

ORIGINAL ARTICLE

Gender difference in allergic airway remodelling and immunoglobulin production in mouse model of asthma

MASAHIDE TAKEDA,¹ MASAKO TANABE,¹ WATARU ITO,¹ SHIGEHARU UEKI,¹ YASUNORI KONNNO,¹ MAMI CHIHARA,¹ MASAMICHI ITOGA,¹ YOSHIKI KOBAYASHI,¹ YUKI MORITOKI,¹ HIROYUKI KAYABA^{1,2} AND JUNICHI CHIHARA¹

¹Department of Infection, Allergy, Clinical Immunology and Laboratory Medicine, Akita University Graduate School of Medicine, Akita and ²Department of Clinical Laboratory Medicine, Hirosaki University Graduate School of Medicine, Hirosaki, Japan

ABSTRACT

Background and objective: Epidemiological studies have shown that the prevalence of adult asthma and severe asthma is higher in women. It has also been reported that female mice are more susceptible than males to the development of allergic airway inflammation and airway hyperresponsiveness (AHR). The influence of gender difference in the pathogenesis of severe asthma, especially airway remodelling in an animal model, has been studied rarely. We investigated gender difference in the development of airway remodelling using a long-term antigen-challenged mouse asthma model.

Methods: Following ovalbumin (OVA)/alum intraperitoneal injection, male or female mice (BALB/c) were challenged with aerosolized 1% OVA on 3 days/week for 5 weeks, and differences in AHR, airway inflammation and airway remodelling between the sexes were investigated.

Results: In OVA-sensitized and OVA-challenged (OVA/OVA) female mice, eosinophils, lymphocytes, T-helper type 2 cytokines and growth factors in bronchoalveolar lavage fluid were increased compared with OVA/OVA male mice. Histological features of airway remodelling were also increased in OVA/OVA female mice. Serum total and OVA-specific immunoglobulin E (IgE) and serum IgA were significantly elevated in OVA/OVA female mice.

Conclusions: These results indicate that female mice experience more airway remodelling compared with male mice. These results suggest the involvement of sex hormones and gender differences in cellular functions in airway remodelling.

Key words: allergy, animal model, asthma, eosinophil, inflammation.

SUMMARY AT A GLANCE

Gender differences in airway remodelling were investigated using long-term antigen-challenged mouse model of asthma. Female mice experienced more airway remodelling. This suggests the involvement of sex hormones and gender differences in cellular functions of asthma induced airway remodelling.

Abbreviations: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; IL, interleukin; OVA, ovalbumin; OVA/OVA, OVA-sensitized and OVA-challenged; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β .

INTRODUCTION

Gender is one of the most fundamental subpopulations for human beings. Therefore, gender differences for disease rate, disease severity and mortality have been reported in a variety of diseases.^{1,2} Only a few studies investigate differences between males and females in basic and clinical research. Clinical data obtained on males are applied to clinical therapy for females.

Asthma is a chronic inflammatory disorder of the airway. Airway hyperresponsiveness (AHR) to non-specific stimuli and mucus production are caused by airway inflammation.³ Persistent airway inflammation is associated with airway remodelling characterized by irreversible airway changes, which is a factor of severe asthma.⁴ Gender differences in the prevalence rate of asthma is recognized. Generally, asthma is more prevalent among boys than girls in childhood, but this is reversed after puberty.^{5,6} Women have a higher disease prevalence and greater severity.^{7,8} The mechanism for gender differences in severe asthma is still unclear. Several studies have reported that female mice are more susceptible to development of allergic

Correspondence: Junichi Chihara, Department of Infection, Allergy, Clinical Immunology and Laboratory Medicine, Akita University Graduate School of Medicine, 1-1-1, Hondo, Akita 010-8543, Japan. Email: chihara@hos.akita-u.ac.jp

Received 11 July 2012; invited to revise 13 August and 12 December 2012; revised 5 November and 17 December 2012; accepted 17 December 2012 (Associate Editor: Darryl Knight).

airway inflammation and AHR than males.^{9,10} We also reported a female preponderance for allergic airway inflammation in animal model of asthma.¹¹ Only a few studies investigate in animal models gender differences in severe asthma, as typified by airway remodelling. In this study, we investigated gender differences in terms of development of airway remodelling using a long-term antigen-challenged mice model of asthma.

METHODS

Animals

Female and male BALB/c mice (8–10 weeks of age) were purchased from Charles River Japan, Inc. (Yokohama, Japan). The mice were maintained on a diet free of ovalbumin (OVA). All experimental animals used in this study were housed under constant temperature and light cycles, and under a protocol approved by the Institutional Animal Care and Use Committee of Akita University Graduate School of Medicine.

Sensitization and airway challenge

Mice (six mice/group/experiment) receiving the following treatments were studied: (i) non-sensitized and non-challenged mice, and (ii) OVA-sensitized and OVA airway-challenged mice (OVA/OVA). OVA/OVA mice were immunized by intraperitoneal injection of 20 µg of OVA (Grade V; Sigma, St. Louis, MO, USA) emulsified in 2.25 mg of alum (AlumImject; Pierce, Rockford, IL, USA) in a total volume of 100 µL on days 0 and 14. Mice were challenged via the airway with OVA (1% in saline) for 20 min on days 28, 29 and 30 by ultrasonic nebulization, and further exposed to aerosolized challenge 3 days per week for 4 additional weeks. AHR and immunological features were assessed 48 h after the last challenge.^{12,13}

Determination of airway responsiveness and measurement of bronchoalveolar lavage fluid cytokine

Resistance of the lung respiratory system (Rl) to methacholine was measured using an invasive system, as previously described.¹² After measurement of pulmonary resistance, the lungs were lavaged via the tracheal tube with saline. The volume of collected bronchoalveolar lavage fluid (BALF) was measured in each sample, and the number of BALF cells was counted. Cytospin slides were stained with May-Giemsa and differentiated in a blinded fashion by counting at least 300 cells under light microscopy. The concentrations of cytokines, growth factors and chemokine in the BALF supernatants, such as interleukin (IL)-4, IL-5, IL-10, IL-13, interferon-γ, platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β and eotaxin, were determined using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA) per the manufacturer's instructions.

Measurement of serum ovalbumin-specific antibodies

Serum total immunoglobulin E (IgE), OVA-specific IgE and IgA antibody levels were measured by enzyme-linked immunosorbent assay 48 h after the last airway challenge, as previously described.¹⁴ The antibody titres of samples were related to pooled standards that were generated in the laboratory and expressed as enzyme-linked immunosorbent assay units per milliliter (EU/mL).

Histological and immunohistochemistry studies

Mice lungs were inflated through a tracheal tube with 2 mL of air and fixed in 10% formaline, and lung tissues were embedded in paraffin blocks. Tissue sections 4-µm thick were affixed to microscope slides and deparaffinized. The slides were stained with haematoxylin–eosin for inflammatory cell infiltration, periodic acid Schiff for identification of mucus-containing cells (goblet cells), Masson trichrome stain for the peribronchiolar collagen layer, and immunostaining for α-smooth muscle actin (DAKO, Glostrup, Denmark), and they were examined under light microscopy and analysed as previously described.¹²

Statistical analysis

All results were expressed as mean ± standard deviation. Significant differences between experimental groups were determined using analysis of variance with post hoc Tukey–Kramer multiple comparison test. The *P*-value for significance was set at *P* < 0.05.

RESULTS

Gender differences in allergic airway hyperresponsiveness and inflammation

There were no significant differences in baseline pulmonary resistance values among all groups. As expected, in OVA/OVA female and male mice, pulmonary resistance was significantly increased in a dose-dependent manner compared with control mice. Compared with OVA/OVA male mice, pulmonary resistance to methacholine showed a non-significant trend to increase in OVA/OVA female mice (Fig. 1a). In OVA/OVA female and male mice, a marked increase of the total cells, eosinophils and lymphocytes were detected in BALF. Compared with OVA/OVA male mice, total cells, eosinophils and lymphocytes were significantly higher in OVA/OVA female mice (Fig. 1b).

Inflammatory cell infiltration into the airway was investigated by histological examination with haematoxylin–eosin staining. In OVA/OVA female mice inflammatory cells in the peribronchial and perivascular areas were significantly increased compared with OVA/OVA male mice (Fig. 2).

Gender difference in formation of airway remodelling

To investigate a gender difference in development of airway remodelling, periodic acid Schiff, Masson

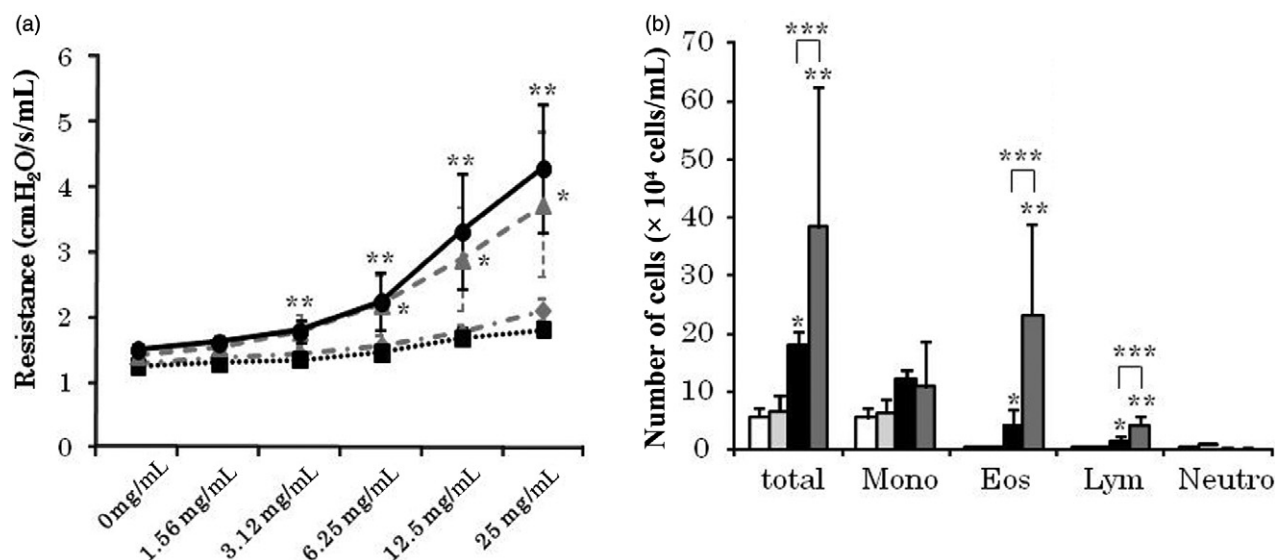


Figure 1 Development of airway hyperresponsiveness (AHR) and inflammatory cell infiltration were increased in ovalbumin (OVA)-sensitized and OVA-challenged (OVA/OVA) female mice. (a) Pulmonary resistance to increasing methacholine doses and (b) inflammatory cell accumulation in bronchoalveolar lavage fluid (BALF) were measured as described in Materials and Methods in non-sensitized and non-challenged (Non/Non) female or male, and OVA/OVA female or male mice. Results for each group are expressed as the mean $\pm$ standard deviation (SD) of six mice in each group. * $P < 0.05$ cf. Non/Non male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice. Mono, monocyte; Eos, eosinophil; Lym, lymphocyte; Neutro, neutrophil. (a) $\circ$ —, Non/Non male; $\bullet$ —, Non/Non female; $\triangle$ —, OVA/OVA male; $\bullet$ —, OVA/OVA female. (b) $\square$, Non/Non male; $\blacksquare$, Non/Non female; $\blacksquare$, OVA/OVA male; $\blacksquare$, OVA/OVA female.

trichrome and immune-staining for α -smooth muscle actin were performed. A significant increase of periodic acid Schiff-positive cells was found in OVA/OVA female mice compared with male mice (Fig. 3). This result indicates that female mice formed more goblet cell hyperplasia than male. A significant increase in the peribronchiolar collagen layer on Masson trichrome stain and the smooth muscle layer on α -smooth muscle actin stain in OVA/OVA female mice compared with OVA/OVA male mice were also noted (Fig. 4).

Gender difference in cytokine, growth factor and chemokine levels in bronchoalveolar lavage fluid

Concentrations of T-helper type 1/T-helper type 2 cytokines, growth factors and chemokines in BALF were measured by enzyme-linked immunosorbent assay (Fig. 5a–h). IL-13, IL-10, TGF- β and PDGF in BALF were significantly increased in OVA/OVA female mice compared with OVA/OVA male mice (Fig. 5c,d,g,h). No significant differences in IL-4, IL-5 and eotaxin in BALF (Fig. 5a,b,f) were observed.

Gender differences in serum total and ovalbumin-specific immunoglobulin E, immunoglobulin G1 and immunoglobulin A

Total and OVA-specific IgE were significantly elevated in OVA/OVA mice compared with control mice. Total and OVA-specific IgE were significantly increased in OVA/OVA female compared with OVA/OVA male mice

(Fig. 6a,b). Serum IgA was significantly increased in OVA/OVA female (Fig 6c) compared with OVA/OVA male mice.

DISCUSSION

In this study, we showed in a mice model of asthma that females were more susceptible to the development of airway remodelling than the males. We also showed that inflammatory cell infiltration into the airway, as well as T-helper type 2 cytokine and growth factor production were increased in the female mice compared with the males. No significant difference AHR to methacholine was noted between OVA/OVA male and female mice.

So far, different murine strains have been used to clarify the gender difference of allergic diseases.^{9,10,15,16} Zhang Y *et al.* reported that BALB/c mice showed stronger OVA-induced allergic responses compared with C57BL/6 mice.¹⁷ Moreover, there were some studies that investigated the gender difference of asthma using BALB/c mice.^{9,10,18} Therefore, we selected BALB/c mice to investigate gender-related differences of airway remodelling in this study.

Although airway remodelling is difficult to define, subepithelial fibrosis, goblet cell hyperplasia and myofibroblast hypertrophy have been identified as components of airway remodelling.⁴ Numerous researches have reported that airway remodelling is orchestrated by cytokines such as IL-13 and growth factors such as TGF- β and PDGF. IL-13, which is mainly produced by lymphocytes, has been shown to

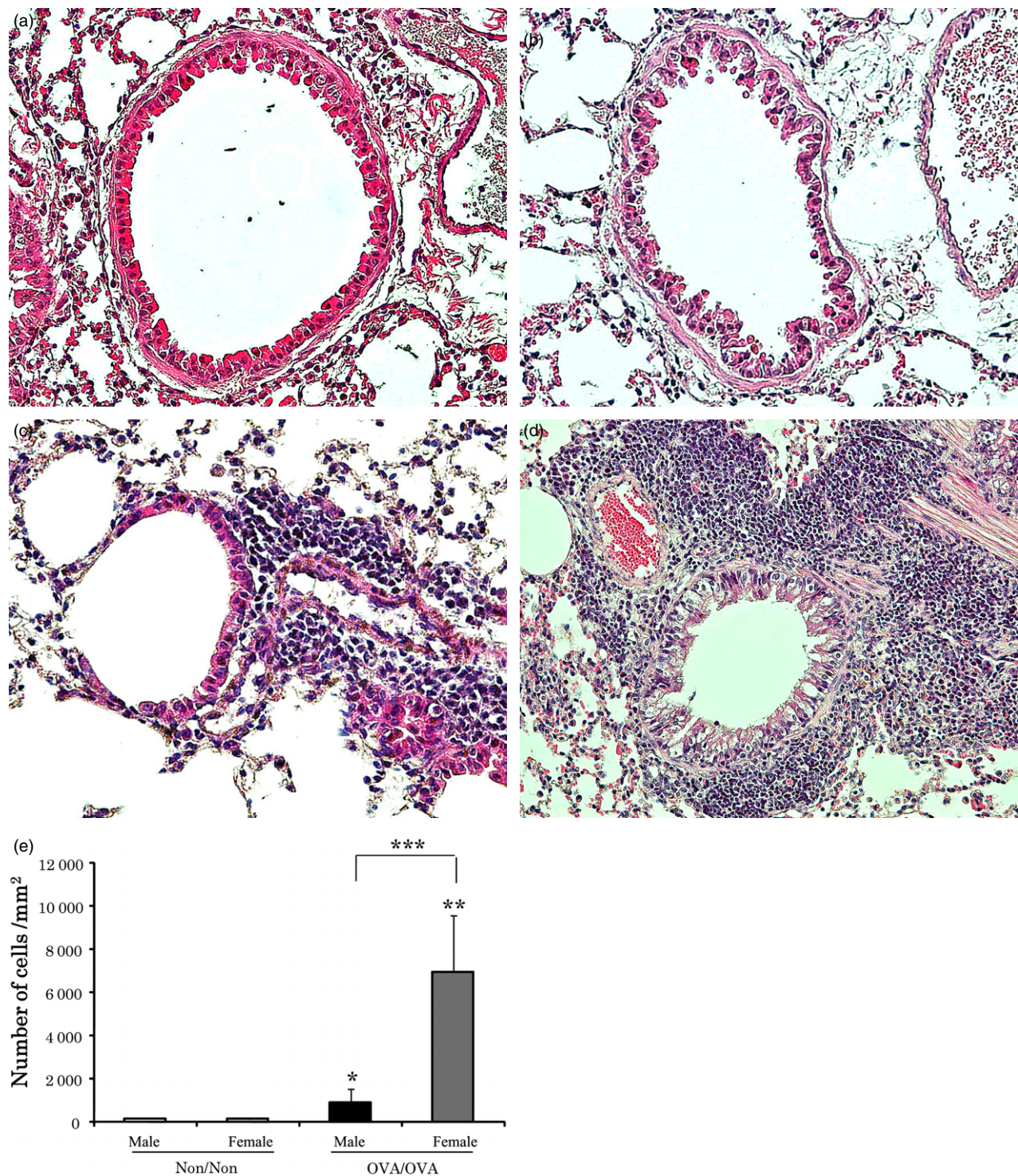


Figure 2 Inflammatory cell accumulation was significantly increased in ovalbumin (OVA)-sensitized and OVA-challenged (OVA/OVA) female compared with male mice. Evidence of inflammatory cell infiltration was investigated by histological examination of hematoxylin–eosin-stained tissue as described in Materials and Methods (final magnification: $\times 400$). (a) Non-sensitized and non-challenged (Non/Non) male mice, (b) Non/Non female mice, (c) OVA/OVA male mice and (d) OVA/OVA female mice. (e) Total inflammatory cell numbers in the peribronchial tissue. Data represent the mean $\pm$ standard deviation (SD). * $P < 0.05$ cf. Non/Non male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice.

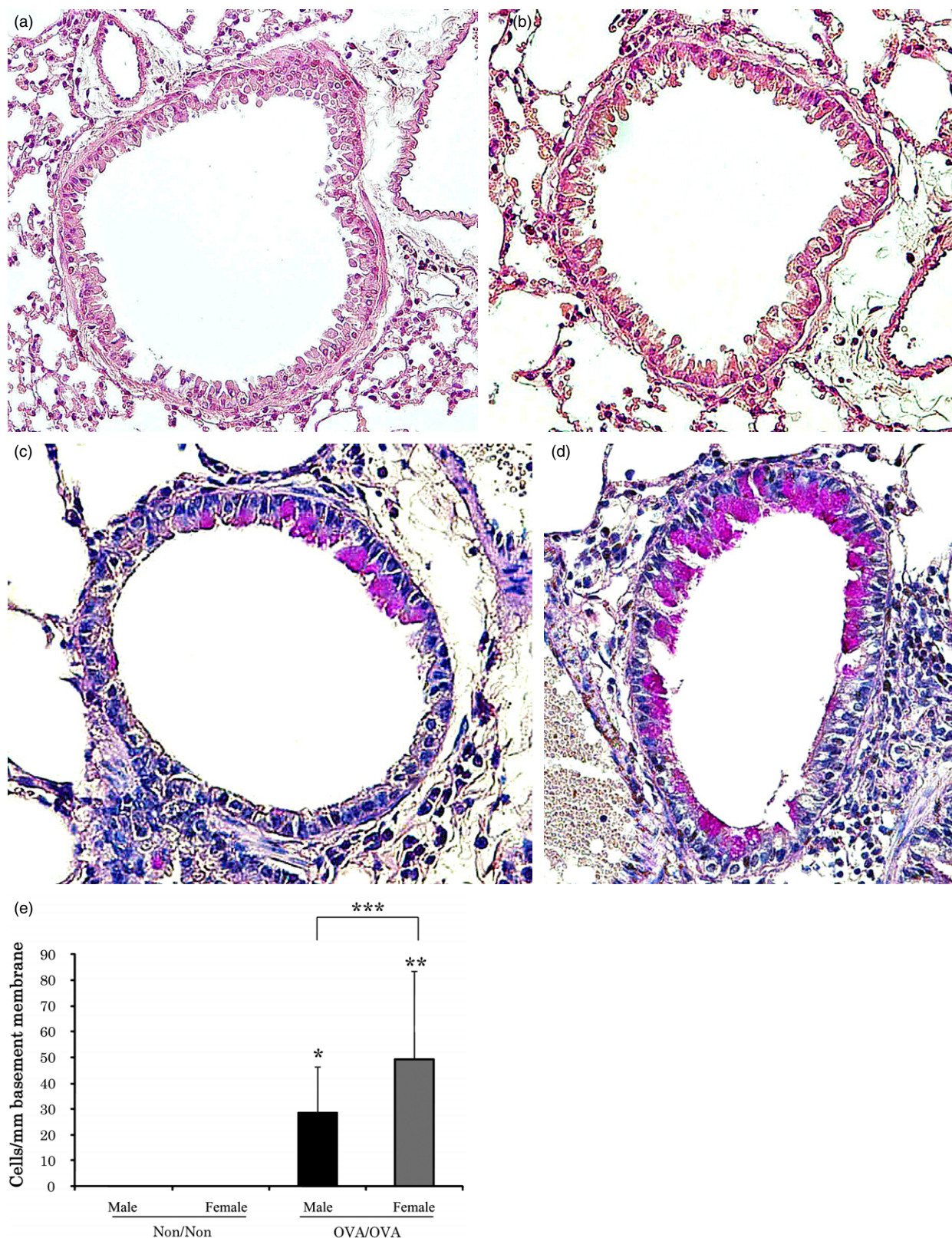


Figure 3 Goblet cell hyperplasia in airway epithelium was significantly increased in ovalbumin (OVA)-sensitized and OVA-challenged (OVA/OVA) female mice. Periodic acid Schiff (PAS) staining was performed to identify mucus-containing cells in the airway epithelium as described in Materials and Methods (final magnification: $\times 1000$). (a) Non-sensitized and non-challenged (Non/Non) male mice, (b) Non/Non female mice, (c) OVA/OVA male mice and (d) OVA/OVA female mice. (e) The number of mucus-positive cells per millimetre basement membrane. Data represent the mean $\pm$ standard deviation (SD). * $P < 0.05$ cf. Non/Non male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice.

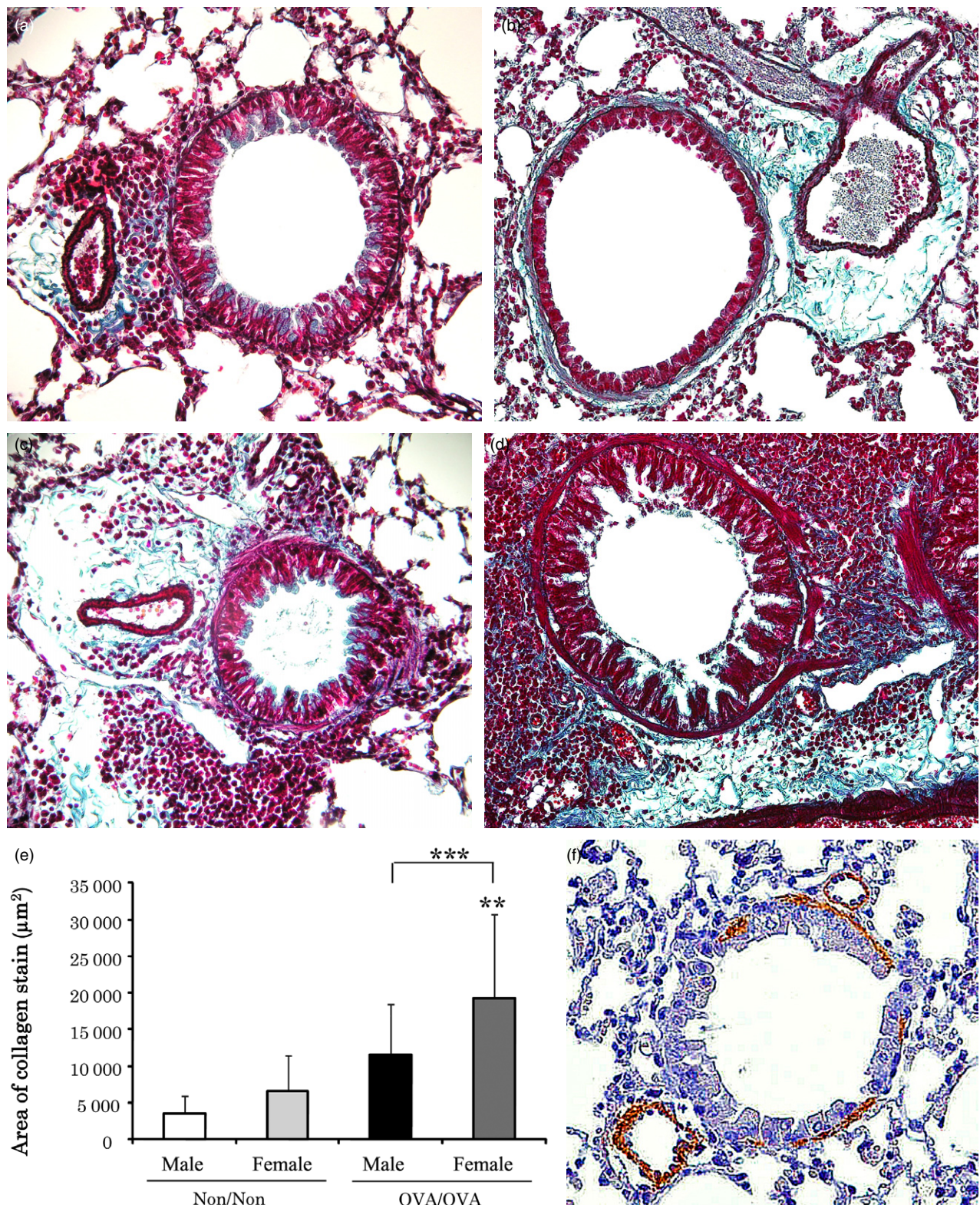


Figure 4 Airway remodelling in mice receiving repeated (4 weeks) airway challenges was significantly increased in ovalbumin (OVA)-sensitized and OVA-challenged (OVA/OVA) female mice. (a–d, e) The area of Masson's trichrome-positive peribronchiolar collagen layer and (f–i, j) α -smooth muscle actin (α -SMA)-stained smooth muscle layer was measured as described in Materials and Methods (final magnification: $\times 400$). (a, f) Non-sensitized and non-challenged (Non/Non) male mice, (b, g) Non/Non female mice, (c, h) OVA/OVA male mice and (d, i) OVA/OVA female mice. Data represent the mean $\pm$ standard deviation (SD). * $P < 0.05$ cf. Non/Non male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice.

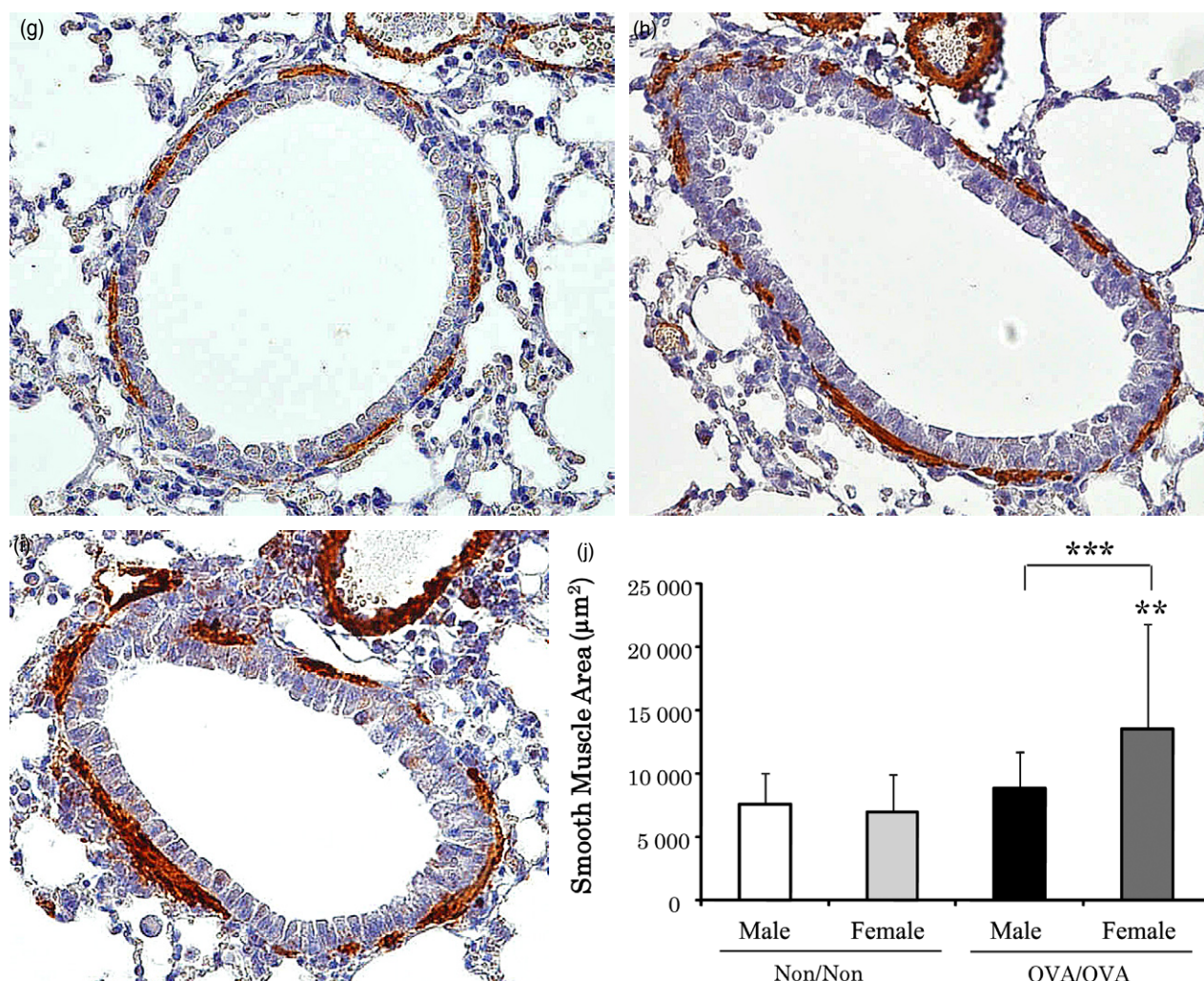


Figure 4 Continued

play a vital role in airway remodelling by regulating goblet cell hyperplasia.¹⁹ TGF- β activates downstream signalling and causes myofibroblast differentiation, leading to tissue fibrosis.²⁰ TGF- β is also involved in migration and proliferation of airway smooth muscle cells, which are thought to contribute to airway remodelling in asthma.²¹ Similarly, PDGF is known to be an important factor in airway remodelling by redistribution of smooth muscle cells during inflammation.²²

In this study, the level of IL-13, TGF- β and PDGF in BALF were significantly increased in OVA/OVA female mice compared with males. These differences correlated with an increased goblet cells hyperplasia, tissue fibrosis and thickness of airway smooth muscle in OVA/OVA female compared with male mice. Taken together, these results appear to correlate with those of previous reports^{19–22} with regards to the mechanisms of airway remodelling; thus, the differences between female and male mice observed in levels of cytokines and growth factors could account for the gender differences in airway remodelling observed here.

Blacquiere M.J. *et al.* investigated gender differences in airway remodelling in two mouse models of asthma using OVA and house dust mite.²³ They reported no gender difference in the development of airway remodelling in these two mouse models. In contrast with our study, Blacquiere M.J. *et al.* did not report an accumulation of lymphocytes, the main source of IL-13, in BALF, and they reported a male predominance for the production of TGF- β in BALF. These divergences with our results could be imputed to different protocols that could have caused a difference in production of T-helper type 2 cytokines and growth factors mainly released from lymphocytes and eosinophils, thus leading to a different response. Data reported by Antunes M.A. *et al.* support this hypothesis.¹⁸

Both TGF- β and PDGF are mainly released from eosinophils and lymphocytes.^{24,25} Hence, eosinophils and lymphocytes are also considered to contribute to airway remodelling. In particular, eosinophil infiltration into lung tissue has been reported to be an important mechanism in airway remodelling.^{26,27} In this study, the number of eosinophils and

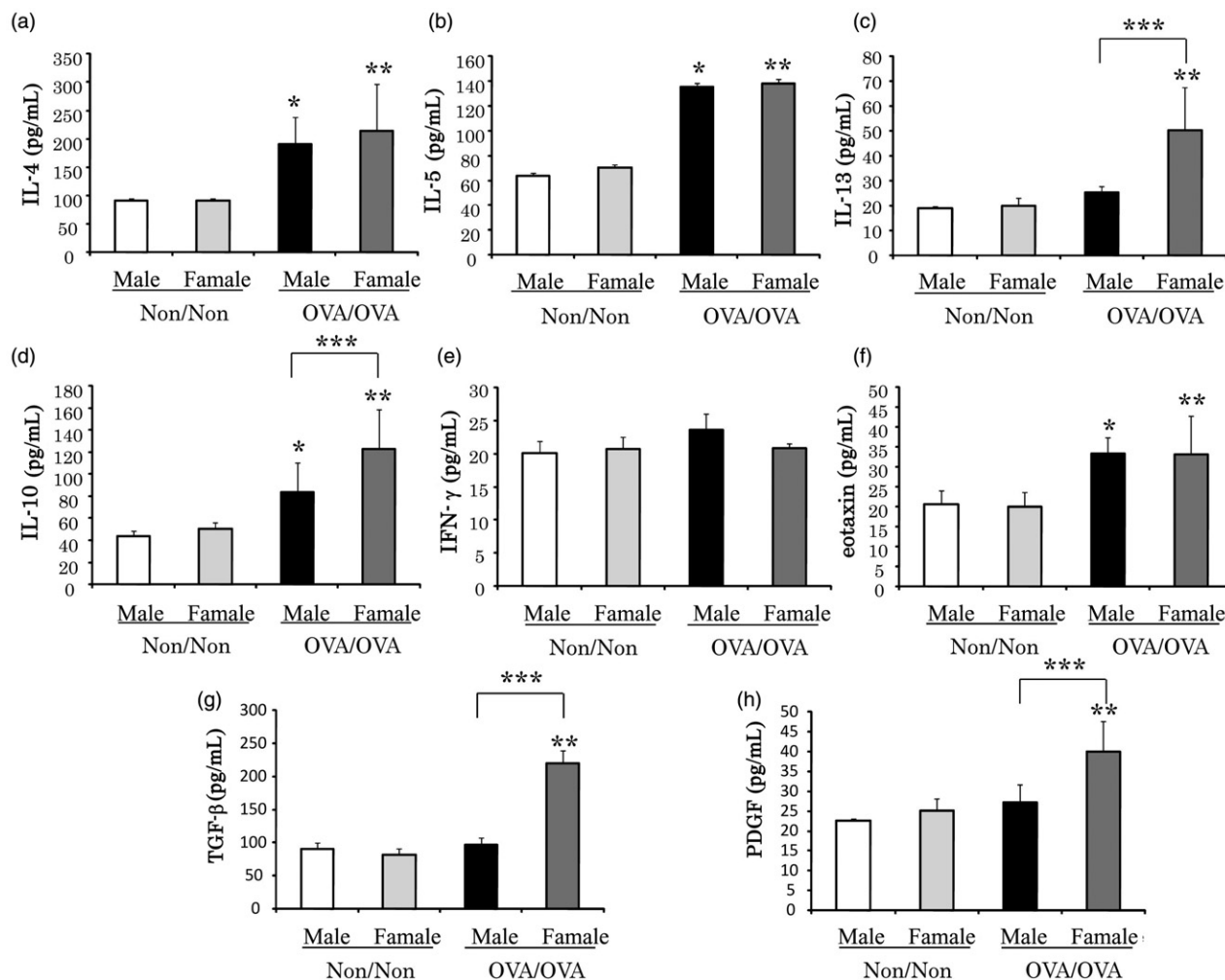


Figure 5 T-helper type 2 (Th2) cytokines and growth factors were increased in ovalbumin (OVA)-sensitized and OVA-challenged (OVA/OVA) female mice compared with male mice. The levels of (a) interleukin (IL)-4, (b) IL-5, (c) IL-13, (d) IL-10, (e) interferon (IFN)- γ , (f) eotaxin, (g) transforming growth factor (TGF)- β and (h) platelet-derived growth factor (PDGF) in bronchoalveolar lavage fluid (BALF) of mice were measured by enzyme-linked immunosorbent assay (ELISA). The results are expressed as the mean $\pm$ standard deviation (SD) of six mice in each group. * $P < 0.05$ cf. non-sensitized and non-challenged (Non/Non) male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice.

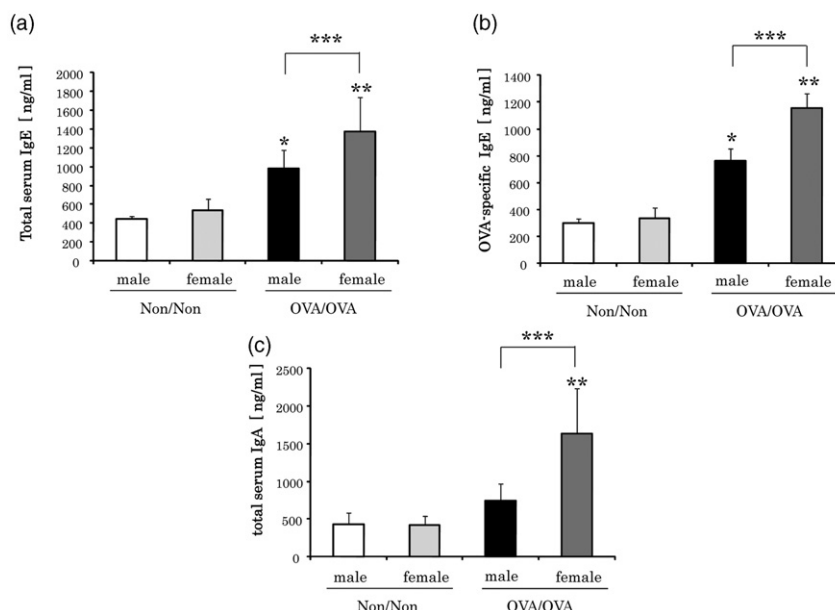
lymphocytes in BALF was significantly increased in OVA/OVA female mice compared with OVA/OVA male mice. These findings may indicate that the significant difference between female and male mice in eosinophil infiltration into lung tissue could be involved in gender differences observed for airway remodelling. Thus, cellular function of eosinophils, such as migration, holds the potential to cause gender differences. Eotaxin is known to be involved in the chemotactic response of eosinophil into lung tissue through CCR3 stimulation.²⁸ However, in this study, the level of eotaxin although significantly increased in OVA/OVA male and female compared with controls was not significantly different between OVA/OVA male and female mice.

The induction phase, which is characterized by IgE production, is an important reaction in allergic diseases, and the establishment of the induction phase leads to stronger responses in the effector phase after

antigen re-exposure.²⁹ In this study, female mice produced more total and OVA-specific IgE than male mice. These results suggest that the detected elevation in inflammatory cell infiltration may be due to the gender difference in the reaction in the induction phase.

The mechanisms underlying the gender difference in the pathogenesis of asthma and allergic response are complex, but they are being increasingly investigated. Hayashi T. *et al.* showed that allergic airway inflammation worsen in castrated OVA/OVA male mice.¹⁰ Roffo-Vasquez Y. *et al.* reported that allergic responses including inflammatory cell infiltration were improved in ovariectomized female mice.³⁰ In addition, Cai Y. *et al.* reported that oestrogen was involved in goblet cell hyperplasia and IL-13 release from mediastinal lymph node cells of allergic mice.³¹ These results indicate that sex hormones play an important role in the formation of airway remodelling.

Figure 6 The levels of serum total immunoglobulin E (IgE), ovalbumin (OVA)-specific IgE and total IgA were significantly increased in OVA-sensitized and OVA-challenged (OVA/OVA) female mice. The levels of serum total IgE, OVA-specific IgE and total IgA were measured, as described in Materials and Methods. (a) The level of serum total IgE in each group. (b) The level of serum OVA-specific IgE in each group. (c) The level of serum total IgA in each group. Results for each group are expressed as the mean $\pm$ standard deviation (SD) of six mice in each group. * $P < 0.05$ cf. non-sensitized and non-challenged (Non/Non) male mice; ** $P < 0.05$ cf. Non/Non female mice. *** $P < 0.05$ between OVA/OVA male and OVA/OVA female mice.



On the other hand, it has been reported that the rate of improvement, exacerbation and stability of asthma status in advanced pregnancy is almost equal.³² Thus, the mechanism underlying the gender difference cannot be explained by sex hormones alone.

We have previously reported that the gender difference in cellular function may be involved in gender difference of airway inflammation using adoptive splenocyte transfer method.¹¹ We have also previously reported that T-helper type 2 cytokine production by splenocytes from OVA-sensitized female mice was significantly increased compared with that from males.¹¹ In the current study, we demonstrated in a mouse model a female predominance for producing IL-13, IL-10, TGF- β and PDGF, and so facilitation a chemotactic response of human eosinophils. Further studies are necessary to elucidate the detailed mechanism underlying the gender difference in allergic responses. Gender difference in cellular functions such as chemotactic activity and cytokine release might have had an impact.

Finally, we did not demonstrate a difference in AHR in our mouse model. Antunes M.A. *et al.*¹⁸ reported that AHR was enhanced in OVA/OVA male mice compared with females despite a female increased airway remodelling. The mechanism of AHR is complex. Several studies found that oestrogen reduces AHR in female mice.^{33,34} Other studies demonstrated that airway remodelling was involved in the enhancement of AHR.^{35–37} We postulate that we did not demonstrate a higher AHR in female mice in our study because of the cross-interaction between the moderating effect of oestrogen and airway remodelling. Further investigations are necessary to explain this different result between Antunes M.A. *et al.*'s and our study.

In summary, we showed that allergic airway inflammation and airway remodelling were significantly increased in OVA/OVA female mice compared with

males. These results are congruent with human epidemiology for asthma, and the gender difference in the formation of airway remodelling may be a factor for female predominance among severe asthma patients. The mechanism for gender difference of airway remodelling is complex, and further studies are needed to elucidate it and to allow the development of gender-specific medicine.

REFERENCES

- 1 Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am. J. Pathol.* 1985; **121**: 531–51.
- 2 Fairweather D, Frischno-Kiss S, Rose NR. Sex differences in autoimmune disease from a pathological perspective. *Am. J. Pathol.* 2008; **173**: 600–9.
- 3 Holgate ST. Airway inflammation and remodeling in asthma: current concepts. *Mol. Biotechnol.* 2002; **22**: 179–89.
- 4 Davies DE, Wicks J, Powell RM *et al.* Airway remodeling in asthma: new insights. *J. Allergy Clin. Immunol.* 2003; **111**: 215–25.
- 5 Carey MA, Card JW, Voltz JW *et al.* It's all about sex: gender, lung development and lung disease. *Trends Endocrinol. Metab.* 2007; **18**: 308–13.
- 6 de Marco R, Locatelli F, Sunyer J *et al.* Differences in incidence of reported asthma related to age in men and women. A retrospective analysis of the data of the European Respiratory Health Survey. *Am. J. Respir. Crit. Care Med.* 2000; **162**: 68–74.
- 7 The ENFUMOSA Study Group. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. *Eur. Respir. J.* 2003; **22**: 470–7.
- 8 Skobeloff EM, Spivey WH, St Clair SS *et al.* The influence of age and sex on asthma admissions. *JAMA* 1992; **268**: 3437–40.
- 9 Melgert BN, Postma DS, Kuipers I *et al.* Female mice are more susceptible to the development of allergic airway inflammation than male mice. *Clin. Exp. Allergy* 2005; **35**: 1496–503.

- 10 Hayashi T, Adachi Y, Hasegawa K *et al.* Less sensitivity for late airway inflammation in males than females in BALB/c mice. *Scand. J. Immunol.* 2003; **57**: 562–7.
- 11 Okuyama K, Wada K, Chihara J *et al.* Sex-related splenocyte function in a murine model of allergic asthma. *Clin. Exp. Allergy* 2008; **38**: 1212–9.
- 12 Takeda M, Ito W, Tanabe M *et al.* Phosphatidyl inositide 3-kinase deficient mice fail to develop allergic airway hyperresponsiveness, inflammation, and remodeling. *J. Allergy Clin. Immunol.* 2009; **123**: 805–12.
- 13 Ito W, Kanehiro A, Matsumoto K *et al.* Hepatocyte growth factor attenuates airway hyperresponsiveness, inflammation, and remodeling. *Am. J. Respir. Cell Mol. Biol.* 2005; **32**: 268–80.
- 14 Lai WQ, Goh HH, Bao Z *et al.* The role of sphingosine kinase in a murine model of allergic asthma. *J. Immunol.* 2008; **180**: 4323–9.
- 15 Yamamoto T, Okano M, Ono T *et al.* Sex-related differences in the initiation of allergic rhinitis in mice. *Allergy* 2001; **56**: 525–31.
- 16 Matheu V, Barrios Y, Arnau MR *et al.* Similar response in male and female B10.RIII mice in a murine model of allergic airway inflammation. *Inflamm. Res.* 2010; **59**: 263–9.
- 17 Zhang Y, Lamm WJ, Albert RK *et al.* Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *Am. J. Respir. Crit. Care Med.* 1997; **155**: 661–9.
- 18 Antunes MA, Abreu SC, Silva AL *et al.* Sex-specific lung remodeling and inflammation changes in experimental allergic asthma. *J. Appl. Physiol.* 2010; **109**: 855–63.
- 19 Zhu Z, Homer RJ, Wang Z *et al.* Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. *J. Clin. Invest.* 1999; **103**: 779–88.
- 20 Horowitz JC, Rogers DS, Sharma V *et al.* Combinatorial activation of FAK and AKT by transforming growth factor- β 1 confers an anoikis-resistant phenotype to myofibroblasts. *Cell. Signal.* 2007; **19**: 761–71.
- 21 Shaoping X, Maria BS, Razao I *et al.* Mechanisms of induction of airway smooth muscle hyperplasia transforming growth factor- β . *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2007; **293**: 245–53.
- 22 Stephen MC, Michael R, Judith LB. Urokinase potentiates PDGF-induced chemotaxis of human airway muscle cell. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2003; **284**: 1020–6.
- 23 Blacqui re MJ, Hylkema MN, Postma DS *et al.* Airway inflammation and remodeling in two mouse models of asthma: comparison of males and females. *Int. Arch. Allergy Immunol.* 2010; **153**: 173–81.
- 24 Elovic A, Wong DT, Weller PF *et al.* Expression of transforming growth factors-alpha and beta1 messenger RNA and product by eosinophils in nasal polys. *J. Allergy Clin. Immunol.* 1994; **93**: 864–9.
- 25 Minshall EM, Leung DY, Martin RJ *et al.* Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am. J. Respir. Cell Mol. Biol.* 1997; **17**: 326–33.
- 26 Lee JJ, Dimina D, Macias MP *et al.* Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004; **305**: 1773–6.
- 27 Humbles AA, Lloyd CM, McMillan SJ *et al.* A critical role for eosinophils in allergic airways remodeling. *Science* 2004; **305**: 1776–9.
- 28 Kampen GT, Stafford S, Adachi T *et al.* Eotaxin induces degranulation and chemotaxis of eosinophils through the activation of ERK2 and p38 mitogen-activated protein kinases. *Blood* 2000; **95**: 1911–7.
- 29 Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 1–5.
- 30 Rifo-Vasquez Y, Ligeiro de Oliveira AP, Page CP *et al.* Role of sex hormones in allergic inflammation in mice. *Clin. Exp. Allergy* 2007; **37**: 459–70.
- 31 Cai Y, Zhou J, Webb DC. Estrogen stimulates Th2 cytokine production and regulates the compartmentalisation of eosinophils during allergen challenge in a mouse model of asthma. *Int. Arch. Allergy Immunol.* 2012; **158**: 252–60.
- 32 Schatz M, Harden K, Forsythe A *et al.* The course of asthma during pregnancy, post partum, and with successive pregnancies: a prospective analysis. *J. Allergy Clin. Immunol.* 1988; **81**: 509–17.
- 33 Matsubara S, Swasey CH, Loader JE *et al.* Estrogen determines sex differences in airway responsiveness after allergen exposure. *Am. J. Respir. Cell Mol. Biol.* 2008; **38**: 501–8.
- 34 Degano B, Pr vost MC, Berger P *et al.* Estradiol decreases the acetylcholine-elicited airway reactivity in ovariectomized rats through an increase in epithelial acetylcholinesterase activity. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 1849–54.
- 35 Boulet LP, Chakir J, Dub  J *et al.* Airway inflammation and structural changes in airway hyper-responsiveness and asthma: an overview. *Can. Respir. J.* 1998; **5**: 16–21.
- 36 Laprise C, Laviolette M, Boutet M *et al.* Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. *Eur. Respir. J.* 1999; **14**: 63–73.
- 37 Leigh R, Ellis R, Wattie JN *et al.* Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. *Am. J. Respir. Crit. Care Med.* 2004; **169**: 860–7.