

Toluene diisocyanate and methylene diphenyl diisocyanate: asthmatic response and cross-reactivity in a mouse model

Lore Pollaris¹ · Fien Devos¹ · Vanessa De Vooght¹ · Sven Seys² · Benoit Nemery¹ · Peter H. M. Hoet¹ · Jeroen A. J. Vanoirbeek¹

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Abstract Both 2,4-toluene diisocyanate (TDI) and 4,4-methylene diphenyl diisocyanate (MDI) can cause occupational asthma. In this study, we optimized our mouse model of chemical-induced asthma in the C57Bl/6 mice strain using the model agent TDI. Furthermore, we validated MDI in this mouse model and investigated whether cross-reactivity between TDI and MDI is present. On days 1 and 8, C57Bl/6 mice were dermally treated (20 µl/ear) with 3 % MDI, 2 % TDI or the vehicle acetone olive oil (AOO) (3:2). On day 15, they received a single oropharyngeal challenge with 0.04 % MDI, 0.01 % TDI or the vehicle AOO

(4:1). One day later, airway hyperreactivity (AHR) and pulmonary inflammation in the bronchoalveolar lavage (BAL) were assessed. Furthermore, total serum IgE levels, lymphocyte subpopulations in auricular lymph nodes and cytokine levels in supernatants of lymphocytes were measured. Both dermal sensitization with TDI or MDI resulted in increased total serum IgE levels along with T and B cell proliferation in the auricular lymph nodes. The auricular lymphocytes showed an increased release of both Th2 and Th1 cytokines. Mice sensitized and challenged with either TDI or MDI showed AHR, along with a predominant neutrophil lung inflammation. Mice sensitized with MDI and challenged with TDI or the other way around showed no AHR, nor BAL inflammation. Both TDI and MDI are able to induce an asthma-like response in this mouse model. However, cross-reactivity between both diisocyanates remained absent.

Lore Pollaris and Fien Devos have contributed equally to this article.

✉ Jeroen A. J. Vanoirbeek
jeroen.vanoirbeek@kuleuven.be

Lore Pollaris
lore.pollaris@kuleuven.be

Fien Devos
fien.devos@kuleuven.be

Vanessa De Vooght
vanessa.devooght@hotmail.com

Sven Seys
sven.seys@kuleuven.be

Benoit Nemery
ben.nemery@kuleuven.be

Peter H. M. Hoet
peter.hoet@kuleuven.be

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Introduction

Diisocyanates, low molecular weight agents containing two highly reactive isocyanate groups, are widely produced and consumed in industrialized countries (Chan-Yeung et al. 2003). Predominantly, 4,4-methylene diphenyl diisocyanate (MDI) and 2,4-toluene diisocyanate (TDI) are used and account together for more than 90 % of the global diisocyanate market. These chemicals are used for the production of polyurethanes, which are mainly present in products such as spray foam insulation, coatings, varnishes and paints (Hettick and Siegel 2011). Nowadays,

¹ Centre for Environment and Health, Department of Public Health and Primary Care, KU Leuven, Herestraat 49 mailbox 706, 3000 Leuven, Belgium

² Laboratory of Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, 3000 Leuven, Belgium

it is recognized that diisocyanates can cause occupational asthma (OA), which is the most common occupational respiratory disease in the industrialized world (Wisnewski et al. 2013b).

The incidence of OA varies depending on the form and type of diisocyanate. Furthermore, it is influenced based on the level and duration of diisocyanate exposure (Wisnewski et al. 2013b). To date, it is well known that diisocyanates are potent sensitizers, with dermal exposure as the leading cause of sensitization. Once immune sensitization to diisocyanates occurs, low airborne levels [below the Occupational Safety and Health Administration (OSHA) established permissible exposure levels] can trigger asthmatic reactions (Bello et al. 2007; Redlich 2010).

Since evidence showed that TDI is an asthmogen, industry started working on ‘safer’ compounds. It is known that the basic structure of the monomer attached to the -NCO group (aliphatic or aromatic), and the number of -NCO groups alters the vapor pressure and reactivity of the monomer (Wisnewski et al. 2013b). The basic structure of TDI consists of one aromatic ring and is highly volatile at room temperature, while MDI is a solid and built upon 2 rings. Because of its lower vapor pressure and therefore safer use, MDI has become the most abundantly produced and consumed diisocyanate (Wisnewski et al. 2011). Although MDI is safer than TDI, human and animal studies show that it also affects the respiratory system (Mapp et al. 1985; O’Brien et al. 1979; Pauluhn 2005; Redlich 2010; Tanser et al. 1973; Wisnewski et al. 2011).

The mechanism for diisocyanates to interact with the immune system is via protein binding, such as albumin. Binding to the ‘self’ protein albumin results into formation of immunogenic haptens which can trigger an immune response, associated with airway inflammation and asthma (Wisnewski et al. 2011, 2013a). At present, it is known that MDI and TDI react with 20 and 37 residues of albumin, respectively. All the residues of albumin to which MDI binds are analogous to those of TDI (Hettick and Siegel 2011).

Cross-reactivity is a phenomenon that occurs in daily practice, via cross-reactive IgEs of homologous proteins, e.g., between mushrooms and airborne molds, or between pollen (bee and weed) and different types of legumes (lupine and peanut) (Choi et al. 2014; Gabriel et al. 2015; van Kampen et al. 2015). Between chemicals cross-reactivity is also described. Patients sensitized to the chemical methylchloroisothiazolinone (MCI) can react to high levels of methylisothiazolinone (MI) and vice versa (Lundov et al. 2011). Since the chemicals MDI and TDI have structural and binding similarities, it is important to study possible cross-reactivity between these diisocyanates. In the past, there was already immunological and respiratory evidence concerning cross-reactivity between MDI and TDI,

although the results remain contradictory and the number of patients included in the studies was limited (Aul et al. 1999; Baur 1983; Grammer et al. 1990; Lushniak et al. 1998; Malo et al. 1983; Mapp et al. 1985; Wass and Belin 1989).

In light of the widely spread combinational use of both TDI and MDI, and to elucidate the contradictory results about cross-reactivity, we optimized our mouse model in the C57Bl/6 mice, tested whether MDI has the same potency as TDI to induce an asthmatic response and investigated possible cross-reactivity between TDI and MDI in our experimental mouse model of chemical-induced asthma.

Materials and methods

Reagents

4,4'-Methylenebis phenyl diisocyanate (MDI) (89 %, CAS101-68-8), 2,4-toluene diisocyanate (TDI) (98 %, CAS584-84-9), acetyl- β -methylcholine (methacholine) and acetone were obtained from Sigma-Aldrich (Bornem, Belgium). Sodium chloride (NaCl) 0.9 % was obtained from B. Braun (Melsungen, Germany). Pentobarbital (Nembutal®) was obtained from Sanofi Santé Animale (CEVA, Brussel, Belgium) and isoflurane (Forene®) from Abbott Laboratories (Louvain-la-Neuve, Belgium). The vehicle acetone olive oil (AOO) used to dissolve MDI and TDI consists of a mixture of 2 volumes of acetone and 3 volumes of olive oil (selection de Almazara, Carbonell, Madrid, Spain) for the dermal sensitization and 1 volume of acetone and 4 volumes of olive oil for the challenge. Concentration of MDI is given as (w/v), while TDI is given as percentage (v/v) in AOO.

Mice

Male C57Bl/6 mice (6–8 weeks old) were obtained from Harlan (Horst, The Netherlands). All mice were housed under a conventional animal house with 12-h dark/light cycles. They were housed in filter-top cages and received lightly acidified water and pelleted food ad libitum.

All experimental procedures performed in mice were approved by the local ethical committee for animal experiments (P118-2011).

Experimental protocols of mouse experiments

On days 1 and 8, mice received dermal applications of 20 μ l of 3 % MDI, 2 % TDI or the vehicle AOO (2:3). On day 15, mice received, under light isoflurane anesthesia, an oropharyngeal instillation of 20 μ l 0.04 % MDI, 0.01 %

TDI or the vehicle AOO (1:4). All experimental groups are indicated with 2 symbols: The first symbol indicates the dermal treatment on days 1 and 8, whereas the second symbol indicates the oropharyngeal challenge on day 15. Each group consists of 7–10 mice.

Airway and lung tissue hyperreactivity measurements

Twenty-four hours after the challenge, airway and lung tissue reactivity to methacholine was measured using a forced oscillation technique (FlexiVent, SCIREQ, Montreal, Canada). Mice were anesthetized with pentobarbital (70 mg/kg body weight, Nembutal®). Applying the ‘quick prime 3’ (QP3) perturbation, mice were exposed to increasing concentrations of methacholine (0, 1.25, 2.5, 5, 10, 20 mg/ml) dissolved in NaCl 0.9 % via an inline nebulizer to measure the airway resistance (Rn), tissue damping (G) and tissue elasticity (H). After each concentration, the QP3 perturbation was performed 5 times spread over 2 min. If the coefficient of determination (COD) of a QP3 perturbation was lower than 0.90, the measurement was excluded and not used to calculate the average. For each mouse Rn, G and H were plotted against methacholine concentration and the area under the curve (AUC) was calculated to perform statistical analysis.

Bronchoalveolar lavage (BAL) and serum sampling

After measuring airway hyperreactivity to methacholine, mice were killed. Blood was sampled from the retro-orbital plexus and centrifuged (14,000g, 4 °C, 10 min), and serum samples were stored at –80 °C until analysis. The lungs were lavaged, in situ, three times with 0.7 ml sterile saline (0.9 % NaCl), and the recovered fluid was pooled. Cells were counted using a Bürker hemocytometer (total cell count), and the BAL fluid was centrifuged (1000g, 10 min). For differential cell counts, 250 µl of the resuspended cells (100,000 cells/ml) were spun (300 g, 6 min) (Cytospin 3, Shandon, TechGen, Zellik, Belgium) onto microscope slides, air-dried and stained (Diff-Quik® method, Medical Diagnostics, Düringen, Germany). For each sample, 200 cells were counted for the number of macrophages, eosinophils and neutrophils.

Lymph node analysis

Retro-auricular lymph nodes, obtained from the same mice, were pooled and kept on ice in RPMI-1640 (1×) + GlutaMAX™-I (Invitrogen, Merelbeke, Belgium). Cell suspensions were obtained by pressing the lymph nodes through a cell strainer (100 µm) (BD Bioscience, Erembodegem, Belgium) and rinsing with 10 ml tissue culture medium (RPMI-1640 (1×) + GlutaMAX™-I).

After centrifugation (1000g, 10 min), cells were counted using a Bürker hemocytometer and resuspended (10^7 cells/ml) in complete tissue culture medium (RPMI-1640 (1×) + GlutaMAX™-I supplemented with 10 % heat-inactivated fetal bovine serum, 10 mg/ml streptomycin/penicillin). Five hundred thousand cells were stained with anti-CD3⁺ (APC), anti-CD4⁺ (APC-Cy7), anti-CD8⁺ (PerCP-Cy5.5) and anti-CD25⁺ (PE) or received a single staining with anti-CD19⁺ (PE)-labeled antibodies, according to standard procedures (BD Biosciences, Erembodegem, Belgium). Percentages of labeled cells were determined by performing flow cytometry (FACSArray, BD Biosciences, Erembodegem, Belgium) on at least 10^5 cells. Cells were seeded into 48-well culture plates at a density of 10^6 cells/ml and incubated in complete RPMI-1640 (1X) + GlutaMAX™-I for 42 h with 2.5 µg/ml of concanavalin A (ConA) (Sigma-Aldrich, Bornem, Belgium). Cell suspensions were then centrifuged (1000 g, 10 min), and supernatant was stored at –80 °C. Concentrations of interleukin-2 (IL-2), IL-4, IL-10, IL-13 and interferon gamma (IFN-γ) were measured via cytometric bead array and analyzed with the FCAPArray version 3 software (BD Biosciences, Erembodegem, Belgium) on the LSR Fortessa (BD Biosciences, Erembodegem, Belgium). Lower detection limits were, respectively, 0.2, 0.3, 9.6, 2.4 and 0.5 pg/ml.

Total serum IgE

The OptEIA Mouse IgE set from Pharmingen (BD Biosciences) was used to measure total serum IgE (diluted 1/70). Measurements were performed according to the manufacturer’s instructions.

Data analyses

The data are presented as means with standard error of the mean (SEM) or as individual mice and group means. Normality of distribution was assessed by the Kolmogorov–Smirnov test, followed by a one-way parametric ANOVA combined with a Bonferroni multiple comparison post hoc test (Graph Pad Prism 5.01. Graphpad Software Inc, San Diego, USA). A level of $p < 0.05$ (two tailed) was considered to be significant.

Results

Airway and lung tissue hyperreactivity

Airway hyperreactivity (AHR), using a methacholine provocation test, was assessed 24 h after the challenge. Figure 1a, c, e shows the mean dose–response curves

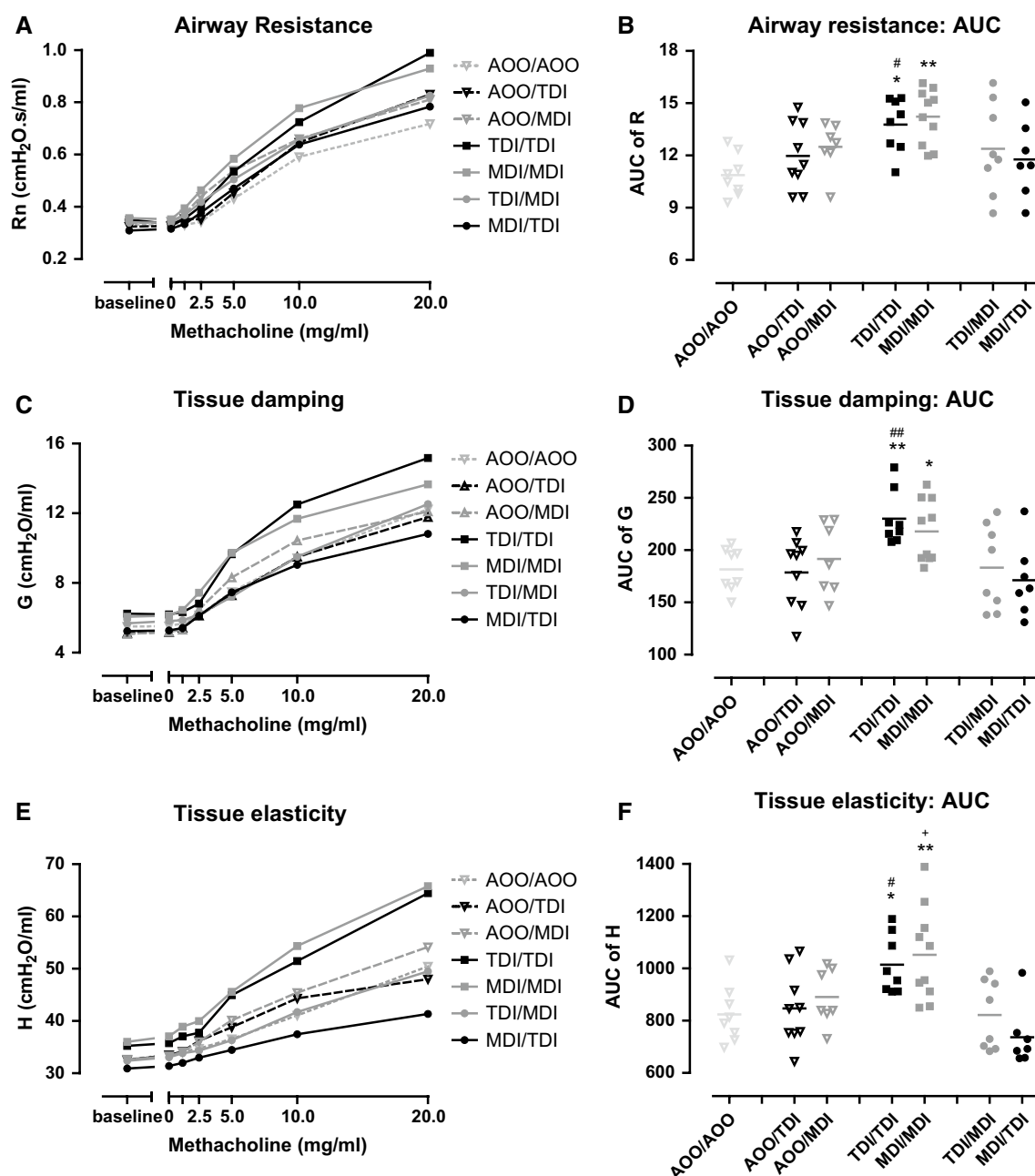


Fig. 1 Airway and tissue hyperreactivity. Methacholine responsiveness was assessed 24 h after the oropharyngeal challenge. The airway (a, b) (Rn—airway resistance) and tissue reactivity (c–f) (G—tissue damping and H—tissue elasticity) to methacholine was measured using a forced oscillation technique. a, c, e Show mean values of Rn, G and H; b, d, f show individual values and the group mean of the area under the curve (AUC) of Rn, G and H against methacholine

concentrations between 0 and 20 mg/ml. Experimental groups are identified by two symbols, the *first symbol* indicates the dermal treatment on days 1 and 8, whereas the *second symbol* indicates the oropharyngeal challenge on day 15. $n = 7–10$ per group. * $p < 0.05$, ** $p < 0.01$ compared to the AOO/AOO control group. # $p < 0.05$, ## $p < 0.01$ compared with the AOO/TDI control group. + $p < 0.05$ compared to the AOO/MDI group

of airway and tissue hyperreactivity response to methacholine provocation, whereas Fig. 1b, d, f shows the area under the curve (AUC) of the individual mice and the group average. Mice fully treated with MDI (MDI/MDI) or TDI (TDI/TDI) show a significant AHR, indicated by significantly increased Rn after methacholine challenge

(Fig. 1a, b), compared to the vehicle-treated control mice (AOO/AOO). The TDI/TDI group was also significantly increased compared to their control group AOO/TDI. Both cross-reactivity groups (TDI/MDI and MDI/TDI) show no AHR compared to all control groups (AOO/AOO, AOO/MDI and AOO/TDI) (Fig. 1a, b). Regarding lung tissue

Fig. 2 Bronchoalveolar lavage (BAL) cell count. Total numbers of macrophages, neutrophils and eosinophils were assessed in BAL 24 h after the oropharyngeal challenge. Experimental groups are as in Fig. 1. $n = 7-10$ per group. Bars show the mean and SEM. $*p < 0.05$, $**p < 0.01$ compared to the AOO/AOO control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the AOO/TDI control group. $^{+}p < 0.05$ compared to the AOO/MDI group

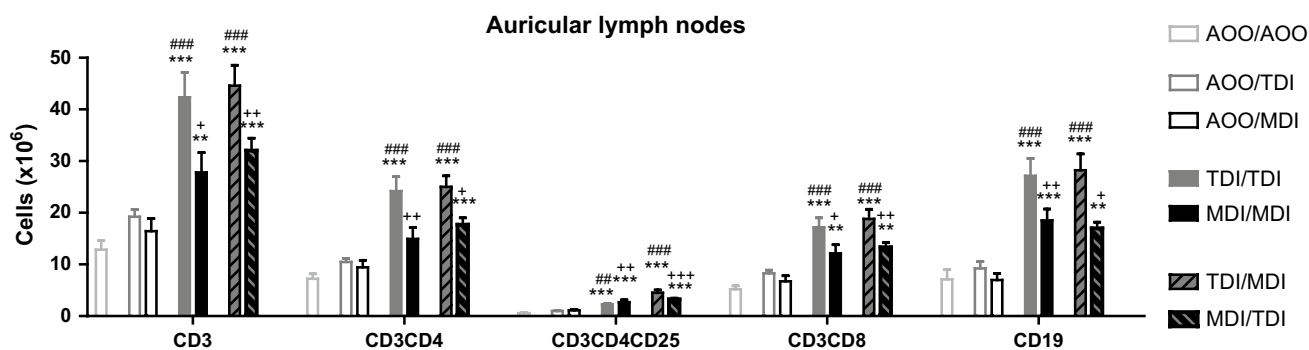
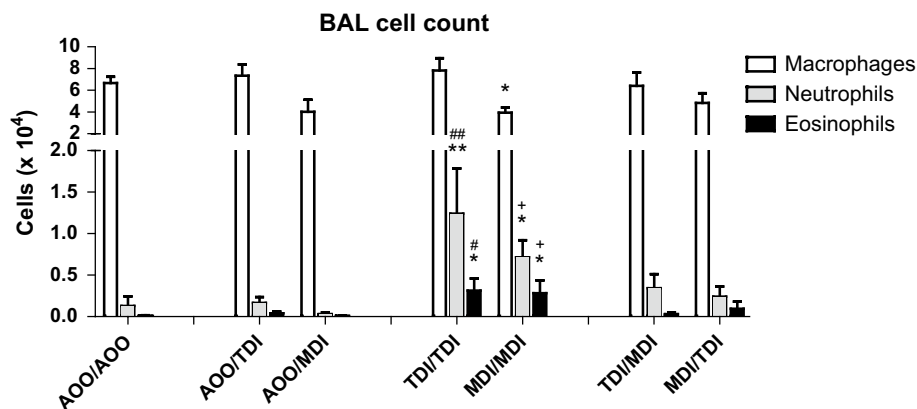


Fig. 3 Lymphocyte subpopulations in auricular lymph nodes. Auricular lymphocytes were stained with anti-CD3⁺ (T lymphocytes), anti-CD3⁺CD4⁺ (Th lymphocytes), anti-CD3⁺CD4⁺CD25⁺ (activated/regulatory Th lymphocytes) and anti-CD3⁺CD8⁺ (Tc lymphocytes) or stained with a single anti-CD19⁺ (B lymphocytes). Experimental

groups are as in Fig. 1. $n = 7-10$ per group. Bars show the mean and SEM. $**p < 0.01$, $***p < 0.001$ compared with the AOO/AOO control group. $^{\#}p < 0.01$, $^{\#\#}p < 0.001$ compared to the AOO/TDI control group. $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ compared to the AOO/MDI group

hypersensitivity, there was a significantly increased tissue damping (G) compared to the vehicle-treated group. The TDI/TDI-treated group was also increased compared to their control group AOO/TDI. Furthermore, there was an increase in tissue elasticity (H) in both the TDI/TDI and MDI/MDI groups, compared to the full control group (AOO/AOO) and their own non-sensitized but challenged control group (AOO/TDI and AOO/MDI, respectively) (Fig. 1c–f).

Bronchoalveolar lavage

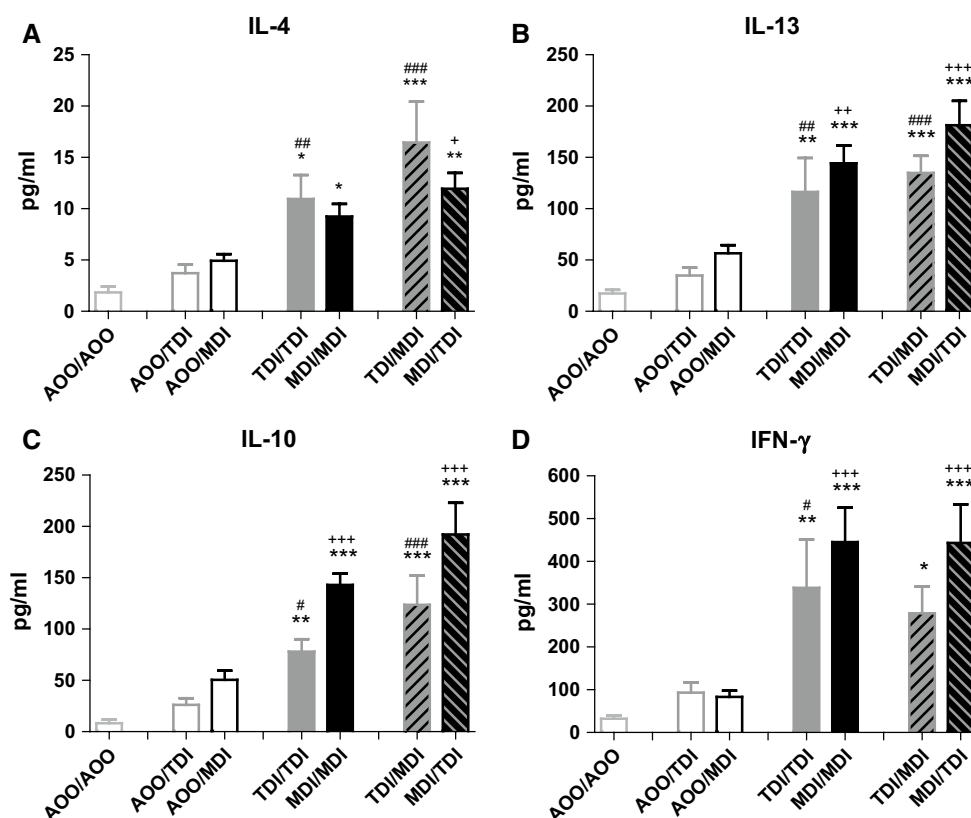
Following AHR measurements, a bronchoalveolar lavage (BAL) was performed. Figure 2 shows the total number of macrophages, neutrophils and eosinophils in the BAL fluid. The total number of macrophages in the completely MDI-treated mice is significantly lower compared to the vehicle-treated control group (AOO/AOO). The total number of neutrophils and eosinophils was significantly increased in completely TDI- and MDI-treated mice, compared to the vehicle-treated group (AOO/AOO) and their own

non-sensitized but challenged control group (AOO/TDI and AOO/MDI, respectively). In the cross-reactivity groups, only a limited inflammation was present, which was not significant.

Auricular lymphocyte analysis

The lymphocyte subpopulation of the auricular lymph nodes was assessed by FACSArray. Figure 3 shows the total number of CD3⁺ (T lymphocytes), CD3⁺CD4⁺ (Th lymphocytes), CD3⁺CD4⁺CD25⁺ (activated/regulatory Th lymphocytes), CD3⁺CD8⁺ (Tc lymphocytes) and CD19⁺ (B lymphocytes) cells in auricular lymph nodes. In the auricular lymph nodes of all mice sensitized with either TDI or MDI, there was a significantly increased number of both T and B lymphocytes, compared to the vehicle-treated group (AOO/AOO) and their own non-sensitized but challenged control group (AOO/TDI and AOO/MDI, respectively). The increase in T lymphocytes was due to a significant increase in all three subpopulations (Th lymphocytes, activated/regulatory Th lymphocytes and Tc lymphocytes).

Fig. 4 Ex vivo cytokine production of auricular lymphocytes. Levels of IL-4 (a), IL-13 (b), IL-10 (c) and IFN- γ (d) were measured by cytometric bead array in supernatant of auricular lymphocytes. Experimental groups are as in Fig. 1. $n = 7$ –10 per group. Bars show the mean and SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the AOO/AOO control group. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to the AOO/TDI control group. + $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$ compared to the AOO/MDI group



Auricular lymphocytes were cultured (42 h) with ConA (2.5 μ g/ml). The levels of ex vivo release of IL-4 (Fig. 4a), IL-13 (Fig. 4b) and IL-10 (Fig. 4c) and IFN- γ (Fig. 4d) in the supernatant are measured using cytometric bead array. Figure 4 shows that all mice sensitized with TDI or MDI have a significant increase in IL-4, IL-13, IL-10 and IFN- γ , compared to the vehicle-treated group (AOO/AOO) and their own non-sensitized but challenged control group (AOO/TDI and AOO/MDI, respectively).

Total serum IgE

Total serum IgE was measured using standard ELISA assays. In Fig. 5, the concentration of total serum IgE is presented. All mice sensitized to TDI and MDI show a significant increase in IgE, compared to the vehicle-treated group (AOO/AOO) and their own non-sensitized but challenged control group (AOO/TDI and AOO/MDI, respectively).

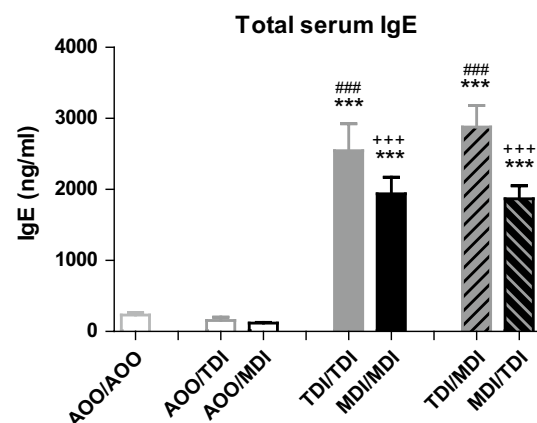


Fig. 5 Total serum immunoglobulin E. Blood was collected 24 h after the oropharyngeal challenge. Total serum IgE was measured using standard ELISA assays. Experimental groups are as in Fig. 1. $n = 7$ –10 per group. Bars show the mean and SEM. *** $p < 0.001$ compared to the AOO/AOO control group. ### $p < 0.001$ compared to the AOO/TDI control group. +++ $p < 0.001$ compared to the AOO/MDI group

Discussion

4,4'-Methylene diphenyl diisocyanate (MDI) and toluene-2,4-diisocyanate (TDI) are widely used chemicals in the polyurethane industry. Both diisocyanates are often

used in the same plants, sometimes even together in the same production process (Woods 1990). Since the structural and binding similarities and the contradictory result in humans concerning cross-reactivity, it is of importance to

test possible cross-reactivity in our mouse model of chemical-induced asthma.

Exposure to diisocyanates is associated with adverse health effects such as sensitization and asthma (Hettick et al. 2012). Our results show that TDI is a potent asthmo-gen, which is in agreement with previous results obtained by our group and others (Hoffmann and Schupp 2009; Matheson et al. 2005; Pauluhn 2014; Scheerens et al. 1999; Selgrade et al. 2006; Tarkowski et al. 2007; Vanoirbeek et al. 2004). Furthermore, we demonstrated that MDI has the same asthmogenic potency as TDI in this mouse model of chemical-induced asthma. Yet, the most important novelty of this study is the fact that in both crossed groups either sensitized with TDI and challenged with MDI or sensitized with MDI and challenged with TDI, no airway/tissue hyperreactivity or BAL inflammatory response could be found, thus showing no cross-reactivity.

Previously, De Vooght et al. (2010) showed that applying the same doses of TDI for sensitization and challenge, which give an asthma-like response in BALB/c mice, does not have the same result in the non-atopic C57Bl/6 mice, which remained similar to the control treatment (De Vooght et al. 2010). Here, we demonstrated that by increasing the dermal sensitization concentration of TDI (0.3 % in BALB/c mice to 2 % in C57Bl/6 mice), we are able to induce an asthma-like response in the C57Bl/6 mouse strain. An advantage is the wide availability of different KO C57Bl/6 mice strains for future mechanistic studies. Furthermore, C57Bl/6 mice may reflect asthma development in non-atopic persons.

Based on the selected doses of MDI and TDI, we established dermal sensitization of the mice. This was demonstrated by the results of the different lymphocyte subpopulations, found in the auricular draining lymph nodes, which were significantly increased in mice sensitized to MDI or TDI. This was indicated by an increase in T helper ($CD3^+CD4^+$), T cytotoxic ($CD3^+CD8^+$), activated/regulatory Th ($CD3^+CD4^+CD25^+$) and B lymphocytes ($CD19^+$). The cytokine release of ConA-stimulated lymphocytes displayed an increased mixed Th1/Th2 cytokine response, which is evidenced by an increased expression of Th2 cytokines IL-13, IL-4, IL-10 as well as Th1 cytokine IFN- γ . This is in agreement with the previous findings (De Vooght et al. 2009; Matheson et al. 2005; Selgrade et al. 2006). The sensitization of the mice is also confirmed by an increase in total IgE after skin exposure of both MDI and TDI. This was also described by other groups for both chemicals (Matheson et al. 2005; Pauluhn 2005; Tarkowski et al. 2007; Wisnewski et al. 2011). The role of IgE in diisocyanate-induced asthma is still under debate (Wisnewski and Jones 2010).

Twenty-four hours after the single challenge, we observed that only mice receiving an antigen-specific

oropharyngeal challenge with either TDI or MDI (TDI/TDI; MDI/MDI) resulted in lung inflammation characterized by mainly neutrophils and eosinophils, which is consistent with previous results using BALB/c mice (De Vooght et al. 2009). The predominant neutrophilic inflammation is also observed in patients with TDI-induced asthma and other TDI-induced animal models of asthma (Matheson et al. 2005; Park et al. 1999; Tarkowski et al. 2007; Vanoirbeek et al. 2004, 2008, 2009). For the first time, we confirm that also MDI induces an asthma-like response in this mouse model, indicated by an increase in eosinophils and mainly neutrophils in BAL fluid. Previously, other animal models showed increased levels of mainly neutrophils or eosinophils in BAL fluid after multiple exposures of MDI (Pauluhn 2005; Wisnewski et al. 2011). This is in accordance with human studies, where MDI has been causally related to induce asthma, with symptoms such as variable airflow limitation, eosinophilic bronchitis and respiratory irritant effects after exposures on the work floor (Hur et al. 2008; Petsonk et al. 2000; Tanser et al. 1973).

Besides the lung inflammation, airway hyperreactivity (Rn) to increasing concentrations of methacholine (AHR) was measured 24 h later after the single challenge. Both TDI/TDI and MDI/MDI groups showed AHR compared to the complete control group. Furthermore, we also showed a significant increase in the TDI/TDI and MDI/MDI groups of tissue damping (G) and tissue elasticity (H), which is indicative for lung tissue hypersensitivity. This can be explained by the presence of epithelial necrosis and epithelial shedding after an oropharyngeal challenge as shown by De Vooght et al. (2009) (De Vooght et al. 2009).

Unlike the TDI/TDI and MDI/MDI group, the cross-reactivity groups (TDI/MDI and MDI/TDI) presented no asthma-like response. Despite successfully sensitized, no statistical changes in airway hyperreactivity and tissue hypersensitivity compared to the vehicle control group could be found. Furthermore, only a nonsignificant lung inflammation was present in the BAL. Yet, some mice in the cross-reactivity groups do show positive responses. This might be due to experimental variation, but it could also be due to actual cross-reactivity as shown for other chemicals (Lundov et al. 2011). Human studies on cross-reactivity currently remain indecisive. Several research groups indicated that respiratory reactions can be elicited by other diisocyanates to which the workers were sensitized (Malo et al. 1983; O'Brien et al. 1979). Using inhalation provocation tests, O'Brien et al. (1979) demonstrated that four persons only exposed to TDI exhibited a bronchoconstrictive response to MDI inhalation (O'Brien et al. 1979). As already indicated, this was also seen in our results, and some mice in the cross-reactivity groups (TDI/MDI and MDI/TDI) showed AHR and neutrophils in BAL 24 h after the challenge. The crossed bronchial responses

may be linked with crossed immunoreactivity. Immunological studies showed mutual cross-reactivity among isocyanate-bound human serum albumin (HSA) to which the workers were not sensitized (Baur 1983; Grammer et al. 1990; Lushniak et al. 1998; Malo et al. 1983; Tee et al. 1998; Wass and Belin 1989). Also Ruwona et al. (2010) showed that murine monoclonal antibodies toward TDI-HSA cross-react with MDI-HSA and HDI-HSA (Ruwona et al. 2010). Furthermore, Lemons et al. (2014) produced a murine IgM mAb with broad specificity toward the most commonly used isocyanates TDI-HSA, MDI-HSA and HDI-HSA (Lemons et al. 2014).

On the other hand, there is evidence in exposed workers that there is no crossed respiratory or immunological reaction to different types of diisocyanates, which is in line with the main findings of this study (Aul et al. 1999; Harries et al. 1979; Mapp et al. 1985). Mapp et al. (1985) demonstrated that in two patients sensitized to MDI, no drop of forced expiratory volume in 1 s (FEV1) was elicited after an inhalation challenge to TDI (Mapp et al. 1985). The absence of respiratory cross-reactivity was also confirmed by immunological evidence. IgG antibodies to MDI, of subjects with asthma induced by MDI, appear to be specific for MDI without cross-reactivity with TDI (Aul et al. 1999). Furthermore, Wisniewski and Liu (2013) developed six different murine monoclonal antibodies which all bind to MDI-conjugated proteins, but not to TDI or HDI protein conjugates (Wisniewski and Liu 2013).

The absence of cross-reactivity can be explained by the different reactivity of the functional group (diisocyanates) of MDI and TDI. Moreover, TDI has a higher affinity for lysine residues (e.g., on albumin), and therefore, MDI binds to less lysine residues compared to TDI (Hettick et al. 2012; Hettick and Siegel 2011). Also, the different stereo isomers of MDI and TDI may lead to a different binding to albumin, which makes it further difficult to generate the same epitope, although not exclusive. Theoretically, MDI and TDI have the ability to cross-react due to the communalities in albumin-binding sites.

In this study, we established three major conclusions. First of all, we successfully optimized our mouse model of chemical-induced asthma in the C57Bl/6 mouse strain. Secondly, we conclude that MDI responses are similar to those of TDI in this model. Thirdly, the most important finding of this study is the absence of cross-reactivity between MDI and TDI.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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