Interleukin-4 and Interleukin-5 Expression in Mice Exposed to 2, 4-Toluene Diisocyanate

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Abstract: Interleukin-4 and Interleukin-5 Expression in Mice Exposed to 2, 4-Toluene Diisocyanate: Kui-Cheng ZHENG, et al. Department of Preventive Medicine, Faculty of Medicine, University of the Ryukyus, Okinawa—This study was aimed at investigating the cytokine status and their gene expressions in airways of asthmatic mouse caused by toluene diisocyanate (TDI). After the mice were exposed to TDI vapor for 5 consecutive days, bronchoalveolar lavage (BAL) was carried out and the cells in the BAL fluid were counted. Lung histological test was conducted to analyse the inflammatory status in the airways. Total serum IgE and IL-4 and IL-5 concentrations in the BAL fluid were determined with ELISA kits. RT-PCR was applied to investigate IL-4 and IL-5 mRNA from BAL cells. The airway cell count and histological analysis showed that TDI exposure resulted in airway inflammatory responses characterized by marked infiltration of eosinophils in the central and peripheral airways. Total serum IgE was significantly increased in the TDI-exposed mice. IL-4 and IL-5 productions in the BAL fluid were significantly enhanced in the mice exposed to TDI, but in BAL cells IL-4 and IL-5 mRNA were slightly enhanced without any significant difference compared to the control mice. These results further support the hypothesis that Th2 cytokines are predominantly produced in TDI-induced airway hypersensitivity, but our data were not able to indicate the exact source of these cytokines and we could not determine whether the change in cytokines in the airways was controlled by the gene transcription level. (J Occup Health 2002; 44: 421–426)

Key words: TDI, Airway hypersensitivity, Cytokine, mRNA, BAL, Mice

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Materials and Methods

Animals

Specific pathogen free ten-week-old female BALB/c mice were used for the experiment. They were kept in a room at constant temperature (25 ± 2°C) and humidity (50% to 70%) with a 12-h light period. Animal care was in compliance with the guidelines of the Policy on Animal
Care and Use of the University of the Ryukyus. After a one week period of adaptation to our laboratory, the mice were randomly assigned to two groups: control and TDI exposure group. Each group contained 8 mice. Both groups were given free access to food and water during the experiment.

**TDI Exposure**

The mice were placed in a 22 l glass chamber and exposed to 2,4-toluene diisocyanate (TDI) vapor, 4 h per day, on 5 consecutive days. The TDI vapor was generated by bubbling dried air through an impinger containing TDI. The air was drawn into the chamber by means of a pump at 23 l/min. The TDI concentration in the chamber was assessed by air sampling from the chamber and analysis by the published method which was a modification of Marcali’s.

Briefly, the air was drawn through an impinger containing an absorber medium made from acetic and hydrochloric acids. Diazotization solution containing sodium nitrite and sodium bromide, sulfamic acid solution, N-1-Naphthylethylenediamine and sodium carbonate solution was added to the absorber medium. A reddish blue solution was formed, which was then measured with a spectrophotometer at 550 nm. The TDI concentration was evaluated from a calibration curve of a series of standard TDI solutions prepared by plotting transmittance against concentration in the chamber was 1.35 ± 0.27 ppm.

**Bronchoalveolar Lavage (BAL)**

On the 7th day, under anaesthesia with sodium pentobarbital, bronchoalveolar lavage (BAL) was performed in the mice. Briefly, the trachea was infused with 1.5 ml of PBS (37°C), the effluent was recovered and centrifuged at 500 g (4°C) and the supernatant (BAL fluid) was collected and stored at −80°C until cytokine determination. The trachea was then infused four times with 2 ml of PBS (37°C), and the recovered fluid was centrifuged at 500 g at 4°C. The cell pellet was resuspended in RPMI 1640 and counted with a hemacytometer. Cell viability was determined with 0.2% trypan blue. To perform the differential cell count, 0.1 ml of cell suspension was smeared on a glass slide and stained with Wright-Giemsa and 300 cells were then examined.

**Total RNA and RT-PCR**

Total RNA from 1 × 10⁶ BAL cells was isolated by using TRIZOL Reagent (GIBCO BRL, LIFE Technologies, USA) according to the manufacturer’s instructions. The RNA pellet was dissolved in RNase-free water and quantitated by absorbance at 260 nm. Both cDNA synthesis and PCR were performed in a single tube by using SUPERSCRIPTTM One-Step RT-PCR with Platinum Taq (GIBCO BRL, LIFE Technologies, USA) according to the manufacturer’s instructions. Briefly, a 50-ul of reaction volume was cycled once at 50°C for 20 min and then at 94°C for 2 min for cDNA synthesis and pre-denaturation. PCR amplification was performed as follows: denaturation at 94°C for 15 s, annealing at 60°C for 30 s and extension at 70°C for 1 min for 36 cycles, followed by a final cycle of 72°C for 10 min. IL-4 primer (3’ GATGTAACCAGGAGCATGTA, and 5’ CTCAGTACTCGAGTATCCA) and IL-5 primer (3’ GACAGCATGACACAGTGA, and 5’ GAAGCTTGCAGGTAATCCAG) were synthesized by Hokkaido System Science. PCR products were run on 2% agarose gels and visualized by ethidium bromide staining. The densities of the PCR products were measured with NIH Image 1.61 software.

**Cytokine Assay**

IL-4 and IL-5 productions in the BAL fluid were quantified with commercial mouse IL-4 and IL-5 ELISA kits (Endogen, Inc, MA, USA), according to the manufacturer’s instructions. The inter-assay and intra-assay CV were <10%. The sensitivities of the assays were <5 pg/ml, <5 pg/ml for IL-4 and IL-5 respectively.

**Total Serum IgE**

The serum was prepared from blood taken from the cervical vein and the total serum IgE concentration was determined with a mouse IgE kit (Mouse IgE EIA kit Yamasa, Yamasa Shoyu Co, Ltd, Japan), according to the manufacturer’s instructions.

**Histopathological Analysis**

The lungs were resected and fixed in buffered formalin, then dehydrated through a series of ethanol solutions, and embedded in paraffin. 2 µm thick sections were sliced and stained with hematoxylin-eosin for examination.

**Statistical Analysis**

Statistical analysis was performed by two-tailed Student’s t-test. Probability values of p<0.05 were accepted as significant.

**Results**

**Respiratory Hypersensitivity Symptoms**

The TDI-exposed mice demonstrated irritability, aggression, sneezing and hyperrhinorrhoea. The mice opened their mouths to gasp for air and exhibited exertional breathing. Prolongation of the expiratory phase was observed in all the TDI-exposed mice. In the control mice, no airway symptoms were observed.

**BAL Cell Counts**

An airway inflammatory reaction was induced in the mice after exposure to TDI. As shown in Table 1, the
that TDI exposure caused airway hypersensitive respiratory tract were then analyzed. The results showed cytokine profiles and their gene expressions in the consecutive days. The airway inflammation and the through exposure to 1.35 mouse airway hypersensitivity model was established change (Fig. 4).

Peripheral airways showed signs of any major pathologic was noted, but in the control mice, neither the central nor central and peripheral airways with numerous eosinophils were significantly higher than in the control mice.

**Histopathological Study**

In the TDI-exposed mice, a marked infiltration of the central and peripheral airways with numerous eosinophils was noted, but in the control mouse, neither the central nor peripheral airways showed signs of any major pathologic change (Fig. 4).

**Discussion**

Exposure of animals to TDI has been established in many laboratories to better understand this low-molecular-weight chemical and occupational respiratory disorders induced by exposure to it\(^{11}\). In this study, a mouse airways hypersensitivity model was established through exposure to 1.35 ± 0.27 ppm of TDI vapor for 5 consecutive days. The airway inflammation and the cytokine profiles and their gene expressions in the respiratory tract were then analyzed. The results showed that TDI exposure caused airway hypersensitive symptoms in mice, and inflammatory events characterized by prominent eosinophil infiltration were seen in both the central and peripheral airways of mice. In TDI-exposed mice, not only serum IgE concentrations were increased, but also IL-4 and IL-5 concentrations in the BAL fluid in vivo were increased. Nevertheless, IL-4 and IL-5 gene expressions in the BAL cells were not significantly increased in TDI-exposed mice compared to control mice.

In our previous study, we reported that Th2 cytokine IL-4 and IL-5 were preferentially secreted in splenocytes and thymocytes in BALB/c mice intranasally sensitized with TDI\(^{12}\). Our further studies on Wistar rats showed that IL-4 and IL-6 were predominantly produced in the airways after intranasal exposure to TDI\(^{11}\). Unlike the exposure method with intranasal administration of TDI solution used in our previous studies, in this study we sensitized the mice with a low concentration of TDI vapor in a chamber. We think that the exposure method used in this study has the advantage that it more closely mimics industrial field exposure. The purposes of this study were to further investigate the cytokine profiles and their gene expressions in an asthmatic mouse model with a different exposure method and a different TDI concentration.

In this study our data indicated that IL-4 and IL-5 in the TDI-exposed mice were significantly produced, further supporting the concept that Th2 type cytokines are predominantly secreted in airways made hypersensitive by TDI exposure. Although both the exposure method and the exposure concentration in this study were different from those in our previous studies, the results of this study were in broad agreement with those of our previous studies. Studies in allergic asthmatic patients have also indicated the existence of a Th2-like cytokine pattern in bronchoalveolar lavage (BAL) fluid and BAL cells. It has also been reported that in BAL fluid and biopsies the relative proportions of cells expressing Th2 cytokines are more significantly increased in symptomatic than in asymptomatic asthmatic patients\(^{12-14}\), implying that Th2 cytokine may be relevant to airway hypersensitivity. Our results in this study are generally consistent with these studies in asthmatics.

Th2 cytokines may favor the induction of acute allergic responses and immediate-type hypersensitive reactions and therefore may play an important role in the inflammatory reactions and airway hypersensitivity in total number of BAL cells in the TDI-exposed group was significantly increased as compared to the control group. The cell differential count revealed that the number of each type of leukocyte, especially the number of eosinophils, in the TDI-exposed mice was significantly higher than that in the control mice.

**IL-4 and IL-5 concentrations in BAL Fluid**

As shown in Fig. 1, the concentration of IL-4 in the BAL fluid in the TDI-exposed mice was significantly increased as compared to that in the control mice. Similarly, the IL-5 concentration in the exposed mice was also significantly higher than that in the control mice.

**IL-4 and IL-5 mRNA from BAL Cells**

As shown in Fig. 2, although IL-4 and IL-5 mRNA levels in the TDI-exposed mice were slighted increased as compared to those in the control mice, these differences in gene transcriptional levels between the two groups did not reach levels of statistical significance (p>0.05).

**Total Serum IgE Production**

Exposure to TDI resulted in a significant increase in total serum IgE. As shown in Fig. 3, the total serum IgE concentrations in the TDI-exposed mice were significantly higher than in the control mice.

**Histopathological Study**

In the TDI-exposed mice, a marked infiltration of the central and peripheral airways with numerous eosinophils was noted, but in the control mouse, neither the central nor peripheral airways showed signs of any major pathologic change (Fig. 4).

**Table 1. Total Numbers of Cells and Differential Cells in BAL Fluid (×10⁵ cell/ml)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cells</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.89 ± 0.43</td>
<td>0.01 ± 0.005</td>
<td>0.02 ± 0.004</td>
<td>0.40 ± 0.06</td>
<td>2.37 ± 0.37</td>
</tr>
<tr>
<td>TDI-exposed</td>
<td>5.34 ± 0.69*</td>
<td>0.17 ± 0.05*</td>
<td>0.09 ± 0.02*</td>
<td>1.74 ± 0.29*</td>
<td>3.19 ± 0.69*</td>
</tr>
</tbody>
</table>

Values are the mean ± SD, each group contains 8 mice, *p<0.01 and #p<0.05 compared to control values.
allergic asthma. IL-4 is known to be essential for the priming of Th2 cell differentiation and for promoting the secretions of IgE antibodies. IgE plays a predominant role in the elicitation of immediate hypersensitivity reactions associated with respiratory allergy\textsuperscript{15, 16}. IL-5 promotes the development, localization, and functioning of eosinophils\textsuperscript{17, 18}, which contribute to airway hypersensitivity by releasing mediators, including major basic proteins, leukotrienes and platelet-activating factors that induce epithelial injury and bronchoconstriction.

In order to determine whether the divergent cytokine secretion patterns observed correspond with cytokine gene expression, in addition to cytokine profiles in airway fluid, we simultaneously investigated cytokine mRNA expression in airway cells of mice after exposure to TDI. The RT-PCR results reported here showed that IL-4 and IL-5 mRNA from all types of uncultured BAL cells were increased in mice exposed to TDI, but these increases did not reach statistically significant levels. The reason why the cytokine mRNA expressions from the BAL cells did not completely parallel the cytokine protein expressions in the BAL fluid might be due to the kinetics of the cytokine gene transcriptions not always coincide with that of cytokine secretions.

Fig. 1. Cytokine concentrations in the BAL fluid. Values are the mean ± SD. Each group contains eight mice. * significantly different from control values at p<0.01.

Fig. 2. Cytokine mRNA from the cells in the BAL fluid. Values are the mean ± SD. Each group contains eight mice.
Fig. 3. Total serum IgE concentrations. Each group contains eight mice. *significantly different from control values at \( p<0.01 \).

Fig. 4. Histological sections of the lungs in control (A and C) and TDI-exposed mice (B and D). A number of eosinophilis (arrows) are accumulated in the central (B) and peripheral (D) airways in a lung of a TDI-exposed mouse. No major pathologic changes are found in a lung of a control mouse. Stained with hematoxylin-eosin, light microscopy; original magnification \( \times 400 \).
It has been hypothesized that the potential cellular sources of cytokines detected in the airways of patients with asthma are derived from cells resident or recruited into the airways (alveolar macrophages, T cells, epithelial cells, mast cells, and neutrophils, etc.)\(^9\). Although the results in this study indicated that Th2 cytokines are predominantly produced in asthmatic airways of animal models exposed to TDI, our data on cytokine mRNA expressions in airway cells could not exactly reveal the source of these cytokines.

The shortcoming of this study was that we did not conduct any analysis of the time courses of cytokine protein and gene expression, so that we could not know their secretion and transcription kinetics and their up and down regulation. In order to better understand the exact cellular source and the kinetics of the cytokines and their role in the airway hypersensitivity induced by TDI, further studies need to be carried out with cell sorting techniques, RT-PCR, and in situ hybridization techniques.

References

3) MH Karol, BA Hauth, EJ Riley and CM Magrem: Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs. Toxicol Appl Pharmcol 58, 221–225 (1981)