Not All Mouse Strains Respond Equal in a Model of Chemical-Induced Asthma

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1 Introduction

Asthma is a chronic inflammatory airway disease that has emerged as a major health concern among industrialized nations (Zhu & Gilmour, 2009). Asthma is a heterogeneous disorder that is characterized by variable airway obstruction, airway inflammation and hyperresponsiveness (Lemanske, Jr. & Busse, 2010). For many asthmatics, the disease has its roots during infancy and early childhood. However, in adult asthma, occupational exposures are estimated to be responsible for 10% to 25% of the asthma prevalence (Mapp et al., 2005). Today, occupational asthma (OA) has become the most common work-related lung disease and can be defined as a disease characterized by variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions attributable to a particular occupational environment and not to stimuli encountered outside the workplace (Bernstein et al., 2006). Depending on their molecular weight, agents causing allergic or immunologically mediated OA can be divided into two categories: a/ biological agents of high molecular weight (HMW) ($> 5kDa$), such as proteins, glycoproteins and polysaccharides b/ chemicals of low molecular weight (LMW) ($< 5kDa$), such as synthetic chemicals, natural compounds, drugs and metals. HMW and certain LMW compounds generally induce OA via IgE-dependent mechanisms, comparable with atopic asthma induced by pollen or house dust mite allergens, whereas many (though not all) LMW compounds (isocyanates, western red cedar) appear to induce OA via pathways that do not involve IgE-dependent mechanisms (Mapp et al., 2005).

Although exposed to similar allergic stimuli, not all humans develop allergic or occupational asthma. The etiology of asthma is complex and is associated with a combination of immune, genetic, environmental and socio-economic factors (Tumes et al., 2007; Zhu & Gilmour, 2009).

The mouse genome has been extensively characterized and exhibits a high degree of homology with its human counterpart. Especially, a high level of resemblance at the immunological level has been found, suggesting that mouse models can yield a relevant insight into human pathology (Willis-Owen & Valdar, 2009). Maximum genetic uniformity can be achieved when using inbred strains. Environmental factors such as exposure to light, diet, air quality and climate can influence the phenotypic outcome of asthma. The advantage of using an animal model is that these confounding factors can be largely controlled in laboratory animal housing situations (Tumes et al., 2007; Wills-Karp & Ewart, 1997). Using different inbred strains of mice gives the opportunity to estimate the influence of the genetic background on the phenotypical outcome of chemical-induced asthma.

Starting from an established mouse model of chemical-induced asthma developed by Vanoirbeek et al. in BALB/c mice, 6 other inbred mouse strains were tested on their sensitizing capacity to toluene-2,4-diisocyanate (TDI) (De Vooght et al., 2009; Vanoirbeek et al., 2004; Vanoirbeek et al., 2006; Vanoirbeek et al., 2008; Vanoirbeek et al., 2009a). By testing different mouse strains we have the opportunity to evaluate the intra- (experimental) and the inter-strain variability, giving us the possibility to select the inbred mouse strain which represents best the phenotype of human OA.

2 Materials and Methods

2.1 Diisocyanates

Diisocyanates are a leading cause of OA and are therefore frequently used in animal models to unravel the mechanisms behind chemical-induced non-IgE-mediated OA. Diisocyanates are LMW agents that contain two highly reactive isocyanate groups (-N=C=O) which can bind to endogenous proteins. These chemicals are widely used in industry for manufacturing polyurethane foams, paints and lacquers (Piirila et al., 2001). The three predominant diisocyanates in use today are toluene diisocyanate (TDI), diphenylmethane diisocyanate (MDI) and hexamethylene diisocyanate (HDI) (Liu & Wisnewski, 2003).

For dermal applications, the vehicle (AOO) for TDI consisted of 2 volumes of acetone (A) and 3 volumes of olive oil (OO). For the oropharygeal aspiration (challenge) the vehicle (AOO) consisted of a mixture of 1 volume of acetone and 4 volumes of olive oil. The vehicle matrix, in which a chemical allergen is dissolved, can have profound effects on the response elicited (Basketter et al., 2001). AOO is one of the standard vehicles for the local lymph node assay (OECD 2002, guideline 429) and it has been proven to be an ideal vehicle for non-aqueous compounds like TDI. We optimized the proportions of A and OO for dermal sensitization and challenge to avoid irritation, inflammation or airway response, in the control group (De Vooght et al., 2009; Vanoirbeek et al., 2004).

2.2 Inbred Mouse Strains

In allergy and asthma research, several protocols for the induction phase and elicitation phase are used as well as different strains of mice. Mostly, these are inbred mouse strains, implying that approximately 99% of all loci are homozygote. So, these mice are genetically identical. Although they are genetically identical within a mouse strain, this is not the case between the mouse strains. The inbred strains of mice have been shown to differ in their ability to mount an allergen-induced asthmatic response. To compare different inbred mouse strains in a mouse model of diisocyanate-induced OA, male BALB/c OlaHsd, A/J OlaHsd, C57Bl/6J OlaHsd, AKR OlaHsd, DBA/2 OlaHsd, CBA/J CrHsd and BP/2IcRj-Biozzi mice (6 weeks old) were used. These mouse strains can be categorized in Th2-biased (BALB/c, BP2 and A/J) or Th1-biased (C57Bl/6, AKR, DBA/2 and CBA) mouse strains and we could hypothesize, based on the known mechanisms of asthma, that the former mouse strains would be more responsive than the latter.

2.3 Mouse Model of TDI-Induced OA

Vanoirbeek et al. and De Vooght et al. developed, optimized and validated a mouse model of chemical-induced asthma (De Vooght et al., 2009; De Vooght et al., 2010; Tarkowski et al., 2007; Vanoirbeek et al., 2004; Vanoirbeek et al., 2006; Vanoirbeek et al., 2008; Vanoirbeek et al., 2009b; Vanoirbeek et al., 2009a). The mouse model consists of dermal sensitizations followed by a single airway challenge. The choice of skin exposure as a sensitization route originates from the fact that several industrial applications of LMW agents provide a high opportunity for skin exposure. Furthermore, it is known that the prevalence of chemical-induced asthma does not tend to decrease despite reductions in airway exposure.

The treatment protocol consist of two dermal applications of TDI in AOO (2:3) on days 1 and 8 on the dorsum of both ears (0.3% TDI), followed by a single airway challenge (day 15) via oropharyngeal aspiration (0.01% TDI) with a lower concentration of TDI in AOO (1:4) (De Vooght et al., 2009).

2.4 Functional, Inflammatory and Immunologic Parameters

2.4.1 Forced Oscillation Technique versus Whole Body Plethysmograph

Pulmonary function analysis is an important tool in the evaluation of mouse respiratory disease models, but the development of these tests has been a great challenge in small animals. At present there are different non-invasive and invasive measurements available (Vanoirbeek et al., 2010). Until recently, unrestrained whole body plethysmography (WBP) was widely used to assess bronchial hyperresponsiveness in conscious, free to move and spontaneously breathing mice. The principle behind the WBP is the measurement of pressure differences, caused by respiration, between a reference chamber and the main chamber containing the animal. Bronchocontriction can cause marked changes in the breathing pattern and this will be reflected in the dimensionless parameter enhanced pause (Penh) (Hamelmann et al., 1997). Although a main advantage is the noninvasive character of the technique, over the years this method has been heavily criticized. This criticism is based on the fact that the pressure differences measured are not solely due to changes in breathing of the animals, but they are largely caused by gas compression and the humidification and heating of air as it moves between the box and the lungs (gas conditioning). Consequently, changes in Penh are only useful as a general indicator that some reaction has taken place in an animal, but may not be correlated with mechanical properties of the lung (Lundblad et al., 2002). Measuring respiratory mechanics can only be achieved with more invasive measurements, for example the forced oscillation technique, in anesthetized and tracheostomized mice. Here the animal's breathing pattern is entirely under the control of the experimenter, and the upper airways are bypassed so that their mechanical properties do not interfere with those of interest (i.e. the lung) (Bates et al., 2009). The forced oscillation measurements can be performed with the FlexiVent (SCIREQ) system. In our research group, mice are anesthetized with an intraperitoneal injection of pentobarbital (70 mg/kg). This anesthetic will, besides sedation, also suppress spontaneous breading needed to perform stable measurements. Subsequently the animals are tracheostomized and connected to a computer-controlled small animal ventilator. The mice are quasi-sinusoidal ventilated with a tidal volume of 10 ml/kg at a frequency of 150 breaths/min and a positive end-expiratory pressure of 2 cm H2O to achieve a mean volume close to that during spontaneous breathing. A broad range of parameters can be measured at baseline or after a methacholine provocation. A methacholine provocation is performed to measure airway hyperreactivity (AHR, 24 hours after the specific challenge) and is achieved by administering increasing concentrations of methacholine (0-10 mg/ml) via an in-line nebulizer which is directly connected to the ventilator. This in-line nebulizer makes it also possible to expose mice to different aerosolized compounds before or during measuring lung mechanics (De Vooght et al., 2009; Vanoirbeek et al., 2010).

2.4.2 Pulmonary inflammation (bronchoalveolar lavage)

Airway inflammation is besides a reversible airway obstruction and non-specific AHR a typical characteristic of (allergic) asthma. In comparison with asthma induced by HMW agents, were eosinophils and lymphocytes are the characteristic cell types present in the bronchoalveolar lavage (BAL) fluid, asthma induced by LMW agents has been associated with an influx of mainly neutrophils and to a lesser extent eosinophils (De Vooght et al., 2009; Lee et al., 2001; Matheson et al., 2001; Scheerens et al., 1996; Tarkowski et al., 2007; Vanoirbeek et al., 2004). The type of inflammation is highly dependent on the used treatment protocol. The duration of exposure (acute or chronic), the route of exposure (intranasal, oropharyngeal, inhalation, intratracheal) as well as the option of conjugating the chemical with serum albumine can influence the inflammatory outcome (Ban et al., 2006; De Vooght et al., 2009; Herrick et al., 2002; Matheson et al., 2005b;

Vanoirbeek et al., 2004; Wisnewski et al., 2011).

The easiest way to assess airway inflammation in mice is to perform a BAL of one or both lungs. This BAL fluid can then be used to verify differences in total or differential cell count as well as differences in cytokine concentrations. Another way is to collect lung tissue samples and measure cytokine concentrations in tissue homogenates or perform lung histology on cryosections or paraffin imbedded slides, depending on the research purpose.

2.4.3 Immunologic Parameters

One of the first steps in the immune response is the recognition of the antigen by antigen presenting cells present in the skin and mucosal sites. When properly stimulated, antigen presenting cells will migrate to the draining lymph nodes where they present antigen and stimulatory signals to T-lymphocytes leading to their activation (Afshar et al., 2008). These lymph nodes are defined as secondary lymphoid organs and provide a supporting framework in which antigens and rare antigen-specific T- and B-lymphocytes can be efficiently brought together to initiate the adaptive response (Phan et al., 2009). Activation of the T-lymphocytes leads to the production of IL-4 and IL-13 that regulates the isotype switch of B-lymphoyctes and their production of immunoglobulins, such as IgE and IgG (Maddox & Schwartz, 2002). Together with IL-9, these cytokines are also important in mast cell development, mucus overproduction and the induction of AHR. Re-exposure to the allergen leads to cross-linking of the allergen on mast cell bound IgE resulting in the release of preformed and newly synthesized mediators and the transcription of cytokines. This will lead to the early-phase of an asthmatic reaction resulting in mucus production, bronchoconstriction, vascular leak, and the initial recruitment of T-lymphocytes in the airways. The early phase of an asthmatic reaction is initiated within minutes, and is subsequently followed several hours later by a late phase reaction distinguished by an influx of neutrophils and eosinophils into the airways. This influx of inflammatory cells is the result of the secretion of IL-3, IL-5 and granulocyte colony stimulating factor (GM-CSF) by mast cells and T-helper (Th) 2 lymphocytes (Afshar et al., 2008; Bloemen et al., 2007).

Although allergen-induced cross-linking of IgE on the surface of mast cells is a cardinal feature to “trigger” allergic asthma, it has been reported that the majority of individuals with isocyanate-induced asthma do not have allergen-specific IgE (Wisnewski & Jones, 2010). It has been suggested that sensitization to isocyanates can be achieved via other immunological mechanisms, such as direct T cell activation, or on the other hand, that IgE goes undetected for largely technical and methodological reasons (Lutz & Palczynski, 2003; Maestrelli et al., 2009). Until now, the role of IgE in the pathophysiology of OA is still uncertain at best.

The assessment of the different subpopulations of lymphocytes in the draining lymph nodes of the ear and their cytokine production, in our mouse model of chemical-induced asthma, gives the necessary information on sensitization status as well as the profile of the activated lymphocytes.

3 Results and Discussion

Seven mouse strains were tested in an established mouse model of chemical-induced asthma (De Vooght et al., 2009; Vanoirbeek et al., 2004; Vanoirbeek et al., 2006; Vanoirbeek et al., 2008; Vanoirbeek et al., 2009a). The main findings of this study were that, in general, Th2-biased mice reproduce better than Th1-

biased mice, the features that characterize human occupational asthma. BALB/c mice showed the most pronounced differences in AHR, airway inflammation and immunologic parameters, compared to the two other Th2-biased mouse strains tested (BP2 and A/J). Th1-biased mice, however, were not completely non-responsive.

Mice are the most commonly used species to develop experimental models of human diseases. Mice are easy to breed, economical to house and relatively easy to work with. Furthermore, the mouse genome has been extensively studied and it exhibits a high degree of homology with the human genome. Further advantages are the wide variety of available immunological and molecular reagents as well as transgenic animals (Dearman & Kimber, 2009; Willis-Owen & Valdar, 2009).

Besides the many advantages of animal models, there is still a lot of controversy concerning the use of animals to study human disease. Thus, mice do not spontaneously exhibit symptoms consistent with asthma. Different treatment protocols have been developed to mimic the phenotypes of human asthma, but no mouse model is currently able to mimic the full range of clinical manifestations. Furthermore, important differences exist in airway development and morphology between humans and mice. Mouse airways have fewer airway generations and do not contain smooth muscle bundles. Nevertheless, although mice cannot be considered as perfect surrogates for humans, they can be used to test hypotheses in a relatively simple controlled system (Dallas M.Hyde et al., 2009; Wenzel & Holgate, 2006; Willis-Owen & Valdar, 2009).

In view of the above considerations, it is important to standardize techniques and protocols used, to be able to compare the results of different research groups. So far, the influence of the genetic background of mice on ventilatory and immunological parameters has not been studied much. Our study is one of the first to have compared multiple endpoints of immune-mediated asthma in a large number of inbred mouse strains. It is surprising how little is known about genetic differences between the commercially available inbred mouse strains, which makes it hard to link phenotypic differences in parameters to genetic variability. So far, our ventilatory, inflammatory and immunologic results can only be linked to a predominant Th1- or Th2-bias.

3.1 Ventilatory Results

Clear differences were found in AHR to methacholine in the different mouse strains. Th2-biased mouse strains showed increases in AHR compared to Th1-biased mice (Fig 1), as already shown by different other research groups in other asthma models (Al Qadi-Nassar et al., 2007; Brewer et al., 1999a; Fukunaga et al., 2007; Van Hove et al., 2009; Zhu & Gilmour, 2009). However, one exception is the AKR strain (Fig 1G and 1H), which although being Th1-biased, responded significantly in terms of increase in AHR (Brewer et al., 1999b). Furthermore, we found differences in baseline reactivity to methacholine between the different Th2-biased mice. BP2 (Fig 1B and 1H) and A/J (Fig 1C and 1H) mice were found to be more sensitive to methacholine provocation than BALB/c mice (Fig 1A and 1H) and this both for the TDI-treated animals and the control mice. Among the three Th2-biased strains, the BALB/c mice presented the best separation between TDI-sensitized plus TDI-challenged animals and the controls.

Differences in baseline AHR can be an intrinsic characteristic of the mouse strain. Differences in alveolar size, lung volume, elastic properties and differences in controlling smooth muscle cells by the autonomic nerve system have been described (Hadeiba et al., 2000; Soutiere et al., 2004; Tankersley et al., 1999).

When we analyse our results of the AHR more in detail, we can conclude that individual adjustments

for methacholine concentrations per mouse strain are preferable. A/J mice reach a plateau at 10 mg/ml methacholine (Fig 1C), while C57Bl/6 mice are still at a submaximum at that concentration (Fig 1D). We chose to perform the methacholine provocation with the same methacholine concentrations for each mouse strain to make comparisons easier.

3.2 Inflammatory Results

In our model, airway inflammation in TDI-induced asthma is characterized by an influx of mainly neutrophils and also some eosinophils (De Vooght et al., 2009; Tarkowski et al., 2007; Vanoirbeek et al., 2008). This study showed that BALB/c mice have the most pronounced airway inflammation of all 7 mouse strains (Fig 2). However, our observations support that there is no consistent relationship between AHR and the influx of neutrophils and eosinophils in the lungs, as already shown by Whitehead et al. (Whitehead et al., 2003).

When using ovalbumin models, C57Bl/6 are often used and they respond with a robust airway eosinophilic response (Herrick et al., 2003; Van Hove et al., 2009). It is conceivable, as suggested by Herrick et al., that the ability to generate airway inflammation after chemical exposure is under different and perhaps tighter genetic control than the ability to mount these responses after exposure to other antigens (Herrick et al., 2003).

3.3 Immunologic Results

Significant increases in the total amount of Th-, T-regulatory-, T-cytotoxic- and B-lymphocytes were found in all Th2-biased mouse strains and for some lymphocyte subpopulations also for the Th1-biased CBA and AKR mice (Fig 3).

Vogelsang *et al.* already showed that there are different amounts of conventional and plasmacytoid dendritic cells and Treg-lymphocytes in blood and spleen of BALB/c vs. C57Bl/10J mice (Vogelsang et al., 2009). This confirms that the genetic background of the mouse strain plays a role in the development of different immunologically linked cell subtypes and probably also in their cytokine production.

Increases in IL-4 were associated with increases in the total amount of B-lymphocytes and with proportional increases in total serum IgE (Fig 4). However, BALB/c mice turned out to be limited producers of total serum IgE compared to the other mouse strains tested. This raises again the question of the relevance of IgE in chemical-induced asthma. For many low molecular weight compounds, specific IgE antibodies have only been found in a small subset of patients (Chan-Yeung & Malo, 1995). The presence of total IgE in serum serves as a marker of prior sensitization in mice, but has limited functional consequences. This confirms the hypothesis of a non-IgE mediated cellular mechanism involved in the development of chemical-induced asthma. In our data the amount of IL-4 does not always correlate with the level of IgE present in the serum. IL-4 plays an important role in the production of IgE, but mediates also other pathways such as the differentiation of Th-lymphocytes, the ability to induce the expression of vascular cell adhesion molecule 1 (VCAM-1) and the production of mucus in the asthmatic airway (Commins et al., 2010). Furthermore, increases in the concentrations of IL-10, IL-13 and IFN-(γ) (Fig 5) were mainly observed in the Th2-biased mouse strains. The primary source of IL-10 are inducible Treg-lymphocytes, but IL-10 can also be produced by B-lymphocytes and monocytes. IL-13 shares much of IL-4's biologic activities. In murine studies IL-13 has an important role in causing mucus hypersecretion and AHR. IFN-(γ) is the most important cytokine responsible for cell-mediated immunity. It is produced by Th1-lymphocytes but is also derived from Tc-

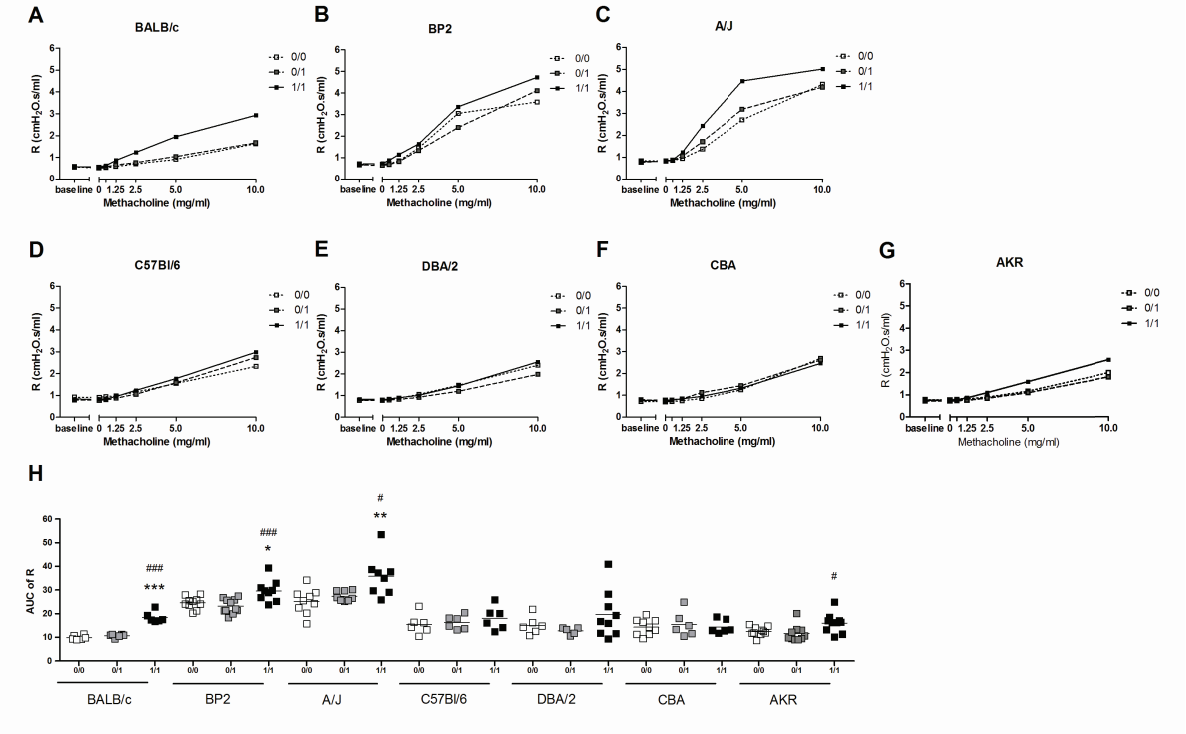

Figure 1: Airway hyperresponsiveness (AHR). The airway resistance (R), after increasing concentrations of methacholine (0-10 mg/ml), was measured 24 hours after the challenge. Figures A) to G) reflect the airway hyperresponsiveness to increasing concentrations of methacholine per mouse strain. Figure H) represents the area under the curve (AUC) of R per experimental condition and per mouse strain. Experimental groups are 0/0, 0/1 and 1/1. The first number identifies the agent used for the dermal applications (sensitizations) and the second number identifies the agent used for the oropharyngeal aspiration (challenge). A treatment with toluene-2,4-diisocyanate (TDI) is shown as 1 and a treatment with the vehicle (a mixture of acetone and olive oil), is shown as 0. Data are presented as mean ± S.D. (A-G) and mean with individual values (H), n = 5-11 per group. According to the D'Agostino & Pearson omnibus normality test, the AUC data of the AHR are not normally distributed and therefore were analyzed with a non-parametric Kruskal-Wallis test followed by a Dunn's multiple comparison test (Graphpad Prism 4.01). * $p < 0.05$, ** $p < 0.01$ and $***p < 0.001$ compared with the 0/0 group, #$p < 0.05$ and ###$p < 0.001$ compared with the 0/1 group.

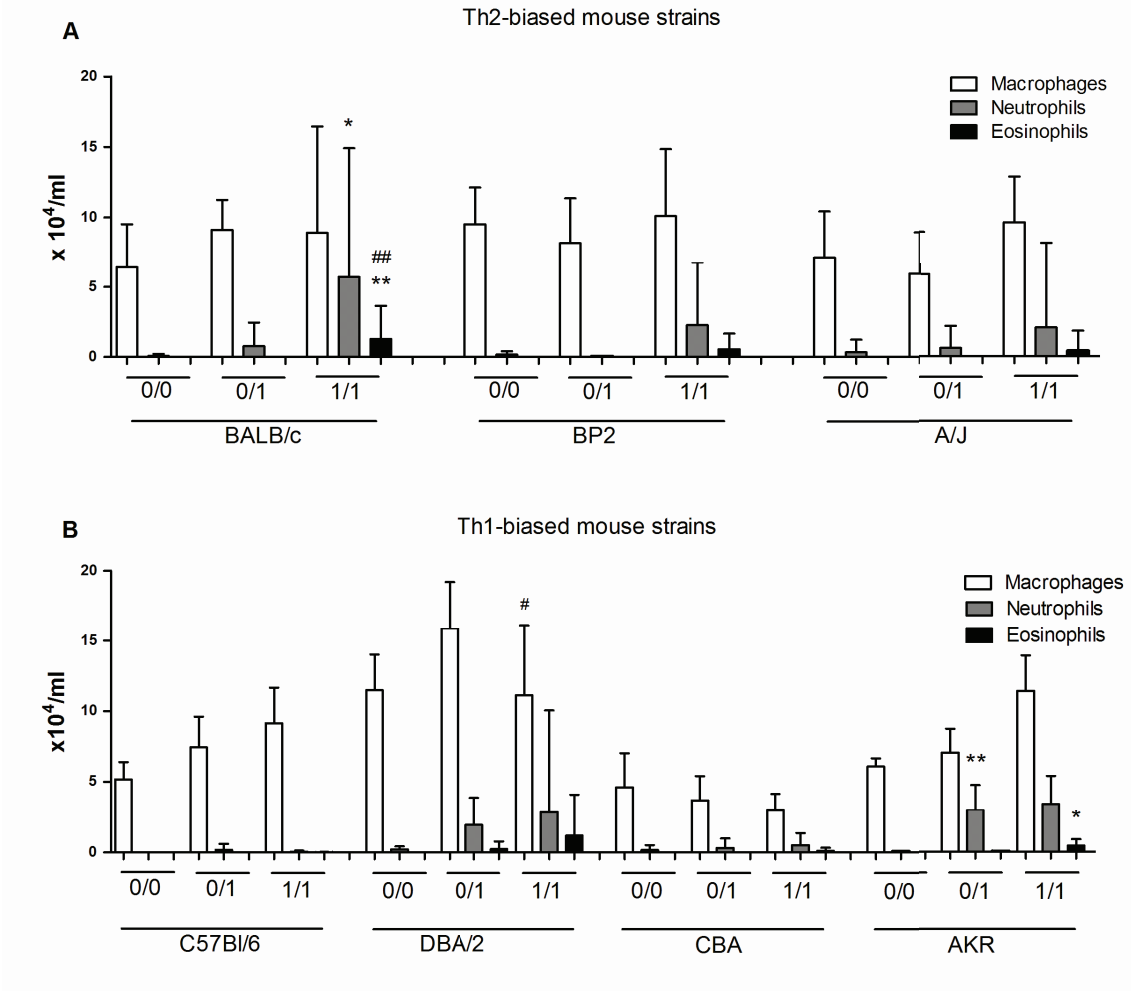

Figure 2: Differential cell count in BAL in different mouse strains. The lungs were lavaged with 3 times 1 ml saline and the number of macrophages, neutrophils and eosinophils was counted in the BAL fluid. Figure A) represents the differential cell count in the Th2-biased mouse strains and figure B) the differential cell count in the Th1-biased mouse strains. Experimental groups are identical to figure 1. Data are presented as means ± S.D., $n = 6 - 14$ per group. The differential cell counts were not normally distributed and therefore were analyzed with a non-parametric Kruskal-Wallis test followed by a Dunn's multiple comparison test. $*p < 0.05$ and $**p < 0.01$ compared to the 0/0 group, # $p < 0.05$ and ## $p < 0.01$ compared to the 0/1 group.

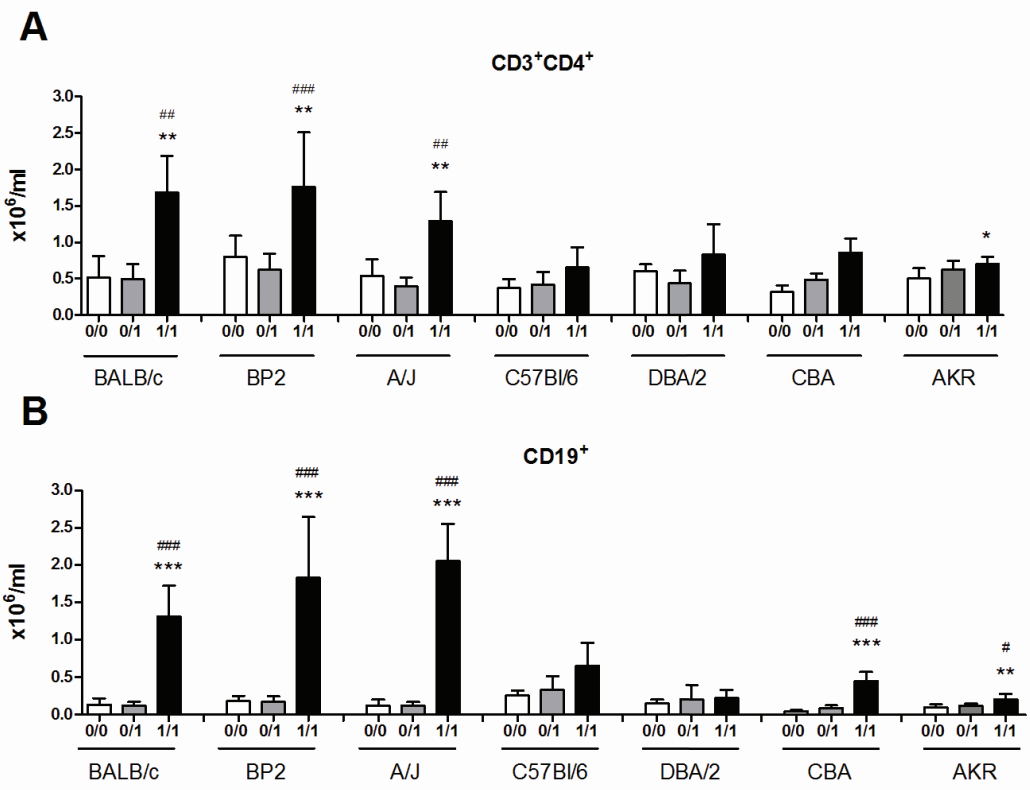

Figure 3: Lymphocyte subpopulations in the auricular lymph nodes of different mouse strains. Auricular lymph nodes were collected and FACS analyses were performed. A) CD3+CD4+ (Th-lymphocytes), B) CD19+ (B-lymphocytes), CD3+CD4+CD25+ (activated/Treg-lymphocytes, data not shown) and CD3+CD8+ (Tc-lymphocytes, data not shown) lymphocytes were characterized. Experimental groups are the same as in figure 1. Data are presented as means ± S.D., $n = 4-9$, $p < 0.05$, $*p < 0.01$ and $**p < 0.001$ compared to the 0/0 group, $\#p < 0.05$, $\#\#p < 0.01$ and $\#\#\#p < 0.001$ compared to the 0/1 group.

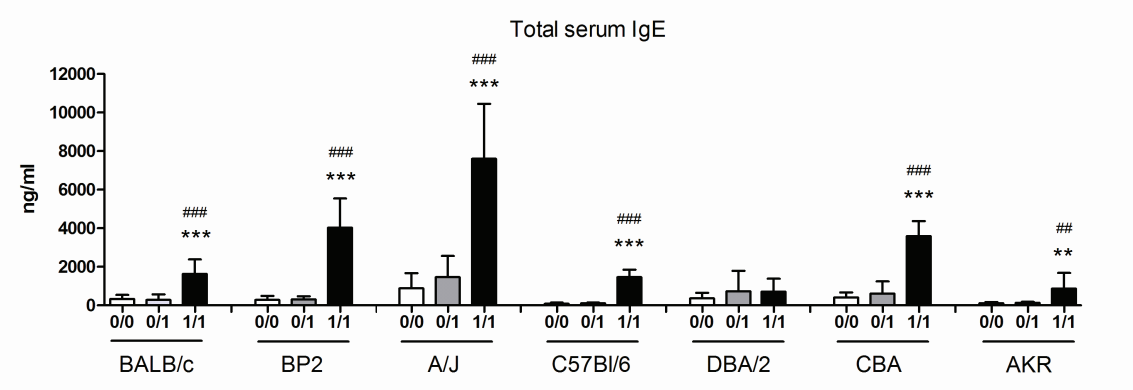

Figure 4: Total serum IgE in different mouse strains. Total serum IgE was measured 24 hours after the challenge. Experimental groups are the same as in figure 1. Data are presented as means ± SD, $n = 6 - 14$. IgE was analyzed with a one-way ANOVA test followed by a Bonferroni's multiple comparison test. $**p < 0.01$ and $***p < 0.001$ compared to the 0/0 group, $\#\#p < 0.01$ and $\#\#\#p < 0.001$ compared to the 0/1 group.

lymphocytes and natural killer cells (Commins et al., 2010).

In an attempt to create a comprehensible overview of all the results combined, we made a radar graph for each mouse strain (Fig 5). In the radar graphs the complete control group (0/0) (blue field) is plotted against the complete TDI-treated group (1/1) (red field). The radar graphs visualize pronounced differences between Th2- and Th1-biased mouse strains. While BALB/c, BP2 and A/J mice gave high values in respiratory, inflammatory and immunological parameters characteristic for occupational asthma, C57Bl/6 and CBA mice showed hardly any responses in our mouse model. In the context of "a mouse model of chemical-induced asthma" both the limited response of the 0/0 group in the BALB/c mice, along with a pronounced response in the 1/1 group, makes the BALB/c mouse strain the most favorable to use, at least in the short term. When we approach the results from a different angle, we can also suggest that C57Bl/6 mice could be a good mouse strain to investigate why most people do not develop allergy and asthma, while some do. The radar graphs show also that TDI sensitization yields a mixed Th1-Th2 profile, as previously shown by us and other research groups, especially in the Th2-biased mouse strains (De Vooght et al., 2009; Matheson et al., 2005b; Matheson et al., 2005a; Tarkowski et al., 2007; Vanoirbeek et al., 2008).

Therefore, in the context of chemical-induced asthma, it is not correct to state that a certain mouse strain is more Th2-biased than Th1-biased.

3.4 Susceptibility Genes

In this experimental setup we did not go in detail into the genetic differences between the different mouse strains. One of the reasons is the lack of proper information about the genetic composition of the different strains. Because genetics probably play an important role in the outcome of an experiment, a short summary is given about what is known in literature concerning susceptibility genes and quantitative trait loci (QTL) in humans and inbred mice (De Vooght et al., 2010).

Although exposed to similar allergic stimuli, not all humans develop allergic or occupational asthma.

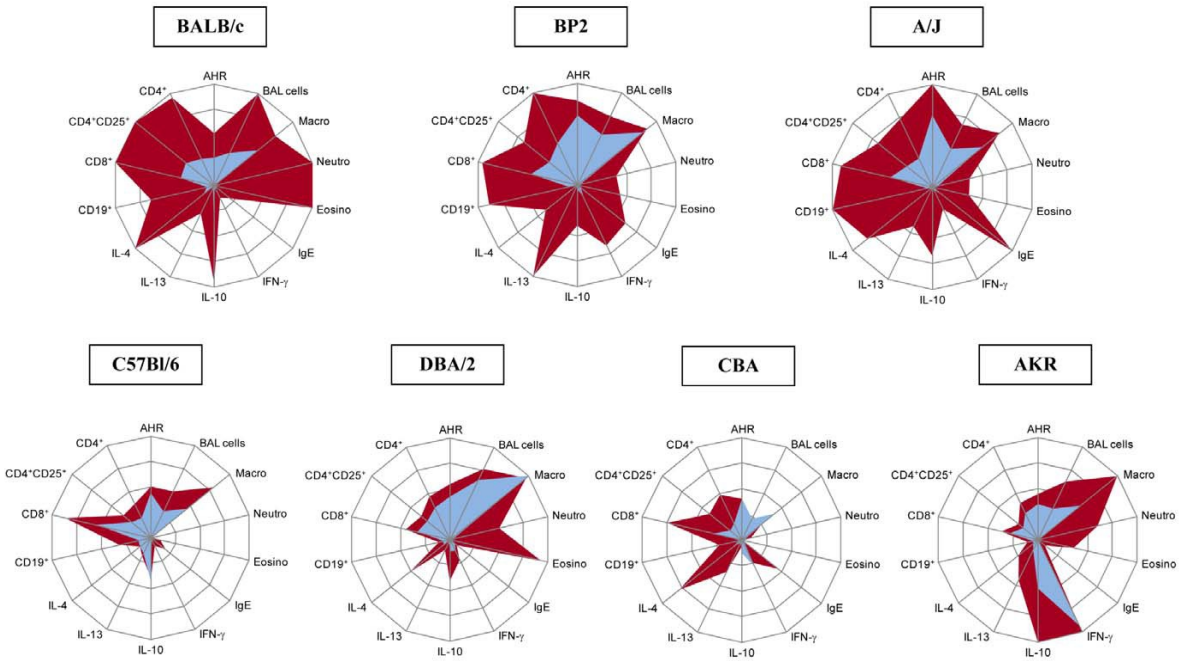

Figure 5: Radar graphs of the different mouse strains. The radar graphs give a visual overview of all results combined for the complete control group (0/0) (blue field) versus the complete TDI-treated group (1/1) (red field). Experimental groups are the same as in figure 1. The lower limit of each axis is always 0. The upper limit of each axis is the maximum average for a specific parameter measured in one strain and is presented as 100%.

The etiology of asthma is complex and is associated with a combination of genetic, immune, environmental and socio-economic factors (Tumes et al., 2007; Zhu & Gilmour, 2009). Involvement of genetic background on chemical-induced asthma has already been shown previously in humans. Associations have been found between diisocyanate-induced OA and glutathione-S-transferase (GST) and N-acetyltransferase (NAT) polymorphisms as well as with human leukocyte antigen (HLA) alleles (Jones et al., 2006; Liu & Wisnewski, 2003; Mapp et al., 2000; Wikman et al., 2002).

The presence of inflammation in the airways is an important biochemical feature of asthma. Enzymes of the GST supergene family are critical in the protection of cells from reactive oxygen species (ROS), key components in inflammation. Wide genetically based individual variations have been observed in the GST enzyme activities, making them candidates as modifiers of susceptibility to diisocyanate-induced asthma. Inability to detoxify ROS could therefore lead to inflammatory processes, activate bronchoconstrictor mechanisms and cause asthmatic symptoms (Piirila et al., 2001). In humans, the GSTM1 null genotype was associated with a two-fold risk of diisocyanate-induced asthma and absence of diisocyanate-specific IgE, the GSTM3 AA genotype with a late reaction in specific challenge testing, and the GSTP1 Val/Val genotype with high total IgE levels. Furthermore, GSTP1 Val/Val genotype was associated with a nine-fold lower risk of asthma than did GSTP1 Ile/Ile (Mapp et al., 2002).

Humans also exhibit large interindividual variation in NAT enzyme activities due to polymorphisms

in NAT1 and NAT2 genes. Diisocyanates may circulate in the body as glutathione conjugates. On the other hand, after inhalation, diisocyanates come into an aqueous environment where they may hydrolyse into diamines, which may subsequently undergo N-acetylation by NAT1 and NAT2. Subjects with slow N-acetylation capacity may be at increased risk because hapten formation would be more favoured. Wikman *et al.* found that the slow acetylator NAT1 genotypes posed a 2.5-fold risk of diisocyanate asthma. The effect of the NAT1 genotype was especially marked for workers exposed to TDI, among whom the NAT1 genotypes posed a 7.8-fold risk of asthma (Wikman et al., 2002).

Besides the metabolizing enzymes GST and NAT, associations have been found with HLA class II proteins encoded on chromosome 6. These are important factors for the specificity of the response to occupational agents. Mapp *et al.* showed a positive association of HLA class II DQA1*0104 and DQB1*0503 with the disease, while DQA1*0101 and DQB1*0501 were significantly more prevalent is asymptomatic exposed subjects (Mapp et al., 2000; Mapp et al., 2005).

Identification of genetic susceptibility loci has been difficult. The genetic mapping of asthma susceptibility in humans has been hampered by factors including variability in clinical phenotype, uncontrolled environmental influences and genetic heterogeneity. Inbred murine models of asthma provide an attractive option to investigate these complexities and genetic analyses of common disease phenotypes in this model system may prove to be more fruitful. Inbred mice show strain-specific variation with respect to various traits (e.g. a phenotypic characteristic) related to asthma (Ackerman et al., 2005). With QTL analysis, different regions of chromosomes that are associated with a particular trait can be found and this can lead to identifying candidate genes. By characterizing strain distribution patterns for asthma related phenotypes, Ewart et al. found in an OVA model that expression of AHR differed between strains and was sometimes discordant with inflammation or serum IgE. This can be compared with the discrepancies we found in our results, suggesting different genetic mechanisms underlying these traits (Ewart et al., 2000).

Although this is only a small selection of susceptibility genes and loci, it reflects well the complexity of allergic and occupational asthma. The few animal studies depicted here can partially explain the differences we found in our results concerning the different mouse strains.

4 Conclusion

In conclusion, this study, based on a comparison of seven different inbred mouse strains in a model of chemical-induced asthma, demonstrates that the genetic background of the different mouse strains has a large impact on the phenotypical outcome of TDI-induced asthma. Caution has to be taken when comparing results from different mouse strains. Furthermore, in this model, BALB/c mice represented best the characteristics of chemical-induced asthma.

Note

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