

Airway Inflammation and Remodeling in Two Mouse Models of Asthma: Comparison of Males and Females

M.J. Blacquière^a M.N. Hylkema^a D.S. Postma^b M. Geerlings^{a, b} W. Timens^a
B.N. Melgert^{a, b}

Departments of ^aPathology and Medical Biology and ^bPulmonology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Key Words

Airway responsiveness • Allergic airway inflammation • Eosinophilic inflammation • House dust mite • IL-4 • IL-17 • IgE • Ovalbumin • Remodeling • Sex

Abstract

Background: Asthma and especially severe asthma affect women more frequently than men. Since asthma severity correlates with remodeling changes in the lung, a female propensity to remodeling could be expected. We studied whether our previous observation that female mice have more pronounced airway inflammation than males is associated with more pronounced remodeling in two models of chronic allergic asthma. **Methods:** Male and female BALB/c mice were (1) sensitized and subsequently challenged with ovalbumin (OVA) for 4 weeks, or (2) exposed to house dust mite (HDM) for 5 weeks. In both models, allergic inflammation, remodeling, antigen-specific IgE and methacholine (MCh) responsiveness were assessed. **Results:** Females had higher antigen-specific serum IgE levels, higher numbers of eosinophils and were more responsive to MCh. In the OVA model, females also had higher levels of Th2 cytokines in lung tissue than males. Both sexes developed similar airway remodeling (smooth muscle layer thickness, collagen III de-

position and goblet cell hyperplasia) in the two models. **Conclusions:** Combining results of an OVA- and a HDM-induced mouse model of allergic airway inflammation, we have shown that more severe allergic inflammation in females is not accompanied with more pronounced airway remodeling.

Copyright © 2010 S. Karger AG, Basel

Introduction

The contribution of remodeling to asthma pathogenesis and its clinical relevance remain controversial. Remodeling in asthma is characterized by structural abnormalities like hypertrophy of airway smooth muscle, subepithelial fibrosis, goblet cell hyperplasia, and proliferation of airway blood vessels and nerves. Airway remodeling is more prominent in patients with severe asthma [1] and inflammation has been regarded as the main pathogenic factor. However, it is not clear whether more inflammation directly leads to more remodeling. It has been suggested that severe asthma is a disease different from mild/moderate asthma and does not necessarily develop from moderate asthma [2].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2010 S. Karger AG, Basel
1018–2438/10/1532–0173\$26.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. B.N. Melgert
Department of Pathology and Medical Biology, EA1
University Medical Center Groningen
Hanzeplein 1, NL-9713 GZ Groningen (The Netherlands)
Tel. +31 50 361 0109, Fax +31 50 361 9911, E-Mail b.n.melgert@med.umcg.nl

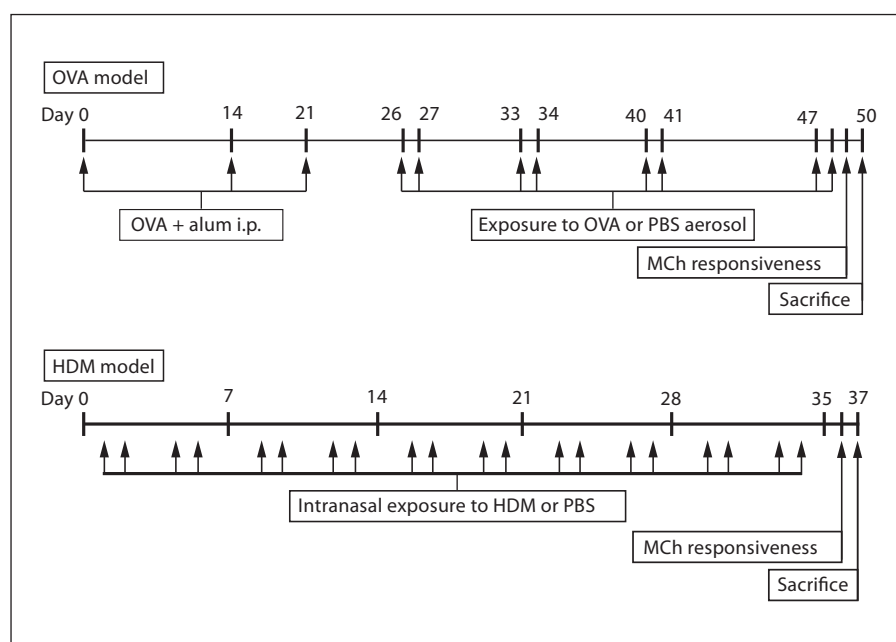


Fig. 1. Experimental design of the study: OVA and HDM model.

The importance of airway remodeling as an early and consistent feature of asthma has been suggested by recent studies in children with asthma [3, 4]. These studies suggest that remodeling starts early during the development of asthma and occurs in parallel with inflammation or is even a prerequisite for persistent inflammation [5, 6].

In adulthood, asthma occurs more frequently among women, and severe asthma is a predominantly female disease [7]. Since asthma severity correlates with remodeling changes in the lung, a female propensity for remodeling could be anticipated. To our knowledge, no studies have addressed this issue yet.

To assess whether more severe airway inflammation leads to more remodeling, we used two mouse models of chronic allergic asthma in which remodeling was defined as goblet cell hyperplasia and depositions of collagen III and α -smooth muscle actin (α -SMA) around airways. We and others recently showed that in a model of ovalbumin (OVA)-induced allergic asthma, female mice develop more severe airway inflammation as compared to males [8–10]. We adapted this model of acute airway inflammation to a model of chronic inflammation to study airway remodeling. In parallel to this model, we ran the clinically more relevant house dust mite (HDM)-induced airway inflammation mouse model to compare responses. First, we confirmed sex differences in airway inflammation in both models and next we investigated the extent of remodeling in male and female mouse lungs.

Materials and Methods

Animals

Male and female BALB/c mice aged 6–8 weeks (Harlan, Horst, The Netherlands) were held under specific pathogen-free conditions in groups of 6–8 mice per cage. The experiments, approved by the local Committee on Animal Experimentation, were performed under strict governmental and international guidelines on animal experimentation.

OVA Model

Male ($n = 6$ per group) and female ($n = 6$ per group) mice were sensitized to OVA on days 1, 14, and 21 by intraperitoneal injections of 10 μ g OVA (Grade V, Sigma Aldrich, Zwijndrecht, The Netherlands) emulsified in 1.5 mg aluminum hydroxide (Aluminject, Pierce Chemical, Etten-Leur, The Netherlands) diluted to 200 μ l with PBS. They were subsequently challenged with 1% OVA aerosols in PBS (OVA mice) or PBS aerosols (PBS mice) for 20 min, twice a week for 4 weeks (fig. 1). On day 49, responsiveness to methacholine (MCh) was tested and the animals were sacrificed on day 50 to collect serum for OVA-specific IgE and lung tissue for histological analysis and cytokine determination.

HDM Model

Male ($n = 8$ per group) and female ($n = 8$ per group) mice were exposed intranasally to 25 μ g (in 10 μ l PBS) purified whole-body HDM extract (*Dermatophagoides pteronyssinus*, Greer laboratories, Lenoir, N.C., USA) 4 times a week during 5 weeks. Control animals were exposed to 10 μ l PBS.

Responsiveness to MCh was tested 1 day after the last intranasal challenge (day 36) and the animals were sacrificed on day 37 to collect serum for HDM-specific IgE and lung tissue for histological analysis and cytokine determination (fig. 1).

Each experiment has been replicated in 6 (OVA model) or 8 (HDM model) mice per exposure group. Due to logistical reasons, these experiments had a staggered design, so in each group, mice were sacrificed at different times.

Assessment of MCh Responsiveness

MCh responsiveness was assessed as changes in enhanced pause (P_{enh}) in conscious, spontaneously breathing animals using a whole-body plethysmography system (Buxco Electronics, Petersfield, UK) as described previously [11]. Recently, there has been a discussion about the accuracy of whole-body plethysmography to measure airway responsiveness. Therefore, we used this technique in conscious mice to assess the response to a nonspecific bronchoconstrictor (MCh) and not as a measure of airway resistance per se. We therefore have chosen the term 'MCh responsiveness' instead of 'airway hyperresponsiveness'.

Measurement of OVA-Specific Serum IgE

OVA-specific serum IgE was measured by ELISA as described previously [8]. Levels of OVA-specific IgE were expressed in arbitrary ELISA units.

Measurement of HDM-Specific Serum IgE

HDM-specific serum IgE was measured by ELISA as described previously [12].

Cytokines

Concentrations of interleukin (IL)-4, IL-5, IL-6, IL-13, IL-17 and interferon- γ (IFN- γ) in lung tissue were measured with a multiplex ELISA system (Lincplex Systems, St. Charles, Mo., USA) on a Luminex 100 system using Starstation software (Applied Cytometry Systems, Sheffield, UK). Lung tissue was homogenized (20% w/v) in 50 mM Tris-HCl buffer containing 150 mM NaCl, 0.002% Tween-20 (pH 7.5) and a protease inhibitor (Sigma Aldrich). This was centrifuged at 12,000 g for 10 min to remove any insoluble material. Supernatants were subsequently stored at -80°C until further analysis.

Transforming growth factor- β_1 (TGF- β_1) in serum was measured with a sandwich ELISA (R&D Systems, Abingdon, UK).

Histology

Frozen lung sections were made in the caudal-cranial axis from the ventral end (the convex side) of the right azygous and diaphragmatic lobe. Sections were made where small/intermediate airways were present and large cartilaginous airways were totally or largely absent in the sectional plane.

Paraffin/Nakane embedded lung sections were made cross-sectionally halfway the left lobe, 45° from the dorsal-ventral axis (to exclude the large cartilaginous bronchi close to the hilum). Sections were made when small/intermediate airways were present and large cartilaginous airways were totally or largely absent in this sectional plane as well.

Eosinophils were identified in 4- μm cryosections by staining for cyanide-resistant endogenous peroxidase activity with diaminobenzidine (Sigma Aldrich) and quantified by morphometric analysis using Leica Qwin image analysis software (Leica Microsystems, Rijswijk, The Netherlands) and expressed as volume percentages. Neutrophils were identified in 4- μm sections of Nakane-fixed lung tissue (OVA model) or 4- μm cryosections (HDM model) with a rat monoclonal anti-GR1 antibody (BD Biosciences)

and visualized with 3-amino-9-ethylcarbazole (AEC, Sigma Aldrich). Positive cells were quantified by manual counting in the whole tissue section and the total tissue area was quantified by morphometric analysis. The numbers of cells were expressed per square millimeter. Positive cells were quantified by morphometric analysis and expressed as volume percentages.

Airway remodeling was defined as increased goblet cell hyperplasia, collagen III deposition and α -SMA expression around airways. Goblet cells were determined in 4- μm sections of frozen lung tissue (OVA model) or formalin-fixed lung tissue (HDM model) by counting cells staining positive for periodic acid-Schiff (PAS). The length of all airways (measured at the basal end of the airway epithelium) in the section was determined, and the total number of PAS-positive cells was expressed per millimeter airway.

Collagen III and α -SMA were identified in 4- μm sections of frozen lung tissue with a polyclonal goat-anti-mouse collagen III antibody (SBA, Birmingham, Ala., USA) and a monoclonal mouse anti-rat α -SMA antibody (Progen, Heidelberg, Germany), respectively. They were visualized with fuchsin chromogen (for collagen III) and AEC for α -SMA, respectively. The surface of positively stained tissue was expressed as square millimeters per millimeter airways in a total lung section. Staining in parenchymal tissue and directly adjacent to blood vessels was excluded from the measurement.

Cartilaginous airways if present were excluded from all analyses. Each analysis was performed blinded by the same observer.

Statistical Analysis

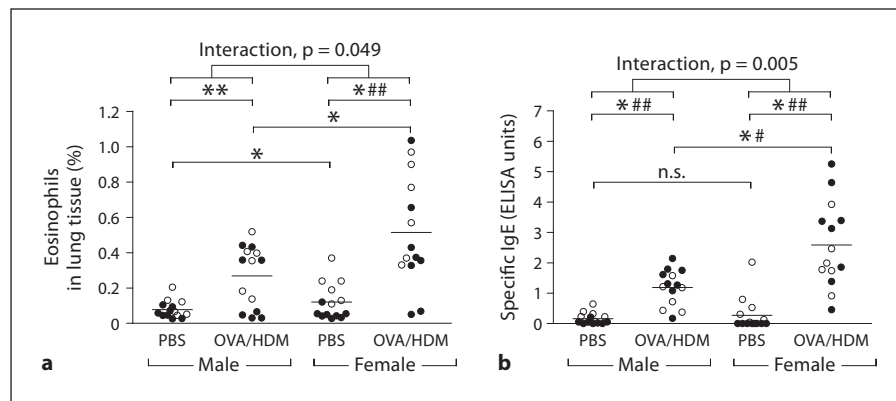
When residuals were not normally distributed, appropriate log10 or 1/x transformation of the data was performed. The interaction of the effect of antigen exposure and the effect of sex difference was investigated with a multiple linear regression model (SPSS 14.0 software, SPSS Inc. Chicago, Ill., USA). Multiple linear regression analysis can distinguish between a positive interaction and a negative interaction. A significant positive interaction (the only interaction type found in our analyses) means that the effect of antigen exposure is greater in females than in males. When no interaction was found, the effect of antigen exposure (OVA/HDM effect) was assessed by linear regression analysis. When no interaction and no effect of antigen exposure were found, the effect of sex was assessed by linear regression analysis. The multiple linear regression analyses were performed for the OVA and HDM models together, except for the cytokines IL-6, IL-13, IL-17, IFN- γ and TGF- β_1 since these cytokines did not fall into equal ranges for the two models. For analysis of MCh responses, the area under the P_{enh} curve was calculated and analyzed with the Mann-Whitney U test. $p < 0.05$ was considered significant.

Results

Females have higher volume percentages of eosinophils in lung tissue and higher levels of antigen-specific IgE in serum after antigen exposure.

OVA and HDM significantly increased the volume percentage of eosinophils in lung tissue (fig. 2a) and levels of antigen-specific IgE in serum (fig. 2b) in male and fe-

Fig. 2. Eosinophils in lung tissue (a) and antigen-specific IgE (b) in serum from male and female mice exposed to PBS or antigen in the OVA model (open symbols) and HDM model (closed symbols). Individual data points and means are shown. 'Interaction' indicates a larger effect of antigen exposure in females as compared to males. OVA/HDM effect, $p \leq 0.001$ (a, b). * $p < 0.05$, ** $p < 0.01$ (OVA model), # $p < 0.05$, ## $p < 0.01$ (HDM model); n.s. = not significant in post-hoc analyses.



male mice (OVA/HDM effect). The response to antigen exposure was larger in female mice (explanation for the interaction between the effects of antigen exposure and sex). No differences in numbers of neutrophils per square millimeter lung tissue were found with respect to antigen exposure or sex (data not shown).

Cytokine Levels

Levels of IL-4 (fig. 3a) and IL-5 (fig. 3b) were measured in equal ranges for the two models and were therefore analyzed collectively. Levels of IL-13, IFN- γ , IL6, IL-17 and TGF- β_1 (fig. 3c–g) were not measured in equal ranges and were therefore analyzed separately for the OVA and the HDM model.

Antigen exposure induced a larger increase in the levels of IL-4 in females than in males (explanation for the interaction between the effects of antigen exposure and sex), but no such interaction was found for IL-5. In addition, OVA exposure increased IL-13 and both OVA and HDM exposure increased levels of IL-6 and IL-17. Furthermore, antigen exposure resulted in a larger increase in IL-6 in the OVA-exposed females and a larger increase in IL-17 in HDM-exposed females compared to males (explanation for the interactions between the effects of antigen exposure and sex).

IFN- γ and TGF- β_1 were not affected by antigen exposure in either sex but were lower in females than in males in the OVA model (sex effect $p = 0.013$ and $p = 0.008$, respectively).

Both Sexes Display Similar Airway Remodeling after Antigen Exposure

Antigen exposure significantly increased the number of goblet cells in the airways (fig. 4a), smooth muscle layer thickness around the airways (fig. 4b) and collagen III

deposition around the airways (fig. 4c). For these three remodeling parameters, no interaction between the effect of antigen exposure and sex was found.

Females Display Increased MCh Responsiveness after Antigen Exposure in Both Models

OVA exposure as well as HDM exposure increased the sensitivity for MCh in females as compared to females exposed to PBS (fig. 5a). This left shift of the curve was not seen in males exposed to OVA or HDM compared to PBS-exposed animals (fig. 5b).

Discussion

This study in two models of chronic asthma confirms earlier findings in *acute* OVA models [8, 10, 13, 14]. We and others previously found that females are more prone to developing a more severe asthma phenotype than males. More importantly, we show that this higher propensity to develop allergic airway inflammation in females is not accompanied with increased airway remodeling.

Two important characteristics of allergic airway inflammation, i.e. eosinophils in lung tissue and antigen-specific IgE in serum, were more pronounced in females than in males in both chronic models. Additionally, the levels of Th2 cytokine IL-4 in lung tissue also showed a larger increase in antigen-exposed females than males. This is in line with the higher levels of antigen-specific IgE in females.

However, when we investigated whether the severity of airway remodeling (goblet cell hyperplasia, smooth muscle layer and collagen III deposition) in male and female mice was associated with the severity of airway inflam-

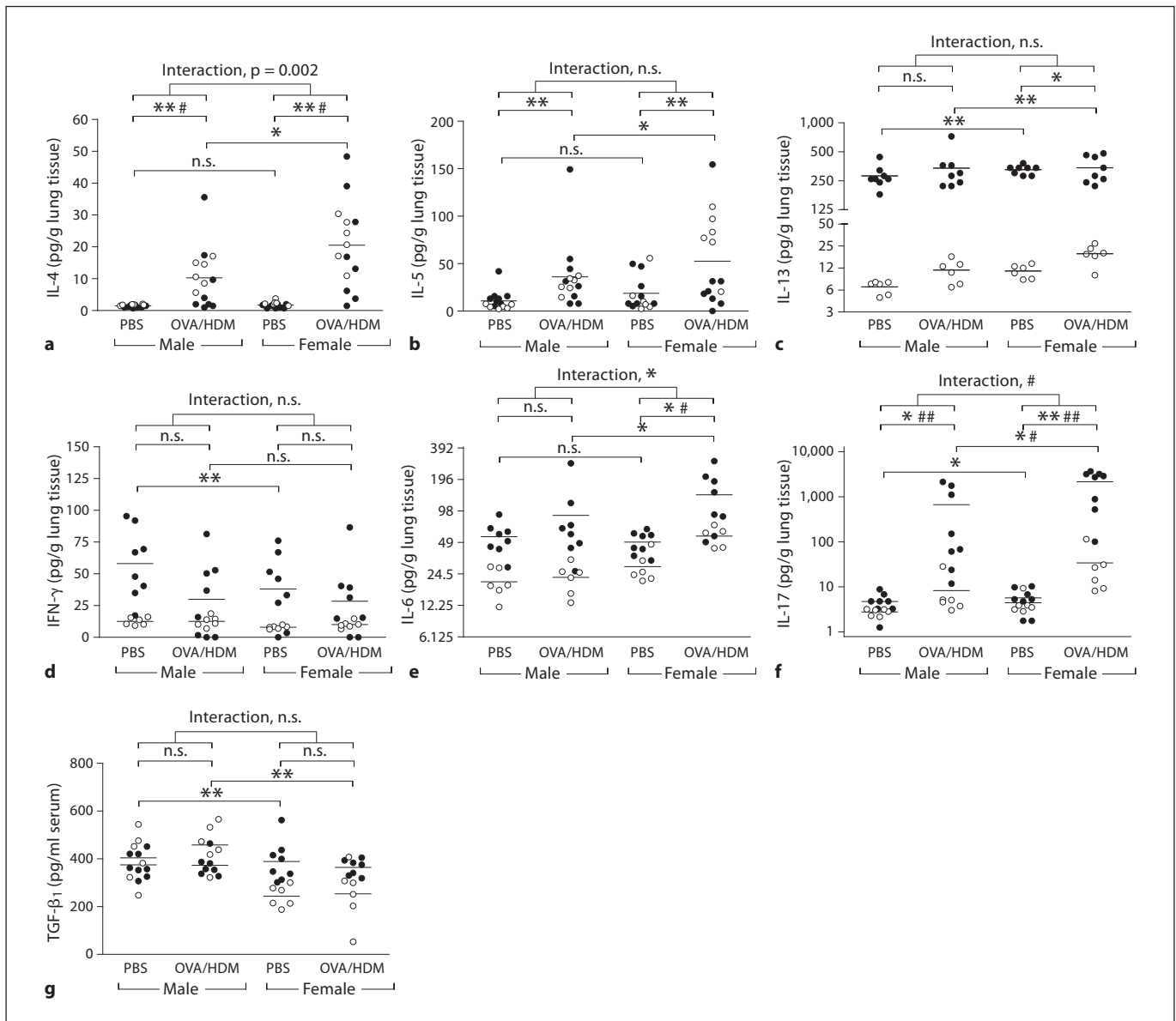


Fig. 3. Levels of IL-4 (**a**), IL-5 (**b**), IL-13 (**c**), IFN- γ (**d**), IL-6 (**e**), IL-17 (**f**) in lung tissue and TGF- β_1 (**g**) in serum from male and female mice exposed to PBS or antigen in the OVA model (open symbols) and HDM model (closed symbols). Individual data points and means are shown. Linear regression analyses for the cytokines IL-13, IFN- γ , IL-6, IL-17 and TGF- β_1 were performed for both models separately. 'Interaction' indicates a larger effect of

antigen exposure in females as compared to males. **a, b** OVA/HDM effect, $p < 0.001$. **c** OVA effect, $p < 0.01$, HDM effect, n.s. **d** OVA and HDM effect, n.s. **e** HDM effect $p < 0.01$. **f** OVA and HDM effect, $p \leq 0.001$. **g** OVA and HDM effect, n.s. * $p < 0.05$, ** $p < 0.01$ (OVA model), # $p < 0.05$, ## $p < 0.01$ (HDM model); n.s. = not significant in post-hoc analyses.

mation, we found that this was not the case. The amount of remodeling in both male and female mice as compared to unchallenged mice was increased, yet there was no difference between male and female mice in this respect. Taken together, our group sizes were sufficiently large to

pick up differences in eosinophilic inflammation, antigen-specific IgE and levels of IL-4 between male and female mice whereas remodeling was similar in males and females or at least differed to a smaller extent between the two sexes. Since there was a trend for an interaction be-

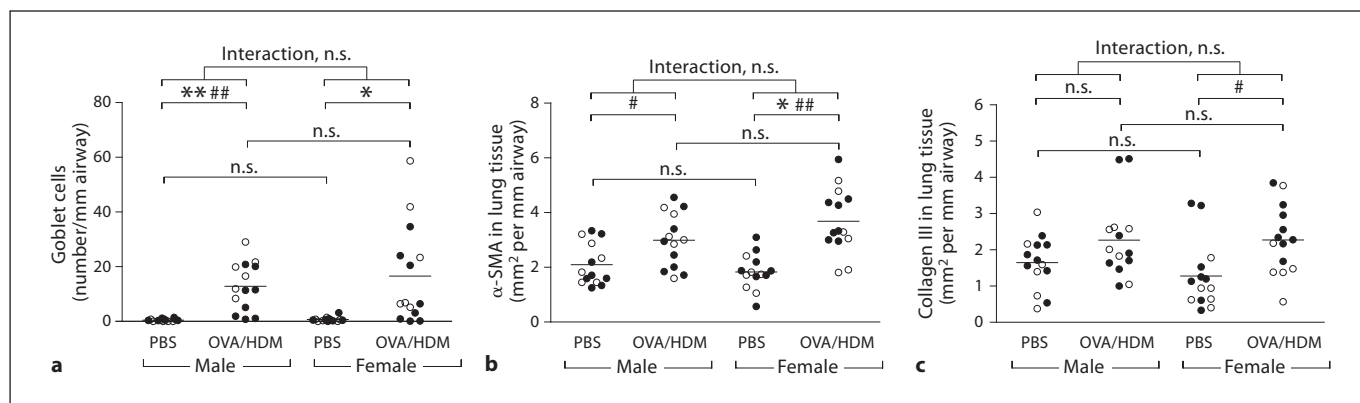


Fig. 4. **a** Goblet cells in the airways. **b** Smooth muscle layer around the airways. **c** Collagen III deposition in lung tissue from male and female mice exposed to PBS or antigen in the OVA model (open symbols) and HDM model (closed symbols). Individual data points and means are shown. OVA/HDM effect, $p \leq 0.002$. * $p < 0.05$, ** $p < 0.01$ (OVA model), # $p < 0.05$, ## $p < 0.01$ (HDM model); n.s. = not significant in post-hoc analyses.

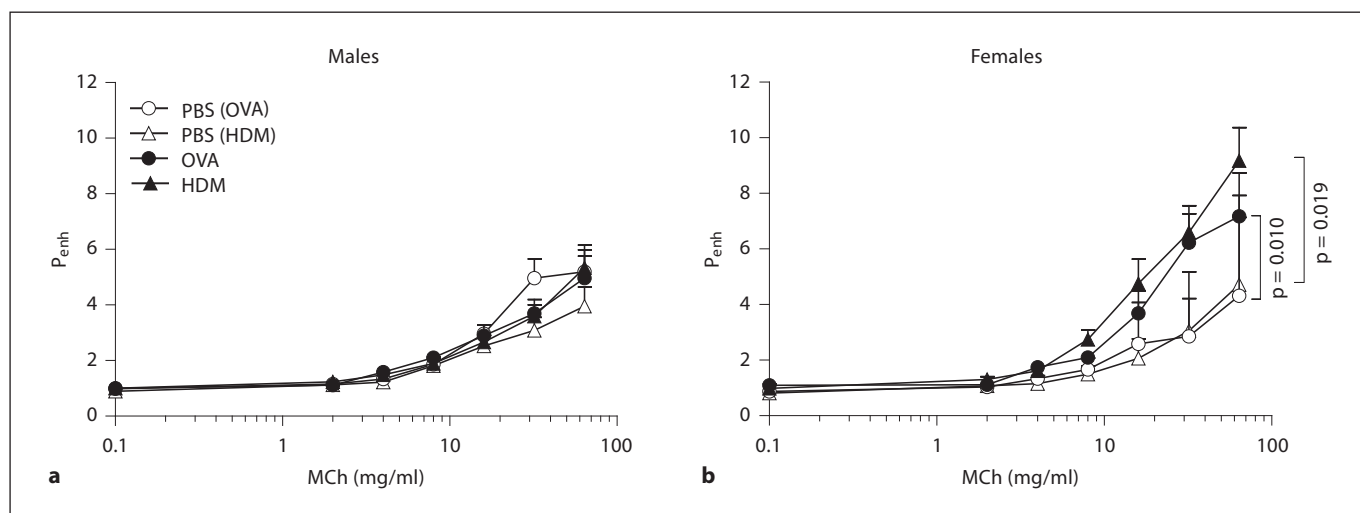


Fig. 5. MCh responsiveness from male (**a**) and female (**b**) mice exposed to PBS or antigen in the OVA model (open symbols) and HDM model (closed symbols). Means $\pm$ SEM are shown. p values expressed in the graph are derived from Mann-Whitney U tests of the area under the P_{enh} curve.

tween the effect of antigen exposure and sex for airway smooth muscle layer, an even larger group size may show that females are more prone to develop airway smooth muscle hyperplasia. However, we do not expect that this will also be the case for goblet cell hyperplasia and collagen III deposition.

Several studies have shown that inflammatory cytokines such as IL-4 and IL-13 can contribute to goblet cell hyperplasia [15, 16] and smooth muscle cell growth in vi-

tro [17]. The lack of an association between goblet cell hyperplasia and airway smooth muscle layer on the one hand and IL-4 on the other hand in this study does not necessarily imply that this cytokine does not contribute to the remodeling process at all in our mouse models of asthma. It is possible that the remodeling parameters we measured can only increase up to a certain maximum beyond which inflammation can increase without further increases in remodeling (ceiling effect). This hypoth-

esis is supported by a study by Payne et al. [18], who showed equal increases in reticular basement membrane thickness (another marker of airway remodeling which is shown to be correlated with collagen III and fibronectin deposition) in children and adults with mild or acute life-threatening asthma, despite changes in eosinophilic airway inflammation, anti-inflammatory therapy or duration of asthma.

Furthermore, we show that antigen exposure results in higher MCh responsiveness in females than in males, and this suggests that MCh responsiveness may be related to airway eosinophilia rather than to airway remodeling (at least in our female mice). A recent study in mice supports this, showing that airway responsiveness and reduced baseline lung function were evident before the onset of collagen deposition and that airway responsiveness together with airway eosinophilia in this model decreased, independently of the persistent peribronchial collagen deposition [19]. Several clinical studies have suggested a relationship between airway responsiveness and reticular basement membrane thickness [20, 21] or airway smooth muscle thickness [22] in biopsies, yet many other studies have not shown consistent relationships between airway remodeling and airway responsiveness, as reviewed in these papers [23, 24]. Therefore, our data in mice are comparable to data in clinical studies showing that airway remodeling may not always contribute to airway responsiveness and that other factors contribute as well. Furthermore, one could interpret from our data that male mice do not develop MCh responsiveness. However, this is not the case since our short-term study provides evidence of MCh responsiveness in male and female mice [8]. This suggests that MCh responsiveness in male mice decreases after long-term OVA or HDM exposure, in contrast to ongoing MCh responsiveness in female mice. This is compatible with previous observations that female mice are more prone to chronic inflammation [8] whereas male mice may be more prone to acute or nonallergic inflammation and subsequent MCh responsiveness [25, 26].

Interestingly, when we investigated the cytokine levels in both models, we found no interactions between the effect of antigen exposure and the effect of sex difference for the Th2 cytokines IL-5 and IL-13, but we observed that the levels of these cytokines were higher in OVA-exposed females than in OVA-exposed males. Post-hoc subgroup analysis with Mann-Whitney U tests confirmed this finding. This indicates that chronic and short-term OVA exposure have similar effects with respect to sex differences [8, 10], but that these effects are

less pronounced. Furthermore, we observed that in the OVA model, cytokine levels of IL-13 and IL-17 were higher and levels of IFN- γ and TGF- β_1 were lower in sensitized and PBS-challenged female mice compared to sensitized and PBS-challenged male mice. This was also confirmed with post-hoc subgroup analyses. Since this difference between PBS-challenged male and female mice was not present in the HDM model, this may indicate that female mice respond differently to the procedure of intraperitoneal sensitization with alum and OVA than male mice with respect to subsequent cytokine production. Whether this is caused by the intraperitoneal injections or by the administration of the adjuvant cannot be established from our data. In an interesting study by Careau et al. [27], intraperitoneal sensitization of rats with an allergen induced phenotypical differences in pulmonary macrophages and these macrophages subsequently had proallergic capabilities. The mechanism behind this remarkable finding was not further investigated, but may be one of the causes for the sex differences found. Especially so as we have just shown that macrophages are probably responsible for the aggravated airway inflammation in female mice in the acute OVA model [10].

Additionally, the lower levels of TGF- β_1 in females may have caused less downregulation of airway inflammation and responsiveness in females but may have had no effect on airway remodeling. This is supported by the finding that TGF- β is an important downregulator of inflammatory responses [28], known to specifically suppress the activity of eosinophils, Th2 cells, B cells and macrophages. The absence of TGF- β signaling increased airway inflammation and airway responsiveness but had no effect on airway remodeling in mice exposed to HDM [29]. Thus, lower levels of this cytokine could induce the higher susceptibility of females to allergic airway inflammation. Our data are in concurrence with data showing that neutralization of TGF- β in female mice increased MCh responsiveness and Th2 inflammation and that intraperitoneal alum + OVA sensitization decreased TGF- β levels in mice [30]. However, this would not explain the sex differences found in the HDM model.

An interesting novel finding was that the increase in IL-6, involved in Th17 differentiation, was larger in females in the OVA model. Furthermore, post-hoc analysis revealed that the level of IL-17, the major product of Th17 cells, was also higher in OVA-exposed females compared with OVA-exposed males, which is consistent with the larger increase in IL-17 in females than in males in the HDM model. IL-17 is known to be associated with airway

responsiveness to MCh [31] and IL-17-expressing cells are increased in sputum and BAL from asthmatic patients [32]. Since Schnyder-Candrian et al. [33] showed that IL-17 is essential in the sensitization phase of establishing allergic asthma, the higher IL-17 levels in females could have made them more susceptible to allergic sensitization and therefore to airway inflammation and MCh responsiveness.

In summary, we have shown increased eosinophilic airway inflammation, IL-4 levels in lung tissue, antigen-specific IgE levels in serum and MCh responsiveness in female mice compared to male mice in two models of asthma. Since airway inflammation is thought to have an effect on airway remodeling, a female propensity for airway remodeling was anticipated. Although we have

shown that airway remodeling is increased in mice with airway inflammation, female mice do not show signs of a more pronounced airway remodeling as compared to males in both models. This indicates that airway inflammation and remodeling are characteristics of asthma that can occur in parallel but may not necessarily be related.

Acknowledgements

This work was supported by grant 3.4.05.041 from the Netherlands Asthma Foundation (B.M.), a Spinoza premium of the Dutch Government (D.P.) and a grant from the J.K. de Cock stichting (M.H.). Nicole Stowell, Anuk Das and Don Griswold (Centocor, Philadelphia, Pa., USA) are kindly acknowledged for performing the Luminex assay for the OVA model.

References

- Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P, Ludwig MS, Martin JG, Hamid Q: Differences in airway remodeling between subjects with severe and moderate asthma. *J Allergy Clin Immunol* 2005;116: 544–549.
- Holgate ST, Holloway J, Wilson S, Bucchieri F, Puddicombe S, Davies DE: Epithelial-mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* 2004;1:93–98.
- Sagliani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK: Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007;176:858–864.
- Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, Zanin ME, Zuin R, Maestrelli P, Fabbri LM, Saetta M: Epithelial damage and angiogenesis in the airways of children with asthma. *Am J Respir Crit Care Med* 2006;174:975–981.
- Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, Sears MR: Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV₁/vital capacity ratio: a longitudinal population study from childhood to adulthood. *Am J Respir Crit Care Med*.
- Cokugras H, Akcakaya N, Seckin, Camcioglu Y, Sarimurat N, Aksoy F: Ultrastructural examination of bronchial biopsy specimens from children with moderate asthma. *Thorax* 2001;56:25–29.
- Almqvist C, Worm M, Leynaert B: Impact of gender on asthma in childhood and adolescence: a GA₂LEN review. *Allergy* 2008;63: 47–57.
- Melgert BN, Postma DS, Kuipers I, Geerlings M, Luinge MA, van der Strate BW, Kerstjens HA, Timens W, Hylkema MN: Female mice are more susceptible to the development of allergic airway inflammation than male mice. *Clin Exp Allergy* 2005;35:1496–1503.
- Corteling R, Trifileff A: Gender comparison in a murine model of allergen-driven airway inflammation and the response to budesonide treatment. *BMC Pharmacology* 2004;4: 4.
- Melgert BN, Oriss TB, Qi Z, Dixon-McCarthy B, Geerlings M, Hylkema MN, Ray A: Macrophages: regulators of sex differences in asthma? *Am J Respir Cell Moll Biol* 2009, Jul 2. Epub ahead of print.
- Melgert BN, Postma DS, Geerlings M, Luinge MA, Klok PA, van der Strate BW, Kerstjens HA, Timens W, Hylkema MN: Short-term smoke exposure attenuates ovalbumin-induced airway inflammation in allergic mice. *Am J Respir Cell Moll Biol* 2004; 30:880–885.
- Blacquiere MJ, Timens W, Melgert BN, Geerlings M, Postma DS, Hylkema MN: Maternal smoking during pregnancy induces airway remodelling in mice offspring. *Eur Respir J* 2009;33:1133–1140.
- Seymour BW, Friebertshauser KE, Peake JL, Pinkerton KE, Coffman RL, Gershwin LJ: Gender differences in the allergic response of mice neonatally exposed to environmental tobacco smoke. *Dev Immunol* 2002;9:47–54.
- Hayashi T, Adachi Y, Hasegawa K, Morimoto M: Less sensitivity for late airway inflammation in males than females in BALB/c mice. *Scand J Immunol* 2003;57:562–567.
- Perkins C, Wills-Karp M, Finkelman FD: IL-4 induces IL-13-independent allergic airway inflammation. *J Allergy Clin Immunol* 2006;118:410–419.
- Park KS, Korfhagen TR, Bruno MD, Kitzmiller JA, Wan H, SE Wert, Khurana Hershey GK, Chen G, Whitsett JA: SPDEF regulates goblet cell hyperplasia in the airway epithelium. *J Clin Invest* 2007;117:978–988.
- Bosse Y, Thompson C, Audette K, Stankova J, Rola-Pleszczynski M: Interleukin-4 and interleukin-13 enhance human bronchial smooth muscle cell proliferation. *Int Arch Allergy Immunol* 2008;146:138–148.
- Payne DNR, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK: Early thickening of the reticular basement membrane in children with difficult asthma. *Am J Respir Crit Care Med* 2003;167:78–82.
- Koerner-Rettberg C, Doths S, Stroet A, Schwarze J: Reduced lung function in a chronic asthma model is associated with prolonged inflammation, but independent of peribronchial fibrosis. *PLoS One* 2008; 3:e1575.
- Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH: Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309–316.
- Lee SY, Kim SJ, Kwon SS, Kim YK, Moon HS, Song JS, Park SH: Relation of airway reactivity and sensitivity with bronchial pathology in asthma. *J Asthma* 2002;39:537–544.
- Woodruff PG, Dolganov GM, Ferrando RE, Donnelly S, Hays SR, Solberg OD, Carter R, Wong HH, Cadbury PS, Fahy JV: Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am J Respir Crit Care Med* 2004; 169:1001–1006.

- 23 Berend N, Salome CM, King GG: Mechanisms of airway hyperresponsiveness in asthma. *Respirology* 2008;13:624–631.
- 24 James AL, Wenzel S: Clinical relevance of airway remodelling in airway diseases. *Eur Respir J* 2007;30:134–155.
- 25 Card JW, Carey MA, Bradbury JA, DeGraff LM, Morgan DL, Moorman MP, Flake GP, Zeldin DC: Gender differences in murine airway responsiveness and lipopolysaccharide-induced inflammation. *J Immunol* 2006;177:621–630.
- 26 Matsubara S, Swasey CH, Loader JE, Dakham A, Joetham A, Ohnishi H, Balhorn A, Miyahara N, Takeda K, Gelfand EW: Estrogen determines gender differences in airway responsiveness after allergen exposure. *Am J Respir Cell Moll Biol* 2008;38:501–508.
- 27 Careau E, Proulx LI, Pouliot P, Spahr A, Turmel V, Bissonnette EY: Antigen sensitization modulates alveolar macrophage functions in an asthma model. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L871–L879.
- 28 Letterio JJ, Roberts AB: Regulation of immune responses by TGF- β . *Annu Rev Immunol* 1998;16:137–161.
- 29 Fattouh R, Midence NG, Arias K, Johnson JR, Walker TD, Goncharova S, Souza KP, Gregory RC, Lonning S, Gauldie J, Jordana M: Transforming growth factor- β regulates house dust mite-induced allergic airway inflammation but not airway remodeling. *Am J Respir Crit Care Med* 2008;177:593–603.
- 30 Alcorn JF, Rinaldi LM, Jaffe EF, van Loon M, Bates JH, Janssen-Heininger YM, Irvin CG: Transforming growth factor- β_1 suppresses airway hyperresponsiveness in allergic airway disease. *Am J Respir Crit Care Med* 2007;176:974–982.
- 31 Barczyk A, Pierzchala W, Sozanska E: Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* 2003;97:726–733.
- 32 Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Page N, Olivenstein R, Elias J, Chakir J: IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001;108:430–438.
- 33 Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, Fossiez F, Ryffel B, Schnyder B: Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med* 2006;203:2715–2725.