［原著］Airway inflammatory and immunological events in a rat model exposed to toluene diisocyanate

Airway inflammatory and immunological events in a rat model exposed to toluene diisocyanate

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ABSTRACT

To investigate the inflammatory and immunological events induced by a short period of repeated exposure to toluene diisocyanate (TDI) in the airway, an animal model, which resembles the industrial field exposure, was established. After Wistar rats were exposed whole bodily to 0.38 and 1.20 ppm 4 h a day for 5 consecutive days in a glass chamber, airway symptoms were observed. Bronchoalveolar lavage (BAL) was performed and cellular contents and cytokine productions from BAL fluid were determined. Lung histopathological changes were also examined. The results showed that exposure to both concentrations of TDI vapor resulted in airway hypersensitivity symptoms. BAL fluid cellular analysis revealed that inflammatory response characterized by marked eosinophil infiltration occurred in the airways. Lung histopathological examination showed that TDI-induced bronchitis occurred in the exposed rats. Cytokine assay demonstrated that IL-4 was significantly produced in the airways of the TDI exposed rats. These findings indicated that a short period of exposure to TDI may cause acute respiratory hypersensitivity in which airway inflammation, represented by eosinophil infiltration, and Th2 cytokines may play an important role. This animal model may be a suitable model for exploring the mechanism underlying TDI-induced occupational airway hypersensitivity. *Ryukyu Med. J., 23(4) 123～131, 2004*

Key words: toluene diisocyanate, airway hypersensitivity, eosinophils, cytokines

INTRODUCTION

Two important types of allergic diseases caused by exposure to exogenous substances at the workplace are allergic contact dermatitis and asthma. Occupational asthma is the most frequently diagnosed form of acute occupational respiratory disease in the industrialized countries[1,2]. Toluene diisocyanate (TDI), a low molecular weight chemical widely used in industries as a polymerizing agent in the production of polyurethane, has been reported to be one of the most important causes of occupational respiratory hypersensitivity[3]. Although the clinical aspects of TDI induced occupational respiratory hypersensitivity have been well defined, the mechanisms involved in the pathogenesis remain unknown. In order to better understand the pathogenesis of TDI induced occupational respiratory hypersensitivity, several animal models have been developed by using cutaneous, intradermal, intranasal, and head only inhalation exposures to TDI[4-9]. However, these methods of exposure to TDI did not closely resemble the exposure condition observed in industrial fields since the individuals are usually whole bodily exposed to TDI atmospheres at the workplace. To establish an in vivo animal model resembling industrial exposure, Wistar rats were placed in a chamber and exposed whole bodily to TDI atmosphere in the present study. Also, it is obscure whether airway hypersensitivity induced by a repeated short period of TDI exposure is caused by immunological and inflammatory response or

simply by TDI's chemical irritant. To elucidate this problem, the rats were sensitized with a repeated exposure to TDI vapor, and the inflammatory and immunological events in the airways were investigated.

Occupational respiratory hypersensitivity has been reported to be a complex pathophysiological event involving the interaction of many cell types and cytokines. Evidence has shown that activation of selected T cells with subsequent eosinophil recruitment and secretion of eosinophil derived mediators may contribute to both epithelial cell damage and airway hyperresponsiveness[10]. Furthermore, studies have suggested that T cell derived cytokines may play a role in the initiation of eosinophilic inflammation[11, 12]. T cell-mediated inflammatory reaction has been hypothesized to be involved in the pathogenesis of TDI induced asthma through an array of cytokines. Therefore, the present study was conducted to further investigate the status and the role of infiltration of inflammatory cells, particularly eosinophils, and the secretion and the role of Th2 cytokines in airway hyperresponsiveness induced by TDI in a rat model exposed to TDI atmosphere.

MATERIALS AND METHODS

Animals

Fifteen 8 weeks old female Wistar rats, weighing 240 ± 30 g obtained from Kyudo Breeding Laboratory (Kumamoto, Japan) were used for the experiment. Upon arrival the rats were kept in a room at a constant temperature (25 ± 2℃) and humidity (50-70%) and at a 12 h light dark cycle. The animals were housed in pathogen free steel mesh cages under environmentally controlled conditions in compliance with the Ryukyus University Policy on Animal Care and Use. Food and water were provided ad libitum through out the experimental period except during the exposure periods. The animals were allowed to acclimatize to our laboratory for a week, and then 5 rats were randomly selected for the control group. The remaining 10 rats were randomly divided into 2 exposure groups (1 and 2) with each exposure group containing 5 rats.

Exposure procedures and quantification of TDI concentrations

The rats were placed in a 22 liters glass chamber with a dynamic adjustable laminar airflow and exposed to TDI vapor, 4 h per day for 5 consecutive days. The TDI atmosphere in the chamber was generated by bubbling air at a rate of 22 liters per minute through a flask containing 10 ml of 2-4 toluene diisocyanate (TDI, Wako Chemical Co., Japan) into the chamber. TDI concentrations were adjusted by varying the airflow rate through the flask. A constant concentration was maintained in the chamber by a constant airflow meter rate during the entire exposure period. Moreover, the concentration was checked every 30 min in order to keep a constant concentration in the chamber. This generation system produced only TDI vapor. The concentrations of TDI atmospheres in the chamber were determined according to Marcali[13], which was modified by NIOSH[14]. Briefly, the air in the chamber was sampled with an impinger containing an absorber medium made from acetic and hydrochloric acids. Several solutions such as diazotization solution containing sodium nitrite and sodium bromide, sulfamic acid solution, N-1-Napthylethylenediamine and sodium carbonate solution, were then added to the absorber medium, respectively. When a reddish-blue colored solution was finally obtained, its transmittance was measured with a spectrophotometer at 550 nm. TDI concentration was evaluated from a calibration curve of a series of standardized TDI solutions prepared by plotting transmittance versus TDI concentrations. The TDI concentrations used were 0.38 ± 0.07 ppm and 1.20 ± 0.22 ppm for exposure groups 1 and 2 respectively. The control group was placed in the same chamber and treated under the same condition with physiological saline instead of TDI in the flask.

Observation of airway hypersensitivity symptoms

After each day's exposure, airway hypersensitivity symptoms, such as the occurrence of sneezing, hyperrhinorrhea, instability and exertional breathing characterized by wheezing and coughing were observed[15].

Bronchoalveolar lavage (BAL)

Twenty-four hours after a 5-day exposure, the rats in both the exposure and control groups were weighed and anesthetized by intraperitoneal injection of 50 mg/kg of sodium pentobarbital. A cervical incision was made and the trachea was isolated. A catheter was then inserted into the trachea and

Table 1 Hypersensitivity symptoms in the airways of wistar rats

Treatment	Hypersensitivity symptoms				
	Sneeze	Hyperrhinorrhea	Cough	Exertional breathing	Instability
Control group	-	-	-	-	-
Exposure group 1					
0.38 ± 0.07 ppm TDI					
Day 1	-	-	-	-	-
Day 2	-	-	-	-	-
Day 3	-	-	-	-	-
Day 4	+	+	+	+	+
Day 5	++	++	++	+++	++
Exposure group 2					
1.20 ± 0.22 ppm TDI					
Day 1	-	-	-	-	-
Day 2	+	+	+	+	++
Day 3	++	++	++	++	++
Day 4	++	++	++	+++	++
Day 5	+++	+++	+++	++++	+++

Symbols represent the severity of the symptoms. −, no ; +, mild; ++, moderate; +++, severe; ++++, very severe.

bronchoalveolar lavage (BAL) was performed. The trachea was slowly infused with 1.5 ml steriled phosphate buffered saline (PBS, 37℃). The effluent (BAL fluid) was recovered (about 0.90 ml, 60%) with gentle aspiration and centrifuged at 500 g (4℃), then the supernatant was collected and stored at −80℃ until cytokines determination. To collect the BAL cells, the trachea was further infused 4 times with PBS (37℃) using 2 ml each time. All the recovered aliquot was then pooled and centrifuged at 500 g (4℃). The cell pellet was resuspended in RPMI 1640 medium (Gibco, Life Technologies, NY, USA) and the total number of cells and their viability were determined using 0.2% trypan blue exclusion method. To perform the differential leukocyte cell count, 0.1 ml of the cell suspension was smeared on a glass slide and stained with Wright-Giemsa. Three hundred nucleated cells were then examined under a microscope. The cells were classified either as macrophages, neutrophils, eosinophils or lymphocytes.

Cytokine assay

IL-2, IL-4 and IL-6 productions in the BAL fluid were quantified respectively with rat IL-2, IL-4 and IL-6 ELISA kits (Endogen, Inc, MA, USA) according to the protocols recommended by the manufacturer. The inter-assay and intra-assay coefficient of variation (CV) were <10%. The sensitivities of the assays were <5 pg/ml, <2 pg/ml and <8 pg/ml for IL-2, IL-4 and IL-6 respectively.

Lung histopathology

Immediately after BAL was performed, 1.5 ml formaldehyde was injected through the catheter into the trachea. The lungs of each rat were resected and fixed in buffered formalin. A section encompassing the maximum cross sectional area of the right lung was taken perpendicular to the major bronchi. The lung tissue was dehydrated through a series of ethanol solutions and then embedded in paraffin. Two μm thick sections were then sliced and stained with hematoxylin eosin for examination. To examine the histopathological changes in the lungs of the rats exposed to TDI, some criteria appropriate for study of respiratory hypersensitivity in animal models reported by Karol were adopted[15]. These criteria included eosinophilic airway inflammation, airway narrowing, airway mucus, airway smooth muscle hypertrophy and airway inflammation edema, etc[15].

Statistical analysis

The cellular contents and cytokine productions are presented as means ± SD. Two-tailed Student's t-test was performed to reveal differences between the means of each exposure group and the control group. Values of $p<0.05$ were considered to be statistically significant.

RESULTS

Hypersensitivity symptoms in the airways

As shown in Table 1, exposure to both 0.38 and

Table 2 Total cell and differential cell counts in the BAL fluid ($x10^5$ cells/ml)

Treatment	Total cells	Differential leucocytes			
		Eosinophils	Neutrophils	Lymphocytes	Macrophages
Control group	15.93±1.24	0.05±0.01	0.26±0.06	1.51±0.41	14.05±1.13
Exposure group 1	25.78±4.62*	0.86±0.29#	1.20±0.39*	2.18±0.75	21.40±3.51#
Exposure group 2	34.18±6.58#	1.51±0.49#	1.63±0.24#	3.16±0.91*	27.68±5.24#

Values are means ± SD. Each group contains 5 rats. *Significantly different from the control group value at $p<0.05$. #Significantly different from the control group values at $p<0.01$.

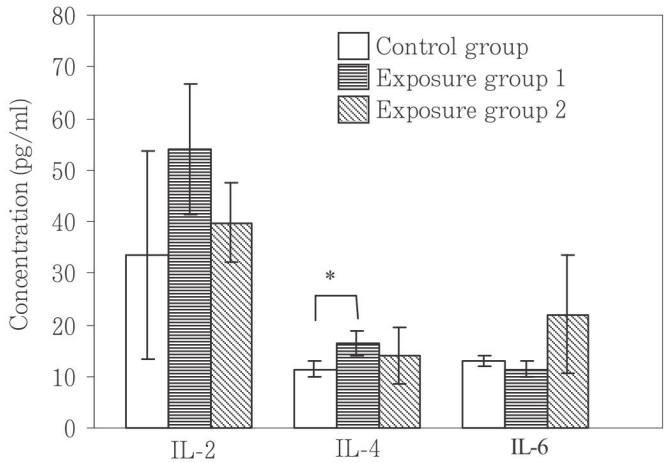

Fig. 1 Cytokine production in the BAL fluid. Values are means ± SD. Each group contains 5 rats. *Significantly different from the control group values at $p<0.05$.

1.20 ppm TDI resulted in airway hypersensitivity symptoms such as sneezing, hyperrhinorrhea, coughing and exertional breathing. When the rats were exposed to 0.38 ppm TDI, the airway symptoms occurred from day 4, and these symptoms became severer after the fifth day's exposure. 1.20 ppm TDI however induced airway symptoms in rats from the second day's exposure, and the symptoms, particularly exertional breathing were much severe, implying that airway hypersensitivity symptoms were aggravated along with the increase of TDI concentration. In the control rats however, there were no obvious respiratory symptoms observed.

Cellular contents in the BAL fluid

BAL fluid cellular analysis demonstrated that exposure to both 0.38 ppm and 1.20 ppm TDI vapors resulted in inflammatory responses in the airways of the rats. As shown in Table 2, a five-day exposure to TDI resulted in a significant increase in the total number of cells and each leukocyte compared to control rats. Eosinophils and neutrophils, in particular, were increased 17.20 and 4.62 times respectively in the rats exposed to 0.38 ppm. Exposure to 1.20 ppm also resulted generally in a further significant in the total number of cells as well as each leukocyte as compared to the control rats. The number of eosinophils and neutrophils in the rats exposed to 1.20 ppm were increased by 30.2 and 6.26 times, respectively.

Cytokines in the BAL fluid

The cytokine production in the BAL fluid was determined with ELISA test. As shown in Fig.1, production of IL-2 in exposure groups 1 and 2 were not significantly different from those in the control group ($p=0.12$ and $p=0.53$, respectively). Similarly, there was no difference between the exposure groups and the control group in IL-6 production ($p=0.16$ and $p=0.23$, respectively). However, IL-4 was significantly increased in exposure group 1 as compared to the control group ($p=0.016$).

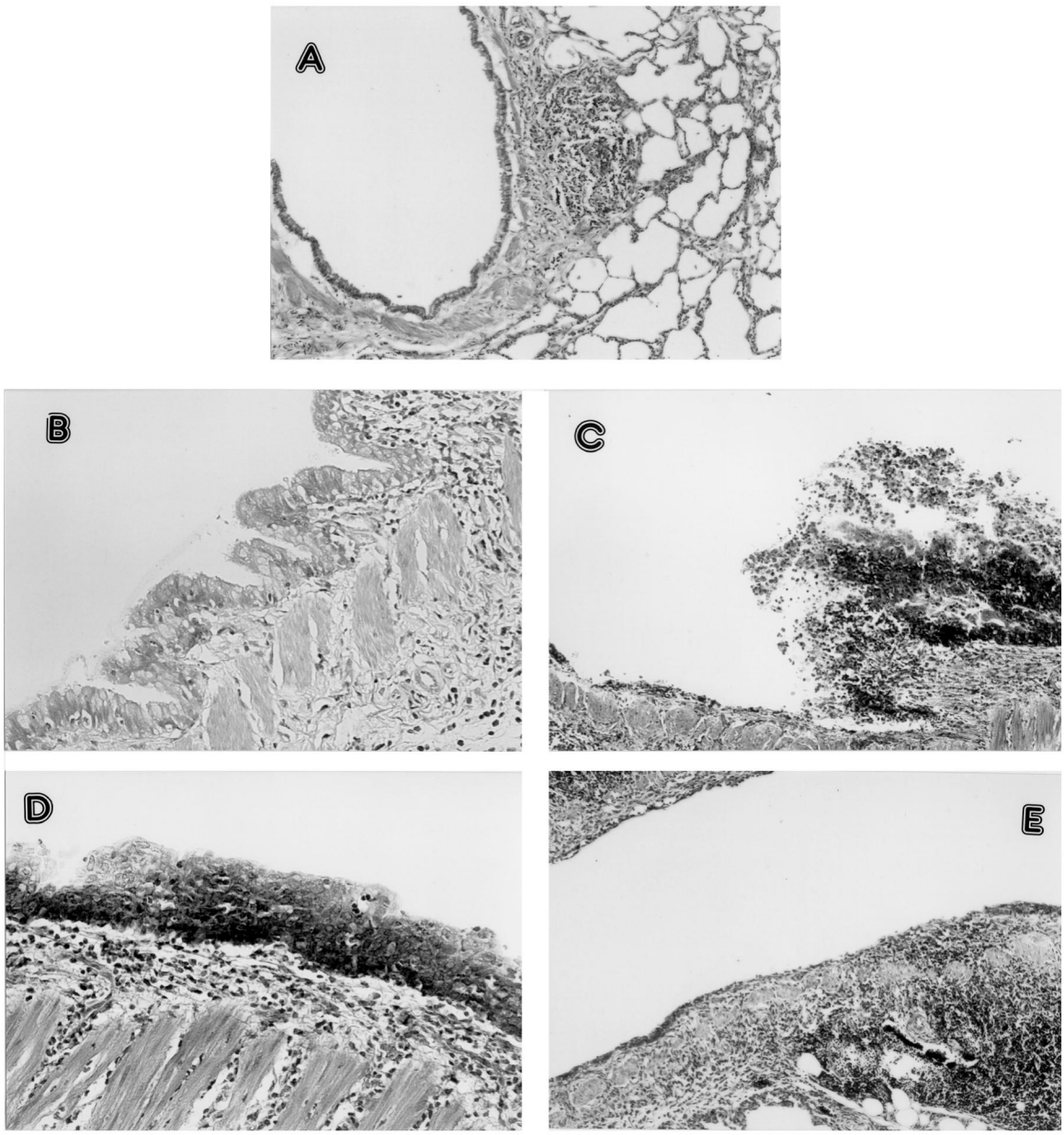

Fig. 2 Light microscopic images of the lungs of the control (A) and TDI-exposed rats (B, C, D and E). In the exposed rats, a few goblet cells are found in the epithelium of the large airways (B), and infiltrations of eosinophils and lymphocytes are present in both the large (B) and small airways (E). Erosion (C) and a few desquamated epithelial cells (D) are also present in the large airways. No obvious pathological changes are found in control rats (A).

Lung histopathological analysis

The histopathological examination showed that inflammatory events occurred in the lungs of the rats exposed to 0.38 ppm and 1.20 ppm TDI (Fig.2). Infiltration of inflammatory cells such as eosinophils, lymphocytes were presented in both the large and small airways of the rats exposed to TDI. A few goblet cells were found in the epithelium, but generally goblet-cell hyperplasia was mild in the large airways of rats. A few desquamated epithelial cells were also presented in the large airways. Squamous metaplasia was observed in the large airways, while erosion was also found in both the large and small airways. Based on these histopathological findings, it was revealed that 5 days' exposure to TDI resulted in chemical bronchitis in the exposed animals.

DISCUSSION

Animal models are extremely important for research and /or testing purposes in the study of industrial chemicals. For occupational pulmonary hypersensitivity, animal models are particularly important because of our current incomplete understanding of the mechanisms involved in chemical sensitization[15]. Several animal models have been developed to understand the mechanisms underlying TDI induce airway hypersensitivity[4-9]. In contrast to animal models previously reported, a Wistar rat model of airway hypersensitivity by whole-bodily exposing the animals to TDI vapor in a glass chamber was established in this study. It was considered that the whole body exposure method used in the present study has an advantage over the other methods in that it closely resembles the individual's exposure to TDI vapor at the workplace. The results from this study show that this exposure method is capable of inducing airway hypersensitivity in the exposed animals implying that this type of animal model can be used for investigating mechanisms underlying TDI induced respiratory hypersensitivity.

It has been reported that not only the exposure method but also the exposure concentrations are important for the development of hypersensitivity symptoms in animal models exposed to TDI[9]. A previous study by Karol has revealed that exposing guinea pigs to 0.12 ppm of TDI vapor did not result in any respiratory hypersensitivity. In contrast, 0.36 ppm or greater resulted in airways hypersensitivity. 2 ppm was pneumotoxic and few pulmonary hypersensitivity reactions were observed[9]. Considering Karol's experiment together with our preliminary ones in rats (data not shown), in the present study we used 0.38 ppm and 1.20 ppm TDI to sensitize the rats. The results show that both 0.38 ppm and 1.20 ppm TDI induced airway hyperresponseness in the exposed rats.

A report by Tanaka *et al*[16] has shown that guinea pigs sensitized and subsequently challenged repeatedly with nasal applications of TDI showed asthma-like symptoms such as exertional breathing, prolonged expiration, and cough immediately after the challenge. In the present study, although the rats were repeatedly exposed to TDI vapor without challenge, they showed airway hypersensitivity symptoms. The results of airway inflammatory cell dynamics by means of BAL fluid, as well as cytokine productions revealed that repeated exposure to 0.38 ppm or 1.20 ppm of TDI resulted in airway hyper sensitivity in which inflammatory and immunological inter-response may be involved. These data were consistent with those in a guinea pig model where the guinea pig was repeatedly exposed to a series of TDI vapor[9]. In that experiment, only head of the guinea pig was exposed to TDI vapors, 3 h per day for 5 consecutive days. An immunologic response (TDI specific IgE) occurred and was exposure concentration dependent. In the present study, although only two concentrations (0.38 ppm and 1.20 ppm) of TDI vapor were used to sensitize the rats, the result revealed that exposure to a higher concentration (1.20 ppm) of TDI vapor resulted in severer immunologic and inflammatory responses characterized with a higher productions of cytokines and a more prominent infiltration of eosinophils in the airways as compared with exposure to a lower concentration (0.38 ppm) of TDI. This supports a concentration dependent relationship between TDI exposure and immuno-inflammatory responses.

Occupational exposure to TDI is usually through cutaneous or respiratory route. The four patterns of respiratory response to TDI have generally been summarized as: chemical bronchitis at high concentrations, an asthma-like condition elicited in sensitized workers by low concentrations of TDI, acute falls in ventilatory capacity during one workshift and chronic decrement in pulmonary function with prolonged exposure. TDI induced chemical bronchitis in animals have been established in some laboratories[17,18]. In a previously reported guinea pig model exposed to 1.4 ppm TDI for 3h per day for 5 consecutive days, the ventilatory response of the animals to a 10% CO_2 challenge was greatly diminished, and the histopathological examination showed that chemical bronchitis was produced in the animals following TDI exposure[19]. In the present study, the histopathological findings indicated that TDI-induced chemical bronchitis occurred in the exposed rats. These results are in accordance with previous ones obtained from guinea pigs[19], further supporting the notion that a short period of exposure to high concentrations of TDI may result in acute chemical bronchitis. Furthermore, these results reveals that the present animal model may be

capable of being used to explore the pathogenesis underlying TDI induced respiratory hypersensitivity.

Typical histopathologic changes related to asthma such as airway narrowing, inflammatory edema or muscular hypertrophy were not observed in this study. The reasons for the absence of these characteristic asthmatic histopathological features might be explained by the shortness of the exposure time. In spite of the lack of these pathological findings, eosinophil infiltration of the airways of the rats further supports previous reports that airway inflammation characterized with eosinophil accumulation may play a critical role in the pathogenesis of occupational airway hypersensitivity.

Some studies have demonstrated a strong cor relation between airway hypersensitivity and eosinophil numbers in biopsies[20], sputum[21] and blood[22]. Eosinophils are important proinflammatory cells in the airway hyperresponse process. These cells release toxic granule proteins such as major basic protein (MBP) and eosinophil cationic protein (ECP)[23,24], which injure the airway epithelium. In addition to the basic proteins they release, the membrane of eosinophils generates phospholipid mediators such as platelet-activating factor (PAF), leukotriene C4 and oxygen metabolites. These lipids have a profound potential to injure the airway epithelium, increase bronchial responsiveness, exacerbate inflammatory response and contract airway smooth muscle[23, 24]. Eosinophils therefore possess properties that can directly and indirectly cause airway obstruction and promote bronchial hyperresponsiveness.

It has also been reported that airway hypersensitivity is a specialized form of cell mediated immunity in which immunological mediators such as lymphokines and cytokines secreted principally by activated CD4+ T lymphocytes orchestrate the accumulation and activation of specific granulocyte effector cells, particularly eosinophils[25]. Bronchial inflammation in airway hypersensitivity may depend in part on the activation of T helper lymphocytes that elaborate proinflammatory cytokines[26,27]. Studies have shown that the Th2 type cytokines interleukin (IL)-4 and IL-5 have an important role in atopic disease and more specifically in atopic airway hypersensitivity[28-30]. IL-4 is involved in the switch of B cells to IgE production[31] and IL-5 has been shown to cause eosinophil infiltration into the airway wall[32]. Both IL-4 and IL-5 have been implicated in the TDI-induced occupational airway hypersensitivity[33]. Animal models exposed to TDI have also revealed the important role of IL-4 and IL-5 in airway hypersensitivity[6]. The significant increase of IL-4 production (but not IL-2 and IL-6) in the airways of TDI-exposed rats in this study further supports the important role of Th2 cytokines in airway hypersensitivity.

The shortcoming of the present study is that IL-5 production was not determined due to the unavailability of commercial rat IL-5 kit at the time the experiment was carried out. Lacking data on IL-5 productions could hamper the explanation of the regulation of eosinophil infiltration and the conclusion of the role of Th2 cytokines in this animal model induced by TDI.

From the results of this study, it can be concluded that a short period of repeated whole body exposure to high concentrations of TDI may result in acute airway hypersensitivity in rats and that eosinophil infiltration and Th2 type cytokines may play an important role in the pathogenesis in this airway hypersensitivity. The animal model used in this study provides the opportunity to further explore the mechanisms underlying TDI induced occupational airway hypersensitivity.

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This animal experiment was done according to the regulation of Japanese laws related to the use of animal for research.

REFERENCES

1) Meredith S.K., Taylor V.M. and McDonald J.C.: Occupational respiratory disease in the United Kingdom 1989: a report to the British Thoracic Society and Society of Occupational Medicine by the SWORD project group. Br. J. Ind. Med. 48: 292-298, 1991.

2) Ross D.J., Sallie B.A. and McDonald J.C.: SWORD's 1994: surveillance of work related and occupational respiratory disease in the UK. Occup. Med. 45: 175-178, 1995.
3) Bernstein I.L.: Isocyanate induced pulmonary diseases: a current perspective. J. Allergy Clin. Immunol. 70: 24-31, 1982.
4) Abe Y., Ogino S., Irifune M., Imamura I., Liu Y.Q., Fukui H. and Matsunaga T.: Histamine content, synthesis and degradation in nasal mucosa and lung of guinea pigs treated with toluene diisocyanate (TDI). Clin. Exp. Allergy. 23: 512-517, 1993.
5) Niimi A., Amitani R., Yamada K., Tanaka K.I. and Kuze F.: Late respiratory response and associated eosinophilic inflammation induced by repeated exposure to toluene diisocyanate in guinea pigs. J. Allergy Clin. Immunol. 97: 1308-1319, 1996.
6) Dearman R.J., Basketter D.A. and Kimber I.: Characterization of chemical allergens as a function of divergent cytokine secretion profiles induced in mice. Toxicol. Appl. Pharmacol. 138: 308-316, 1996.
7) Patterson R., Zeiss C.R. and Harris K.E.: Immunologic and respiratory responses to airway challenges of dogs with toluene diisocyanate. J. Allergy Clin. Immunol. 71: 604-611, 1983.
8) Karol M.H., Hauth B.A., Riley E. J. and Magreni C.M.: Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs. Toxicol. Appl. Pharmacol. 58: 221-230, 1981.
9) Karol M.H.: Concentration dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicol. Appl. Pharmacol. 68: 229-241, 1983.
10) Bentley A.M., Maestrelli P., Saetta M., Fabbri L.M., Robinson D.S., Bradley B.L., Jeffery P.K., Durham S.R. and Kay A.B.: Activated T lymphocytes and eosinophils in the bronchial mucosa in isocyanate induced asthma. J. Allergy Clin. Immunol. 89: 821-829, 1992.
11) Maestrelli P., Di Stefano A., Occari P., Turato G., Milani G., Pivirotto F., Mapp C.E., Fabbri L.M. and Saetta M.: Cytokines in the airway mucosa of subjects with asthma induced by toluene diisocyanate. Am. J. Respir. Crit. Care Med. 151: 607-612, 1995.
12) Mattoli S., Colotta F., Fincato G., Mezzetti M., Mantovani A., Patalano F. and Fasoli A.: Time course of IL1 and IL6 synthesis and release in human bronchial epithelial cell cultures exposed to toluene diisocyanate. J. Cell. Physiol. 149: 260-268, 1991.
13) Marcali K.: Microdetermination of toluene diisocyanates in atmosphere. Anal. Chem. 29: 552-558, 1957.
14) NIOSH US department of health, education and welfare, Washington DC: 2-4-toluene diisocyanate (TDI) in air. In: Manual of analytical methods, 2nd ed. 1: 1411-1418, 1977.
15) Karol M.H.: Animal models of occupational asthma. Eur. Respir. J. 7: 555-568, 1994.
16) Tanaka K., Kawai M. and Maekawa N.: Experimental model of asthma by toluene diisocyanate (TDI). Bull. Chest. Dis. Res. Inst. Kyoto Univ. 16: 1-9, 1983.
17) Niewenhuis R., Scheel L., Stemmer K. and Killens R.: Toxicity of chronic low level exposures to toluene diisocyanate in animals. Am. Ind. Hyg. Assoc. 26:143-149, 1965.
18) Thompson G.E. and Scheel L.D.: Alteration of lung pathology from diisocyanate by glycemic or sensitizing agents. Arch. Environ. Health. 16: 363-370, 1968.
19) Wong K.L., Karol M. H. and Alarie Y.: Use of repeated CO_2 challenges to evaluate the pulmonary performance of guinea pigs exposed to toluene diisocyanate. J. Toxicol. Environ. Health 15: 137-148, 1985.
20) Moqbel R., Barkans J.R., Bradley B., Durham S.R. and Kay A.B.: Application of monoclonal antibodies against major basic protein (BMK-13) and eosinophil cationic protein (EG1 and EG2) for quantifying eosinophils in bronchial biopsies from atopic asthma. Clin. Exp. Allergy. 22: 265-273, 1992.
21) Frigas E., Loegering D.A., Solley G.O., Farrow G.M. and Gleich G.J.: Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo Clin. Proc. 56: 345-353, 1981.
22) Durham S.R. and Kay A.B.: Eosinophil bronchial hyperreactivity and late asthmatic reactions. Clin. Allergy 15: 411-418, 1985.
23) Gleich G.J.: The eosinophil and bronchial asthma: current understanding. J. Allergy Clin. Immunol. 85: 422-436, 1990.
24) Weller P.F.: The immunobiology of eosinophils.

N. Engl. J. Med. 324: 1110-1118, 1991.
25) Kay A.B.: Asthma and inflammation. J. Allergy. Clin. Immunol. 87: 893-910, 1991.
26) Walker C., Virchow J.C. Jr., Bruijnzeel P.L.B. and Blaser K.: T cell subsets and their soluble products regulate eosinophilia in allergic and non-allergic asthma. J. Immunol. 146: 1829-1835, 1991.
27) Azzawi M., Bradley B., Jeffery P.K., Frew A.J., Wardlaw A.J., Knowles G., Assoufi B., Collins J.V., Durham S. and Kay A.B.: Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am. Rev. Respir. Dis. 142: 1407-1413, 1990.
28) Daher S., Santos L.M., Sole D., De Lima M.G., Naspitz C.K. and Musatti C.C: Interleukin-4 and soluble CD23 serum levels in asthmatic atopic children. J. Invest. Allergol. Clin. Immunol. 5: 251-254, 1995.
29) Seder R.A., Paul W.E., Davis M.M. and Fazekas de St Groth B.: The presence of IL4 during in vitro priming determines the lymphokine producing potential of CD4+ T cells from T cell receptor transgenic mice. J. Exp. Med. 176: 1091-1098, 1992.
30) Bjorksten B., Borres M.P. and Einarsson R.: Interleukin-4, soluble CD23 and interferon-gamma levels in serum during the first 18 months of life. Int. Arch. Allergy immunol. 107: 34-36, 1995.
31) Le gros G., Ben-Sasson S.Z., Seder R., Finkelman F.D. and Paul W.E.: Generation of interleukin-4 (IL-4) producing cells in vivo and in vitro: IL-2 and IL-4 are regulated for in vitro generation of IL-4 producing cells. J. Exp. Med. 172: 921-929, 1990.
32) Campbell H.D., Tucker W.Q., Hort Y., Martinson M.E., Mayo G., Clutterbuck E.J., Sanderson C.J. and Young I.G.: Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). Proc. Natl. Acad. Sci. USA. 84: 6629-6633, 1987.
33) Maestrelli P., Occari P., Turato G., Papiris S.A., Di Stefano A., Mapp C.E., Milani G.F., Fabbri L.M. and Saetta M.: Expression of IL-4 and IL-5 proteins in asthma induced by toluene diisocyanate (TDI). Clin. Exp. Allergy 27: 1292-1298, 1997.