suggested that more than 50% of these patients complained of persistent asthmatic symptoms,⁶ we hypothesized that self-antigen-driven immune responses might contribute to the development of persistent airway inflammation in airway mucosa, although the target antigens of these autoantibodies are still unknown. Our previous investigation also identified CK18 of bronchial epithelial cells as a possible autoantigen in nonallergic, nonoccupational asthma using immunoblot analysis.⁷ In this study, our objective was to elucidate the possible existence of an isocyanate-induced, asthma-associated autoantigen from the bronchial epithelial cells, which may be associated with TDI-induced asthma development.

MATERIALS AND METHODS

Sixty-eight patients with TDI asthma were enrolled and classified as group 1. Their bronchial sensitizations were

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Table 1. Demographic Data of the Study Patients

	TDI-induced asthma (n = 68)	Allergic asthma (n = 40)	Unexposed healthy controls (n = 80)	P value
Age, mean $\pm$ SD, y	41.5 $\pm$ 9.9	36.8 $\pm$ 9.6	36.4 $\pm$ 14.2	.61
Initial PC ₂₀ , mean $\pm$ SD, mg/mL	7.98 $\pm$ 1.8	11.2 $\pm$ 4.0	ND	.11
Sex, M/F	46/22	23/17	40/40	.44
Smoking, yes/no	21/47	15/25	20/60	.11
Atopy, yes/no	21/47	40/0	NA	

Abbreviations: NA, not applicable; ND, not done; PC₂₀, provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20%; TDI, toluene diisocyanate.

confirmed by positive responses to TDI bronchoprovocation tests. As control subjects, 40 patients with allergic asthma sensitive to house dust mite allergen (group 2) and 80 unexposed healthy control subjects (group 3) were enrolled. Atopy was determined by a positive skin test response to at least 1 common inhalant allergen, including house dust mite, tree and pollen mixtures, mug wort, ragweed pollens, and *Alternaria* (Bencard, Munich, Germany). The demographic data of the 3 study groups are compared in Table 1. There were no significant differences in age, sex, and other clinical parameters among the study participants. Serum samples from group 1 and 2 patients were collected before the treatments; all of these patients stopped using inhaled or oral steroids for 4 weeks before the study. They underwent an interview, chest radiography, skin prick test with common inhalant allergens, lung function measurement, and inhalation challenge with both methacholine and TDI. All the patients gave their informed consent, which was regulated by the Institutional Review Board of Ajou Medical Center, Suwon, Korea.

Bronchial Challenge Test With Both Methacholine and TDI

The methacholine bronchial challenge test was performed in patients in groups 1 and 2 according to the method previously described.² Briefly, aerosols were generated by a nebulizer connected to a dosimeter driven by compressed air (DeVilbiss Co, Doylestown, PA). Five inhalations of isotonic sodium chloride solution at 5-minute intervals were taken followed by a series of successively doubled doses of methacholine (0.075 to 25 mg/mL) provocation concentration that caused a decrease in forced expiratory volume in 1 second (FEV₁) of 20% (PC₂₀) was observed or the maximum dose given. The FEV₁ was measured 5 minutes after the beginning of each set of inhalations of aerosolized methacholine. The methacholine PC₂₀ level was determined by interpolation from the dose-response curve. The TDI bronchial challenge test was performed according to the protocol described in the previous report.²

Preparation of TDI-HSA Conjugate

2,4-TDI-HSA conjugates were prepared as described previously³ by using a modification of Tse and Pesce's method. 2,4-TDI was added to 90 mL of 1% HSA in phosphate-buffered saline (PBS) with constant stirring. Aliquots were taken 5, 10, 20, 30, and 40 minutes after the reaction. Ten milliliters of 1% HSA solution was used as an unconjugated

control HSA. Ammonium carbonate (2 mol/L) was added to each aliquot to terminate reactions. All reactive samples were centrifuged at 3,000g 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 modified Gutmann assay, 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, St. Louis, MO.

Culture of Bronchial Epithelial Cells Beas-2B With TDI-HSA Conjugate and Peripheral Blood Mononuclear Cells of TDI Asthma

Beas-2B cells were cultured as described in the previous report.⁸ The Beas-2B cell line, derived from human bronchial epithelium transformed by an adenovirus 12-SV40 hybrid virus, was purchased from the American Type Culture Collection. Cells were cultured in BEGM medium (Clonetics, Walkersville, MD) containing bovine pituitary extract, insulin, hydrocortisone, and gentamicin at 37° C with 5% carbon dioxide in humidified air. To observe TDI-induced response, epithelial cells were incubated with 50 μ g/mL of TDI-HSA conjugate for 24 hours and compared with those with mock HSA conjugate (mock conjugate: HSA alone without TDI conjugation) incubation.

Complementary DNA Microarray Preparation

Microarray analysis was performed according to the previous report.⁹ In brief, a set of 7,448 sequence-verified human complementary DNA (cDNA) clones was purchased from Research Genetics (Huntsville, AL). Plasmid DNA was isolated using a plasmid kit (Millipore, Bedford, MA), and open reading frames were polymerase chain reaction (PCR) amplified using a pair of universal primers, 5'-CTGCAAGGC-GATTAAGTTGGGTAAC-3' and 5'-GAGCGGATAACAATTTCACACAGGAAACAGC-3', under the following conditions: initial denaturation at 94° C for 2 minutes, followed by 30 cycles of 94° C for 45 seconds, 55° C for 45 seconds, and 72° C for 2 minutes, and a final extension step at 72° C for 10 minutes. The PCR amplification products were examined by 1% agarose gel electrophoresis, purified using Sephadex G-50 columns, dried, and resuspended in

50% dimethyl sulfoxide solution. Prepared DNAs were spotted using an OmniGrid Micro-arrayer (GeneMachines, San Carlos, CA) onto silanized glass slides (CMT-GAPS; Corning, Charlotte, NC).

Total RNA Isolation, Target Preparation, and Hybridization

Beas-2B cells cultured to 70% confluence were treated with TDI-HSA or mock conjugate for 24 hours, and total RNA was purified from the cells using TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The cDNA targets were then synthesized from total RNAs in the presence of either cyanine 3 deoxyuridine 5-triphosphate (Cy3-dUTP) (for media treatment) or cyanine 5 dUTP (Cy5-dUTP) (for TDI-HSA conjugate treatment) and applied to the cDNA microarray. RNA from the untreated cells was labeled with Cy3-dUTP (NEN Life Science Products, Boston, MA) and then used as a tester. The labeled cDNAs were then purified through a microcon-30 column and resuspended in 80 μ L of hybridization solution (3 $\times$ 0.1 M sodium citrate with 3% NaCl and 0.3% sodium dodecyl sulfate [SDS]). The target mixture was denatured at 100°C for 2 minutes and applied to the DNA chip at 65°C for 16 hours in a humidified chamber. The hybridized slide was washed in 2 $\times$ sodium chloride–sodium citrate solution (SSC) for 2 minutes, 0.1 $\times$ SSC and 1% SDS for 5 minutes, and 0.1 $\times$ SSC for 5 minutes and then spin-dried before scanning at room temperature.

Data Acquisition and Analysis

Microarray scanning and normalization of the data were performed using a GenePix 4000B scanner and the GenePix Pro 3.0 software package (<http://www.axon.com>). Poor quality spots (sum of median, <500) were filtered from the raw data before analysis. For each hybridized spot, the gene expression value (ratio of median in GenePix pro 3.0), a ratio of Cy5 fluorescence intensity minus Cy5 background intensity to Cy3 fluorescence intensity minus Cy5 background intensity, is calculated and represents a fold gene expression change for each gene. The cDNA microarray experiments were repeated twice at each condition, and the average of 2 gene expression values for each gene was used for the analysis.

IgG Immunoblot Analysis of Bronchial Epithelial Cell Lysate Using Anti-CK19 Antibody

Cultured bronchial epithelial cells were lysed by the addition of lysis buffer containing 10-mmol/L TRIS-hydrochloride, 10-mmol/L EDTA, and 0.4-mmol/L phenylmethane sulphonylfluoride (pH 7.4) and separated by discontinuous SDS-polyacrylamide gel electrophoresis (PAGE) using an 8% resolving gel (pH 8.8) and a 4% stacking gel (pH 6.8). After electrophoresis, proteins were transferred onto a polyvinylidene difluoride membrane (Millipore) and blocked with 5% nonfat milk. After the transfer, the membrane strips were probed with monoclonal antibody to CK19 (Sigma) at 1 in 100 dilution for 2 hours at room temperature. After washes, the membrane was incubated with alkaline phosphatase–conjugated goat anti-mouse IgG (1:1,000, Sigma) for 1 hour

at room temperature. After a final washing, the membrane was stained with a substrate solution (nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl phosphate; Sigma), and the intensity was measured using a densitometer and presented as the relative intensity.

Specific IgG and IgE Antibody to TDI-HSA Conjugate by Enzyme-Linked Immunosorbent Assay

Serum-specific IgE and IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA) as described previously.³ In brief, 1 μ g of TDI-HSA conjugate or mock conjugate was dissolved in sodium bicarbonate buffer (pH 9.5) and coated on the ELISA plate individually (Corning, New York, NY) at 4°C. After washing with PBS-Tween 20 (PBS-T), the plates were blocked with 350 μ L of blocking buffer (PBS containing 1% bovine serum albumin and 0.1% Tween 20). Diluted serum (50 μ L) from patients 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 37°C in both TDI-HSA- and HSA-coated wells and washed 3 times. Peroxidase-conjugated anti-IgG (1:2,000 vol/vol, 50 μ L, Sigma) or biotin-conjugate anti-IgE antibody (1:200 vol/vol, Sigma) was added into each well and incubated for 1 hour at room temperature, then 50 μ L of substrate solution dissolved in substrate buffer (Sigma) was added. After 15 minutes of incubation at 37°C, absorbance values were read using an ELISA reader at 405/450 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 cutoff value (0.18 for IgG, 0.13 for IgE) was determined as mean $\pm$ 2-fold SD of the absorbance value from 80 unexposed healthy controls.

Specific IgG Antibody to CK19 by ELISA

Serum specific IgG level was detected by ELISA. A total of 50 μ L of diluted (1:200) patient's serum or negative control serum, which was determined to be the optimal concentration in the preliminary experiment, was added to each well of ELISA plates that had been coated with 100 ng per well of CK19 (Research Diagnostics, Flanders, NJ) dissolved in coating buffer (0.1-mol/L sodium bicarbonate buffer, pH 9.5) and blocked with 200 μ L of blocking buffer (PBS containing 10% fetal bovine serum). After 2 hours of incubation at 4°C, the plate was washed 3 times with PBS-T. Then biotin-conjugated anti-human IgG (50 μ L, Sigma) diluted to 1:2,000 (vol/vol) with 5% bovine serum albumin PBS-T was incubated for 1 hour at 37°C and washed with PBS-T. Then streptavidin-peroxidase conjugated anti-IgG (1:1,000, Sigma) was incubated for 30 minutes and B solution (3,3',5,5'-tetramethyl benzidine, Sigma) added as the substrate solution. Reactions were stopped with the addition of 2.5N H₂SO₄. The optical density of the solution was determined at 450 nm by using an ELISA reader. The absolute value of CK19 level was presented as arbitrary units (AU) based on a standard curve, which was derived from serial dilution of the pooled sera with high specific IgG level to CK19. The positive cutoff

value was determined as mean $\pm$ 2-fold SD of the absorbance values from 80 unexposed healthy controls. However, when we repeated the same experiment with anti-CK14 antibody using the ELISA system, no specific bindings were observed; therefore, we did not extend ELISA for IgG to CK14.

Association With Other Autoantibodies

We measured antinuclear antibody (ANA), IgG, and IgA to transglutaminase (TG) in the same set of serum samples from TDI-induced asthma patients using diagnostic kits (BL Diagnostika, Hamburg, Germany). They were compared with the prevalences of IgG to CK19. The ELISA procedures and their interpretation were performed according to manufacturer's guidance.

Statistical Analysis

The *t* test was applied to compare the mean levels of IgG to CK19 between groups 1 and 2 and groups 2 and 3. The χ^2 test was applied to compare the prevalence of IgG to CK19 with specific antibodies to TDI-HSA conjugate or other autoantibodies. Analysis of variance was applied to compare demographic data among the 3 groups. *P* < .05 was regarded as statistically significant.

RESULTS

Microarray of Cultured Bronchial Epithelial Cells

Table 2 shows the microarray result of bronchial epithelial cells, Beas-2B incubated with TDI-HSA, or mock conjugate. Among various proteins, compared with those exposed to mock conjugate, increased expression of *CK14* and *CK19* genes (more than 2-fold), which are major skeletal proteins of

Table 2. The Genes Showing 2-Fold Increased Expressions in Cultured Bronchial Epithelial Cells after Treatment of Toluene Diisocyanate–Human Serum Albumin Conjugate

Up-regulated (genes)	Fold change
Small inducible cytokine subfamily D	13.68
RNA cyclase homology	9.88
Integrin, α V	6.76
Forkhead box O1A	5.09
Interleukin 6 signal transducer	4.62
Integrin β -3 binding protein	3.72
Megakaryocyte stimulating factor	2.95
HIR (histone cell cycle regulation)	2.56
Cell division cycle 42 (GTP-binding)	2.55
Keratin 19	2.51
Interferon, α -inducible protein	2.4
PPAR (γ) angiopoietin-related protein	2.3
Jun B proto-oncogene	2.27
Fn14 for type 1 transmembrane protein	2.24
Thrombospondin 1	2.2
Myxovirus resistance 1, homology	2.18
Interferon-stimulated protein, 15 kDa	2.17
Keratin 14	2.13
Nonmetastatic cells 4, protein express	2.06
Tax interaction protein 1	2.06

epithelial cells, were noted after the incubation of TDI-HSA conjugate.

IgG Immunoblot of Epithelial Cell Lysate Reacting With Monoclonal Antibody to CK19

Figure 1 shows SDS-PAGE findings of bronchial epithelial cells, which were cultured under 2 different conditions: incubation with TDI-HSA or incubation with mock conjugate. There were no remarkable differences in protein bands within the epithelial cell lysates; however, IgG immunoblot analysis using monoclonal antibody to CK19 demonstrated that compared with mock conjugate incubation expression of CK19 protein increased with addition of TDI-HSA conjugate (Fig 1).

ELISA Results for IgG to CK19

Figure 2 shows specific IgG bindings to CK19 by ELISA in 3 study groups. Serum IgG levels to CK19 were significantly higher in group 1 (mean, 2,287.4 AU) compared with groups 2 (1,622 AU) and 3 (1,132.4 AU; *P* < .05; Fig 2). The prevalence of IgG to CK19 was significantly higher in group 1 (38.2%) than in groups 2 (12.5%) and 3 (1.3%; *P* = .008). Prevalence of IgG to CK19 was significantly associated with that of specific IgG to TDI-HSA conjugate (*P* = .02) not with specific IgE to TDI-HSA conjugate (Table 3). No significant associations were found between IgG to CK19 and sex, atopy, and smoking status in TDI asthma patients (data not shown).

Association With Other Autoantibodies

IgG and IgA antibodies to TG and ANA were measured in serum samples of the same set of TDI-induced asthma patients and compared with the prevalences of IgG to CK19. A significant association was found with IgG to TG (*P* = .05), whereas no significant associations were found with other autoantibodies (*P* > .05; Table 4).

DISCUSSION

The location of lung epithelial cells at the air-lung interface can make these cells targets for injury after TDI exposure. It

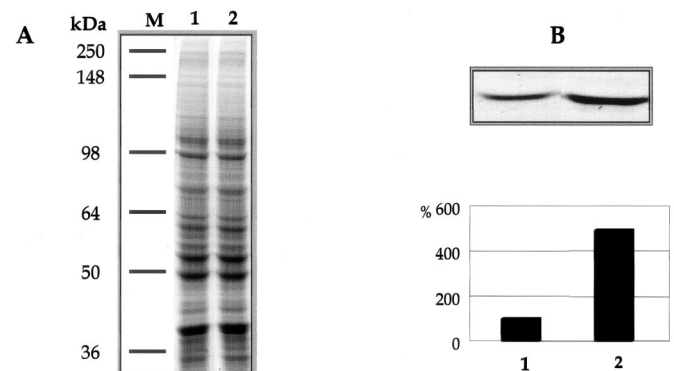


Figure 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (A) and IgG immunoblot (B) analysis of the bronchial epithelial cell lysate cultured with 2 different conditions. M indicates marker; 1, mock conjugate; and 2, toluene diisocyanate–human serum albumin conjugate exposure.

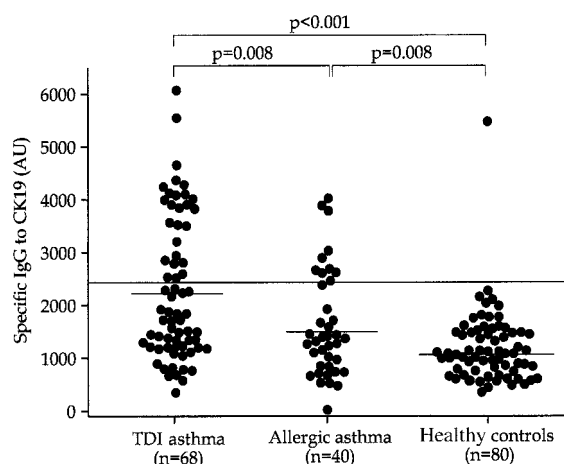


Figure 2. Comparison of specific IgG level to cytokeratin 19 (CK19) in serum samples of toluene diisocyanate-induced asthma, allergic asthma, and healthy controls.

Table 3. Association between Specific IgG to CK19 and Specific IgE and IgG Antibodies to TDI-HSA Conjugate

	Specific IgG*		Specific IgE†	
	Positive	Negative	Positive	Negative
Specific IgG to CK19				
Positive	14	11	1	24
Negative	11	30	4	37

* $P = .02$ by Fisher exact test.

† $P = .64$ by Fisher exact test.

Table 4. Association of Prevalence between Antibodies to TG, ANA, and Cytokeratin 19

	Specific IgG to cytokeratin 19*		P value
	Positive	Negative	
IgG to TG*			
Positive	11	8	.05
Negative	15	34	
IgA to TG			
Positive	0	1	>.99
Negative	26	40	
ANA			
Positive	3	2	.34
Negative	23	39	

Abbreviations: ANA, antinuclear antibody; TG, transglutaminase.

* $P \leq .05$ indicates a significant association was found.

has been suggested that the conjugation of lung epithelial cell proteins with isocyanates may have important implications in immune sensitization to elicit cellular immune responses and the subsequent development of isocyanate-induced asthma.² Regarding the role of CK in isocyanate asthma, CK18 has been identified as a major hexamethylene diisocyanate-bind-

ing protein in human bronchial tissue after diisocyanate exposure, and this conjugate could induce T-cell proliferation of sensitized asthma patients.¹⁰ In this study, the microarray results using bronchial epithelial cells demonstrated increased expression of *CK14* and *CK19* genes after TDI exposure. Serum IgG to CK14 was not detected by ELISA. However, IgG immunoblot confirmed protein expression of CK19, which increased after TDI-HSA conjugate incubation. Furthermore, higher levels of circulating IgG to CK19 were detected in serum samples of TDI asthma patients than in allergic asthma patients and unexposed controls. Furthermore, in this study, some allergic asthma patients had IgG to CK19 in their sera, and we need further studies to investigate its clinical significance.

Usually, intracellular and/or structural proteins such as CK can gain access to the immune system following cell death or damage to the epithelial layer. CK19 is a cytoskeletal protein found primarily in epithelial cells that line the lower respiratory tract.^{11,12} From these findings, we suggest that TDI exposure may augment CK19 protein expression, which can be an epithelial self-antigen to induce autoantibody production in TDI-exposed asthma patients.

Furthermore, to support our hypothesis of a possible involvement of an autoimmune mechanism in TDI asthma, we measured ANA, IgG, and IgA to TG in the same set of study patients. It is interesting that circulating IgG not IgA to TG was detectable in 28% of TDI asthma patients. Autoantibodies against tissue TG have been known as pathonomic markers for celiac disease. Dietary gluten peptide can act as a substrate for the deamidase activity of tissue TG. To the best of our knowledge, there have been few studies to suggest a role of TG in asthmatic patients, although TG expressions were noted in the bronchial epithelial tissue using the immunohistochemical technique.¹³ One possibility is that epithelium of asthmatic patients are more susceptible to apoptotic stimuli,¹⁴ which may induce TG-induced cross-linking, and in vitro evidence demonstrated that tissue TG could release CKs from epithelial cells, because CK is one of the substrates for TG.¹⁵ In this study, TDI asthma patients with higher CK19 antibodies had higher levels of serum IgG to TG with significant associations. From these findings, we speculate that in TDI asthma, bronchial epithelial cells, if "damaged" by TDI conjugation, may induce activation of the endogenous pathways, including TG activation, which may contribute to the release of epithelial self-antigens, including CK19 and TG with the subsequent productions of IgG antibodies to CK19 and TG. Further studies may shed light on possible mechanisms of CK antibodies in TDI asthma.

CONCLUSION

We found that CK19 protein can be a self-antigen associated with immune responses following TDI exposure, since TDI exposure may affect the expression or function of CK19. Such a phenomenon could contribute to the development of TDI asthma in eliciting autoantibody production. Further

studies are needed to determine whether these antibodies to CK19 can be used as diagnostic markers for TDI asthma.

REFERENCES

1. Park HS, Cho SH, Hong CS, Kim YY. Isocyanate-induced occupational asthma in far-east Asia: pathogenesis to prognosis. *Clin Exp Allergy*. 2002;32:198–204.
2. Wisnewski AV, Redlich CA. Recent developments in diisocyanate asthma. *Curr Opin Allergy Clin Immunol*. 2001;1:169–175.
3. Park HS, Kim HY, Nahm DH, Son JW, Kim YY. Specific IgG, but not specific IgE, antibodies to toluene diisocyanate-human serum albumin conjugate are associated with toluene diisocyanate bronchoprovocation test results. *J Allergy Clin Immunol*. 1999;104:847–851.
4. Wagner V, Tomsikova A, Sach J, Jankova J, Novackova D. Autoimmune mechanism in diseases of the respiratory tract. *Acta Allergol*. 1965;20:1–9.
5. Girard JP, Dami J. Antigenic properties of lung tissue: presence of specific antibodies in certain chronic respiratory disorders. *Poumon Coeur*. 1973;29:267–270.
6. Park HS, Lee SK, Kim HY, Nahm DH, Kim SS. Specific immunoglobulin E and immunoglobulin G antibodies to toluene diisocyanate-human serum albumin conjugate: useful markers for predicting long-term prognosis in toluene diisocyanate-induced asthma. *Clin Exp Allergy*. 2002;32:551–555.
7. Nahm DH, Lee YE, Yim EJ, et al. Identification of cytokeratin 18 as a bronchial epithelial autoantigen associated with nonallergic asthma. *Am J Respir Crit Care Med*. 2002;165:1536–1539.
8. Lee YM, Park HS, Lee SK, Kim HA, Nahm DH. Exposure to TDI induces IL-8 production from bronchial epithelial cells: effect of proinflammatory cytokine. *J Korean Med Sci*. 2003;18:809–812.
9. Yang S, Kim JS, Oh TJ, et al. Genome-scale analysis of resveratrol-induced gene expression profile in human ovarian cancer cells using a cDNA microarray. *Int J Oncol*. 2003;22:741–750.
10. Wisnewski AV, Lemus R, Karol MH, Redlich CA. Isocyanate-conjugated human lung epithelial cell proteins: a link between exposure and asthma? *J Allergy Clin Immunol*. 1999;104:341–347.
11. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell*. 1982;31:11–24.
12. Blobel GA, Moll R, Franke WW, Vogt-Moykopf I. Cytokeratins in normal lung and lung carcinomas, I: adenocarcinomas, squamous cell carcinomas, and cultured cell lines. *Virchows Arch B Cell Pathol Incl Mol Pathol*. 1984;45:407–429.
13. Martinet N, Bonnard L, Regnault V, et al. In vivo transglutaminase type 1 expression in normal lung, preinvasive bronchial lesions, and lung cancer. *Am J Respir Cell Mol Biol*. 2003;28:428–435.
14. Montefort S, Herbert CA, Robinson C, Holgate ST. The bronchial epithelium as a target for inflammatory attack in asthma. *Clin Exp Allergy*. 1992;22:511–520.
15. Mowat AM. Coeliac disease—a meeting point for genetics, immunology, and protein chemistry. *Lancet*. 2003;361:1290–1292.

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