

Rats Repeatedly Exposed to Toluene Diisocyanate Exhibit Immune Reactivity against Methyl Isocyanate-Protein Conjugates

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Key Words

Occupational asthma · Methyl isocyanate · Isocyanic acid · Toluene diisocyanate · Cross-reactivity · Immunoglobulin G · Bronchoconstriction

Abstract

Background: Volatile monoisocyanates are formed through thermal degradation when products containing polyurethane are heated. Repeated exposure to diisocyanates, such as toluene diisocyanate (TDI) are a well-known cause of occupational asthma. However, although monoisocyanates are abundant in occupational settings, there are few data concerning their ability to provoke immune reactions and asthma. We compared immune reactivity and respiratory disease following single or repeated inhalation exposures to the monoisocyanates methyl isocyanate (MIC) and isocyanic acid (ICA) with the effects of TDI. **Methods:** Isocyanates were administered either as single vapor exposures or as repeated intranasal instillations in rats. Adverse health effects were monitored by analyzing airway inflammation, respiratory function and weight gain. Immune reactivity caused by repeated exposures was studied by analysis of isocyanate-specific antibodies and airway infiltration of immune competent cells. **Results:** Repeated exposures to TDI induced airway infiltration of neutrophils and lymphocytes, while neither MIC nor ICA provoked a detectable inflammatory response. Antibodies against isocyanate-albumin conjugates

were detected in serum after both exposures to TDI and MIC, but not to ICA. TDI-exposed rats also displayed IgG antibodies against MIC-albumin conjugates. Even though MIC did not induce airway inflammation, single exposure provoked an increase in airway resistance and repeated exposures caused weight loss similar to that of TDI. **Conclusions:** Airway exposure to TDI produces an antibody response not only against TDI but also against MIC-protein conjugates. This indicates that immune reactivity against abundant monoisocyanates in occupational environments can occur in individuals pre-sensitized with low abundance but highly sensitizing diisocyanates.

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Introduction

Diisocyanates are used in the manufacture of polyurethane products such as rigid foams, elastomers, glues, and lacquers, and are a well-known cause of occupational asthma [1]. It is reported that up to 10% of workers repeatedly exposed to toluene 2,4-diisocyanate (TDI) develop asthma [2]. In spite of this, the mechanisms for sensitization and asthma are poorly understood. Although diisocyanate-induced asthma shares many features with atopic asthma, several differences have been reported. For example: (1) absence of antigen-specific IgE in many patients [3, 4], (2) a significant inflammatory contribution of neu-

trophils [5, 6] and CD8⁺ T lymphocytes [7, 8], and (3) a mixed Th1/Th2 cytokine profile instead of a Th2-biased T lymphocyte response [9–11]. Like other low molecular weight chemicals, TDI is not antigenic per se but reacts spontaneously with a variety of proteins, such as albumin, keratin, hemoglobin, and ciliated cell tubulin to form immunoactive hapten-protein conjugates [12–14].

The risk of being exposed to isocyanates is not confined to manufacturing of polyurethane products where isocyanates are handled directly. Large amounts of isocyanates are also emitted through thermolysis, when products that contain polyurethane are heated above 200°C, for example during welding, cutting and grinding [15]. New chemical analysis methods have revealed that the vast majority of isocyanates emitted through thermal decomposition are low molecular weight monoisocyanates, in particular methyl isocyanate (MIC) and isocyanic acid (ICA) [16]. In these studies, many of the air samples collected in the breathing zone of the workers contained monoisocyanate levels exceeding 20 ppb, the Swedish threshold limit value for maximum exposure. Near the welding spot the levels could be as high as 300 ppb for ICA and 34 ppb for MIC. The acute toxic properties of MIC have been well studied since the 1984 chemical disaster in Bhopal, India. It mainly exerts its effects on the respiratory tract, resulting in pulmonary edema, bronchial necrosis, interstitial deposits and small airway obstruction [17]. Data regarding toxicity and possible health effects of ICA are not available since the compound was only recently identified as the dominant isocyanate in air samples after thermal degradation of polyurethane [18]. Despite the abundance of monoisocyanates in occupational environments, little is known about their potency in inducing immunotoxic reactions after repeated inhalation exposures. Single exposure to MIC caused production of isocyanate-specific IgG, IgE and IgM antibodies in serum from the Bhopal victims, but since the antibodies were undetectable 1 year after the accident they were considered to have little clinical significance [19]. However, evidence of monoisocyanate immune reactivity has recently been reported, showing that repeated dermal exposures to p-tolyl isocyanate induced increased levels of the Th2 characteristic cytokines IL-4, IL-10 and IL-13 in mice, equal to that of known respiratory sensitizers [20].

Whether the highly volatile monoisocyanates MIC and ICA can evoke an immune response following airway exposure is not known. To investigate the ability of gaseous monoisocyanates to provoke immune reactivity and respiratory disease, we performed repeated airway exposures to MIC and ICA in rats and compared the re-

sponsiveness and adverse health effects with those of TDI. The immune reactivity was monitored by analysis of isocyanate-albumin conjugates in serum and infiltration of immunocompetent cells to the airways. To determine the influence on health status we analyzed inflammatory response in airways, weight loss and effects on respiratory parameters such as airway resistance, compliance, respiratory frequency and tidal volume.

Material and Methods

Chemicals

MIC was purchased from Eastman Chemical Company (Kingsport, Tenn., USA), TDI and dibutylamine (DBA) from Aldrich Chemie GmbH (Munich, Germany), and tetrahydrofuran (THF) from J. T. Baker (Deventer, The Netherlands). Ethyl acetate was purchased from Lab-Scan (Dublin, Ireland) and dried with molecular sieve (4 Å; Fluka AG, St. Gallen, Switzerland) before use. ICA was prepared by modification of a method described by Linhard [21]. A solution of isocyanic acid was prepared by pyrolysis of cyanuric acid at 400–500°C in a stream of argon. In order to avoid polymerization, the gas outlet was allowed to pass through a gas absorption flask containing ethyl acetate or THF, cooled to –78°C.

Animals

Male Wistar rats (130–150 g) were obtained from Scanbur BK AB (Sollentuna, Sweden) for repeated exposure experiments. Single exposure experiments were performed on female Fischer rats (F344, 180–200 g), obtained from Charles River, Germany, to be able to compare the results with previous studies [22, 23]. Upon arrival the animals were quarantined for at least 2 weeks. The animals were housed 5 per cage under standard laboratory conditions and permitted access to food and water ad libitum. The study was approved by the Animal Research Ethical Committee, Umeå, Sweden, in compliance with Swedish law.

Experimental Design

In order to investigate the effects of repeated exposure to isocyanates, male Wistar rats were given repeated intranasal instillations with isocyanate dissolved in ethyl acetate according to Zheng et al. [24]. In brief, rats were instilled with 5 µl of a 0.7 M isocyanate solution into each nostril for 5 consecutive days (days 0–4) under light enfluran (Efrane®; Abbott, Solna, Sweden) anesthesia. Control animals were instilled with ethyl acetate. Ten days later the instillations were repeated over 5 days (days 14–18), and at day 30 the animals were challenged intranasally with instillation of a 0.35 M solution of the isocyanate. The rats were sacrificed at day 31 and samples were collected for analysis. During the experiment period the rats were weighed every third or fourth day and changes in their general condition were observed.

Analysis of Leukocytes in Bronchoalveolar Lavage Fluid

At day 31 the animals were sacrificed, 18–20 h after the intranasal challenge, and bronchoalveolar lavage (BAL) was performed using 5 × 5 ml aliquots of ice-cold Hanks balanced salt solution. The obtained cells were centrifuged (400 g, 10 min, 4°C) and fi-

nally resuspended in PBS before the total amount of leukocytes was counted using trypan blue exclusion in a Bürker chamber. Differential count of leukocytes in BAL fluid was performed in a blinded fashion using duplicate Cytospin (Cytospin 3; Shandon, Runcorn, UK) preparations of 30,000 cells stained with May-Grünwald Giemsa, counting 300 cells per slide.

Flow Cytometry Analysis

Lymphocytes in BAL fluid were analyzed with flow cytometry using the monoclonal antibodies CD3-PE, CD3-FITC, CD4-FITC, CD8-PerCP, CD45RA-PE and NKR-P1A-PE (BD Biosciences Pharmingen, San Diego, Calif., USA). Isotype-matched antibodies were used as negative controls. For staining, 250,000 cells were distributed into tubes and washed in PBS containing 0.1% FBS and 2% milk powder. Non-specific binding was blocked by incubating the cells with 4 μ l rat Fc Block™ (BD Biosciences Pharmingen) and 15 μ l FBS for 5 min on ice. Monoclonal antibodies were added and the cells were incubated for 30 min in the dark. Red blood cells were lysed using FACSe Lysing Solution and the lymphocytes were fixed with BD CellFIX (BD Biosciences, San Jose, Calif., USA) before analysis on BD FACSort. B cells were defined as CD45RA⁺ and CD3⁻, CD4 cells as CD4⁺, CD8 cells as CD8⁺ and CD3⁺, NK cells as NKR-P1A⁺ and CD3⁻. The results are presented as total numbers of the different lymphocytes.

Analysis of Isocyanate-Specific IgG Antibodies in Serum

Serum samples were obtained from the aorta 18–20 h after intranasal challenge with isocyanates. TDI- and MIC-specific IgG antibodies were analyzed with enzyme-linked immunosorbent assay (ELISA) using isocyanate-albumin conjugates. The conjugate was prepared according to a method previously described by Scheerens et al. [25]. In brief, 4.8 μ l TDI was added to 2.5 mg rat serum albumin (RSA) dissolved in 0.05 M sodium phosphate buffer (pH 7.4). The mixture was stirred for 4 h at 37°C and the reaction was stopped by adding 2 μ l monoethanolamine. After 1 h the solution was filtered and dialyzed and finally the total protein content was quantified using Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif., USA). Conjugates with MIC were prepared in the same way except that 1.6 μ l of MIC was added to 2.5 mg RSA and the mixture was stirred for 4 h while kept on ice [19]. For the ELISA, Nunc-Immuno Plates with Max Sorb surface (Tamro MedLab AB, Mölndal, Sweden) were coated with 50 μ l isocyanate-RSA conjugate (50 μ g·ml⁻¹) overnight in 4°C and blocked with 250 μ l 1% BSA in PBS for 1 h. The plates were incubated with 50 μ l of serum (diluted 1:10,000 or 1:10) for 2.5 h and bound isocyanate-specific IgG-antibodies were detected after incubation with 50 μ l biotin-labeled anti-IgG polyclonal antibody (0.5 μ g·ml⁻¹, KPL, Gaithersburg, Md., USA), by using a ready-to-use streptavidin-peroxidase system (Roche Diagnostics, Basel, Switzerland) together with tetramethylbenzidine substrate, according to the manufacturer's instructions. The soluble product was analyzed after 40 min at 620 nm in a Thermo Labsystems iEMS ELISA reader (Vantaa, Finland). The background levels of the ELISA as determined in wells coated with non-conjugated RSA, to which all substrates and serum from each sample were added, was subtracted from all data.

Measurement of Respiration and Airway Resistance

Respiration was recorded during inhalation of vaporized MIC or ICA, in order to further investigate their toxic effects. The

method for measurement of respiratory parameters and the instrumental setup has previously been described in detail by Koch et al. [26]. Briefly, conscious rats were placed in head-out plethysmographs, each connected to a pneumotachograph, and mounted in Battelle nose-only inhalation chambers (RCC Ltd., Geneva, Switzerland). During 30 min exposures to different concentrations (5–45 ppm) of isocyanates the respiratory frequency, tidal and minute volumes were monitored online using the Lfx-system (Mumed Systems, London, UK). Data from the last 10 min of exposure were presented as a percentage change in respiration compared to individual start values. Control animals received air or solvent. For measurement of airway resistance and compliance, rats were anesthetized by an i.p. injection of 120 mg·kg⁻¹ thiobutabarbital (Inactin®, RBI, Natick, Mass., USA). A catheter (Optiva 2, 18G) was inserted into the intra-pleural space for registration of the intra-pleural pressure. The animals were then placed in the plethysmograph and airway mechanics (pulmonary resistance and dynamic compliance) and respiration (tidal volume, respiratory rate and minute volume) were recorded online with 2 pneumotachographs, one connected to the intra-pleural catheter and the other to the plethysmograph. The animals were breathing spontaneously and were exposed for 5 min to air followed by a 30-min exposure to MIC (4.9 ppm), ICA (42 ppm) or THF. Data were presented as a percentage change in resistance compared to individual start values.

The isocyanate vapor was generated by injecting monoisocyanates dissolved in THF (0.75–3.0 mg·ml⁻¹) into a filtered, dried and preheated air stream (15–25 l·min⁻¹, Rh <10%, 25°C) with a peristaltic pump (IPC-16; Ismatec SA, Glattbrugg, Switzerland). The test atmosphere was directed to the inhalation chamber and the concentration of MIC and ICA in the atmosphere was analyzed with a modified DBA-method according to Karlsson et al. [18]. In short, duplicate air samples were collected in the breathing zone of the animals during exposure, using impinger flasks containing internal standards and 10 ml of 0.01 mol·l⁻¹ DBA in toluene. The obtained isocyanate-DBA derivatives were washed with 0.02 M acetic buffer and the upper phase was analyzed with GC-EI-SIR (Hewlett-Packard 5890 Series II gas chromatograph with Hewlett-Packard 5972 mass selective detector).

Statistics

All results were analyzed using 1-way or 2-way ANOVA with Dunnett's or Bonferroni post-hoc corrections versus control animals. *p* values of 0.05 or less were considered significant. All values are reported as mean $\pm$ SEM. Statistical calculations were carried out using GraphPad PRISM 3.02 (GraphPad Software Inc., San Diego, Calif., USA).

Results

Characterization of Airway Leukocyte Infiltrates

Only rats receiving repeated intranasal instillations of TDI displayed an increase of leukocytes in BAL fluid (fig. 1). Differential count of the BAL cells revealed significantly increased numbers of neutrophils (32%) and lymphocytes (1.3%) compared to rats exposed to solvent

Table 1. Number of lymphocytes in BAL fluid from rats repeatedly exposed to isocyanates

Group	T cells	CD8 cells	CD4 cells	B cells	NK cells
Control	4,036 ± 661	843 ± 167	3,109 ± 655	948 ± 181	572 ± 131
TDI	18,985 ± 3,387*	4,131 ± 495*	15,239 ± 3,790*	12,350 ± 2,509*	15,950 ± 2,435*
MIC	1,614 ± 1,199	271 ± 172	1,332 ± 916	366 ± 112	146 ± 24.3
ICA	1,536 ± 159	437 ± 15.0	1,194 ± 110	998 ± 521	224 ± 60.8

* $p < 0.001$ vs. control group exposed to ethyl acetate, using 1-way ANOVA with Dunnett's multiple comparisons test ($n = 4-7$).

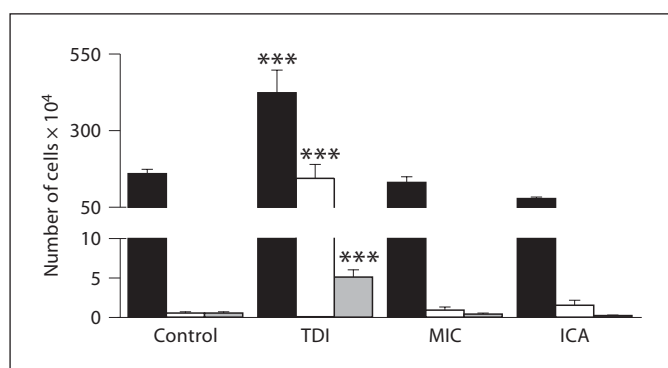


Fig. 1. Number of total leukocytes (black bar), neutrophils (white bar) and lymphocytes (grey bar) in BAL fluid from rats ($n = 4-7$) given repeated intranasal instillations of TDI, MIC, ICA or vehicle alone. *** $p < 0.001$ vs. control, using 1-way ANOVA with Dunnett's multiple comparisons test.

alone. A flow cytometry analysis of the lymphocytes showed a significant increase of B, NK and T cells, the later consisting of both CD8⁺ and CD4⁺ cells (table 1).

Isocyanate-Specific Antibodies in Serum

Serum levels of IgG antibodies, specific to TDI- and MIC-protein conjugates (fig. 2) were analyzed in rats after repeated exposure to isocyanates. Instillation of TDI caused up to 10-fold increases of TDI-specific IgG levels compared to instillations of solvent (fig. 2a). No TDI-specific antibodies were detected in rats exposed to MIC (fig. 2a). Repeated exposure to MIC resulted in MIC-specific IgG antibody production (fig. 2b), but with lower titers than seen for TDI-specific IgG antibodies after TDI treatment (fig. 2a). Notably, reactivity towards MIC-RSA conjugate could not be detected after instillations with ICA, but in serum from TDI-exposed rats the reactivity was even higher than in the MIC treated group (fig. 2b).

Table 2. Effects on respiration in rats after inhalation of monoiso-cyanates

Group	Conc. ppm	Frequency % ¹	Tidal volume, % ¹	Minute volume, % ¹
Control	–	98.0 ± 5.7	88.6 ± 3.3	86.0 ± 3.3
MIC	8.4	73.2 ± 4.4*	72.8 ± 4.7	53.2 ± 5.2***
	13.0	69.6 ± 8.4***	71.4 ± 3.8*	49.4 ± 5.8***
	25.0	40.2 ± 2.8***	67.8 ± 5.8*	27.6 ± 2.9***
ICA	43.7	98.8 ± 2.6	71.8 ± 7.5	70.4 ± 6.4***

* $p < 0.05$; *** $p < 0.001$, both vs. control group exposed to air, using 2-way ANOVA with Bonferroni multiple comparisons test ($n = 5$).

¹ Percent of baseline values.

Capture ELISA analysis of total IgE production in serum did not reveal any changes after repeated exposure to any isocyanate. TDI-specific IgE levels were also measured in rats exposed to TDI, but no difference was observed compared to the controls when using a capture ELISA with a biotin-labeled TDI-RSA conjugate for detection (data not shown).

Effects of Isocyanates on General Condition and Respiration

Rats repeatedly exposed to TDI or MIC intranasally displayed wheezing respiration, extensive sneezing as well as swelling and redness of the nose, while there were no visible symptoms after exposure to ICA or the solvent ethyl acetate. In addition, TDI and MIC exposure caused extensive weight loss in rats (fig. 3) during the 2 instillation periods, but this was not observed after repeated exposure to ICA. In order to further investigate the toxic

Fig. 2. Individual serum levels of TDI specific IgG (**a**) and MIC specific IgG (**b**) analyzed with ELISA coated with isocyanate-conjugated RSA. Serum was diluted 1:10,000 for TDI specific IgG analysis and 1:10 for MIC specific antibody analysis. Mean values are indicated with a bar. *** $p < 0.001$ vs. control group, using 1-way ANOVA with Dunnett's multiple comparisons test.

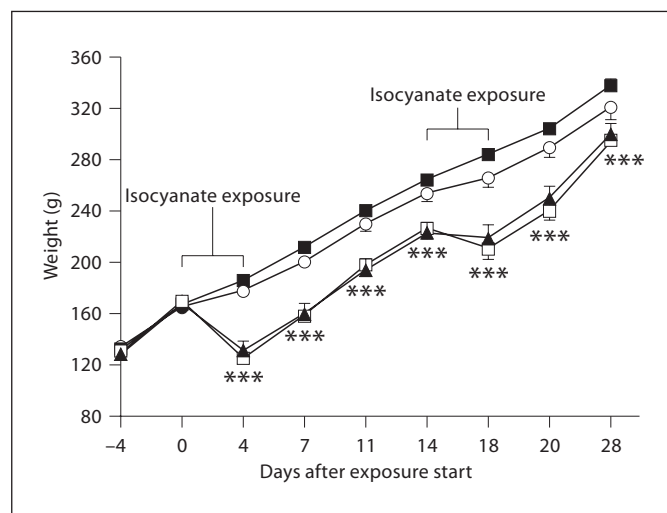
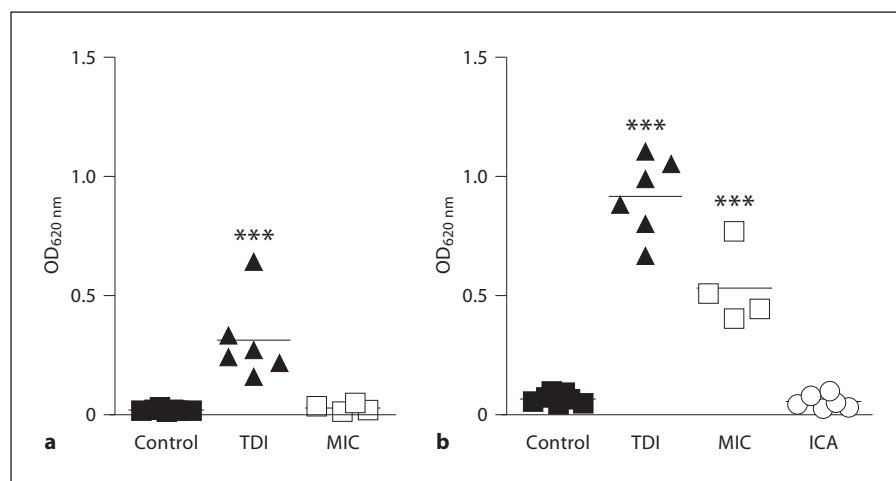


Fig. 3. Weight curves from rats repeatedly exposed to isocyanates. Rats ($n = 4-7$) were given 5 daily intranasal instillations of TDI ($\blacktriangle$), MIC ($\square$) or isocyanic acid ($\circ$), on days 0-4 and days 14-18. Controls ($\blacksquare$) were instilled with vehicle alone. *** $p < 0.001$, TDI and MIC vs. control, using 2-way ANOVA with Bonferroni multiple comparisons test.

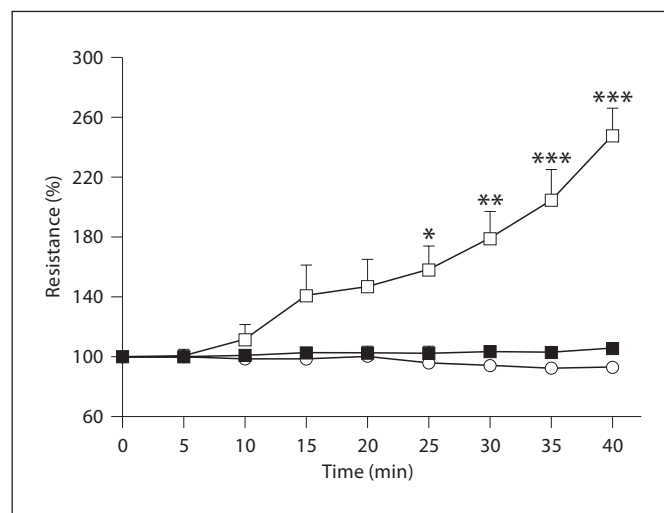


Fig. 4. Pulmonary resistance in anaesthetized rats ($n = 5$) during exposure to MIC 4.9 ppm ($\square$), isocyanic acid 42 ppm ($\circ$) or solvent control ($\blacksquare$). Values are expressed as percentage change in respiration compared to normal values recorded during exposure to air ($t = 0-5$ min). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$, using 2-way ANOVA with Bonferroni multiple comparisons test.

effects of monoisocyanates, respiration was measured during inhalation of vaporized MIC or ICA. For MIC, the respiratory frequency and minute volume were significantly reduced at concentrations of 8.4 ppm (table 2). At 13 ppm the minute volume was reduced below 50% of baseline values (0.834 ± 0.046 ml, 134 ± 3.51 min⁻¹ and 110 ± 6.25 ml·min⁻¹ for tidal volume, frequency and minute volume respectively), while the frequency was reduced to the same level only at the highest tested dose, 25

ppm. The respiratory response to ICA was considerably lower and no significant effects were observed at concentrations up to 44 ppm (table 2). Based on these respiratory data, additional groups of rats were exposed to 4.9 ppm MIC, 42 ppm ICA or solvent (THF) over 30 min in which pulmonary resistance and compliance were recorded (fig. 4). Since the invasive measurement of respiratory effects is more sensitive than the non-invasive registration of respiratory frequency and volume, we used

doses causing a minor decrease in minute volume for this experiment (5% of normal start values). Immediately after start of exposure, airway resistance increased in animals exposed to MIC. The increase was statistically significant after 20 min, reaching 250% of start values at the end of exposure (0.76 ± 0.11 and 1.90 ± 0.16 cm H₂O ml⁻¹ s, for start and end values respectively). In contrast, inhalation of ICA did not result in any detectable increase of pulmonary resistance when compared to control animals. Compliance was unaffected in all groups throughout the recording period.

Discussion

Despite the abundance of isocyanates in occupational settings due to thermal degradation of polyurethane products, there are very few data concerning their sensitizing properties. We demonstrated that repeated intranasal exposure to TDI induces airway inflammation in rats, in agreement with previously reported findings by Zheng et al. [24] in a similar model for TDI sensitization. However, we detected a predominance of neutrophils and lymphocytes in BAL fluid 20 h after challenge, while Zheng et al. also observed an eosinophilic response. This discrepancy may be explained by a difference in number of instillations due to a slightly shorter protocol in our study. Other experimental studies have supported the notion that neutrophils are an important component in airway inflammation in rats after repeated airway exposures to isocyanates [27, 28]. From clinical studies, it is reported that induced sputum from patients diagnosed with occupational asthma caused by low-dose exposure to isocyanates increased the number of neutrophils rather than eosinophils [29], indicating a prominent presence of neutrophils also in human isocyanate-induced airway disease. In concordance with previous animal studies of TDI-induced asthma [30–32], we demonstrated increased numbers of lymphocytes including B cells, NK cells, CD4⁺ and CD8⁺ T cells in BAL fluid from rats repeatedly exposed to TDI, confirming cell mediated and innate immune activation in these animals.

In humans, albumin has been identified as the major isocyanate-conjugated protein formed in BAL fluid after inhalation-challenge with aerosols of hexamethylene diisocyanate [33]. The interaction of albumin-conjugates with the immune system leading to diisocyanate-induced hypersensitivity reactions is, however, not fully understood. In this study, we demonstrated TDI-RSA reactive IgG antibodies in serum, indicating an IgG immune re-

activity in animals exposed to TDI. The lack of detectable serum production of isocyanate-specific IgE indicates that the immune response favors the development of IgG antibodies. Previously it was shown that the development of IgE antibodies following TDI exposure is dependent on exposure concentrations of the isocyanate (i.e. a strong immune response against TDI does not lead to increased serum IgE [30]). TDI-specific IgE antibodies are undetectable in at least a proportion of patients diagnosed with diisocyanate asthma, and it is debated whether this is due to the technical difficulty of detecting a specific IgE response, or if the disease can be mediated by IgE-independent immunologic or non-immunologic mechanisms [1, 4, 34, 35]. Repeated exposure to MIC also resulted in specific IgG antibody production, although the response was weaker than to TDI. Since MIC-exposed animals did not exhibit any detectable inflammatory response, this observation points out that the induction of isocyanate-specific antibodies is not sufficient for the development of inflammatory responses in airways. Similar lack of linkage between serum antibodies and airway disease has been previously reported from studies of mice subchronically exposed to TDI. Those mice displayed airway eosinophilia as well as TDI-specific IgG antibodies, but serum transfer to naïve mice failed to induce airway inflammation [30], further supporting the idea that the systemic humoral immune response is not sufficient for disease induction.

One hypothetical explanation for the difference in immune reactivity and inflammatory response between TDI and MIC exposures could be the ability of TDI to cross-link proteins with the 2 reactive isocyanate groups [36], thereby causing a more fundamental conformational change than MIC and hence exhibiting greater immunogenic properties. Our data demonstrated that IgG antibodies elevated in TDI-exposed animals also display reactivity against MIC-RSA conjugate, while exposure to MIC did not give rise to IgG antibodies with a similar reactivity against TDI-RSA. This apparent cross-reactivity might be due to a capacity of TDI to form 2 different types of hapten conjugates, of which one is similar to the conjugate formed by MIC. However, the basis for this cross-reactivity remains unclear. Human studies have previously shown cross-reactivity between IgG antibodies from workers exposed to methylenediphenyl diisocyanate and serum albumin conjugates with other diisocyanates, such as TDI and hexamethylene diisocyanate [36]. In addition, IgG and IgE antibodies reactive both against methylenediphenyl diisocyanate and the monoisocyanate p-TMI have been reported in some foundry

workers [37]. However, the environments in occupational settings are complex and since no exposure measurements were made in those studies, it cannot be excluded that the workers were exposed to a mixture of mono- and diisocyanates. However, from our data it is not likely that the antibodies raised against the isocyanate-conjugates exclusively recognized the isocyanate (NCO) group, since MIC-RSA specific antibodies did not react with TDI-RSA, an observation supported by similar studies in guinea pigs [19].

Although MIC did not provoke a detectable inflammatory response in lower airways, it was evident from our data on weight loss and observations of airway irritation that this monoisocyanate exerts symptoms of respiratory toxicity similar to that of TDI. Single-dose exposures demonstrated that MIC but not ICA caused a severe decline in respiratory function at doses below 10 ppm. We also showed that this respiratory depression was due to an instant bronchoconstriction leading to increased airway resistance. From these results we conclude that MIC and TDI share some properties in terms of respiratory toxicity. Previously it was reported that TDI-induced airway hyperreactivity is dependent on tachykinins [38], suggesting a plausible mechanism for induction of immediate bronchoconstriction by diisocyanates [25, 39, 40]. Moreover, a connection between release of tachykinins, immunoglobulin production, leukocyte recruitment and activation has been established in earlier studies [41, 42], indicating that diisocyanate-induced respiratory toxicity could trigger the subsequent immune response. It is possible that the bronchoconstriction and immune responses induced by MIC are also mediated by tachykinins, although it remains to be demonstrated whether monoisocyanates can stimulate the release of

neuroactive peptides in a similar way to TDI. Regarding ICA, the lack of airway inflammation and isocyanate-specific antibodies in serum may thus reflect the inability of the compound to elicit a pulmonary response upon inhalation, an effect that could be partially due to rapid hydrolysis of the compound in the upper airways.

In conclusion, our results indicate that the monoisocyanates MIC and ICA are relatively poor sensitizers when compared to the diisocyanate TDI since they do not provoke any detectable airway inflammation. A contribution of MIC to isocyanate-induced disease in occupational environments cannot, however, be ruled out since this monoisocyanate caused symptoms of lung irritation, bronchoconstriction and hapten-carrier immune response in serum. Furthermore, TDI exposure elicits immune reactivity that can cross-react with MIC-protein conjugates suggesting a plausible risk of exacerbation of airway responses in occupational asthmatics pre-sensitized to TDI. Further studies are needed to clarify the nature of the cross-reactive TDI-induced antibodies, as well as the clinical implications. Our results imply that monoisocyanates should be taken in account when assessing health risks since workers often are exposed to a mixture of monoisocyanates and diisocyanates during work where polyurethane products are thermally degraded [16, 43, 44].

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