

# Contribution of CD4<sup>+</sup> T Cells and Dendritic Cells to Female-Dominant Antigen-Induced T Helper Type 2 Cytokine Production by Bronchial Lymph Node Cells

Kaori Okuyama<sup>a</sup> Masatoshi Suenaga<sup>a</sup> Shyunya Furuki<sup>a</sup> Tasuku Kawano<sup>a</sup>  
Yuichi Ohkawara<sup>a</sup> Motoaki Takayanagi<sup>a</sup> Toshiaki Kikuchi<sup>b</sup> Isao Ohno<sup>a</sup>

<sup>a</sup>Department of Pathophysiology, Tohoku Pharmaceutical University, and <sup>b</sup>Department of Pulmonary Medicine, Tohoku University Hospital, Sendai, Japan

## Key Words

Bronchial asthma · Sex difference · T helper type 2 cytokines · Dendritic cells · CD4<sup>+</sup> T cells

## Abstract

**Background:** After puberty, asthma severity is higher in women than in men. The underlying mechanisms of this gender difference are not fully understood. In murine models of allergic asthma, more severe airway inflammation in female mice is associated with higher levels of T helper type 2 (Th2) cytokines. The aim of this study was to investigate the contributions of CD4<sup>+</sup> T cells and dendritic cells (DCs) to the differences in Th2 cytokine production between sexes.

**Methods:** Bronchial lymph node (BLN) cells from ovalbumin (OVA)-sensitized male and female C57BL/6 mice were stimulated with OVA and anti-CD3/CD28 antibodies. The CD4<sup>+</sup> T cells and DCs purified from BLN cells were cocultured with OVA in a sex-matched or mismatched fashion. The CD4<sup>+</sup> T cells were also stimulated with anti-CD3/CD28 antibodies. The concentrations of interleukin (IL)-5, IL-4, IL-13 and interferon (IFN)- $\gamma$  in the culture supernatants were measured. **Results:** The concentrations of IL-5, IL-4 and IL-13, but not IFN- $\gamma$ , were significantly higher in female BLN cells stimulated with

OVA than in male BLN cells. Sex differences were also observed in the CD4<sup>+</sup> T cells stimulated with anti-CD3/CD28 antibodies, whereas only IL-4 was significantly different in the BLN cells stimulated with antibodies. IL-5 production by OVA-stimulated male and female CD4<sup>+</sup> T cells, but not IL-4 or IL-13 production, was significantly increased in the coculture with female DCs when compared to the male DCs. **Conclusions:** The differences in Th2 cytokine production between sexes by the BLN cells may be attributable, at least in part, to the differing functions of CD4<sup>+</sup> T cells and DCs between sexes.

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## Introduction

Allergic asthma is a chronic inflammatory disease that results from dysregulated CD4<sup>+</sup> T helper 2 (Th2)-biased immune responses directed against innocuous antigens. The hallmarks of the disease include inflammatory cell infiltration (e.g. eosinophils and mast cells) into the airways, mucus production and variable airflow obstruction with airway hyperresponsiveness. The Th2 cytokines including IL-4, IL-5 and IL-13 play an important role in these processes [1, 2].

The prevalence and severity of asthma are higher in women than in men. This disparity occurs at the time of puberty [3–5], which strongly suggests that sex hormones play a role in the pathogenesis of the disease [6, 7]. Sex differences in cytokine production have been reported in humans. Girón-González et al. [8] reported that the production of Th2 cytokines by phytohemagglutinin-stimulated peripheral blood mononuclear cells was significantly higher in healthy women than in men. Furthermore, in atopic asthmatics, an increased accumulation of IL-13<sup>+</sup> T cells was observed in peripheral blood leukocytes of women when compared with men after TCR stimulation [9]. In contrast, Zhang et al. [10] reported that CD4<sup>+</sup> T cells from healthy women produced higher levels of IFN- $\gamma$  after TCR activation compared with men. However, the underlying mechanisms of gender differences in the prevalence and severity of asthma are not fully understood.

Using murine models of allergic asthma, we and other investigators have demonstrated that adult female mice have a higher susceptibility to allergic airway inflammation; this is associated with higher amounts of Th2 cytokines [11–13]. In addition, we have demonstrated sex differences in antigen-specific immune responses under sex hormone-free conditions [11, 14]. We observed greater antigen-induced Th2 cytokine production, greater Th2 cell differentiation and higher expression of the Th2 master transcription factor GATA-3 in bronchial lymph node (BLN) cells from sensitized female mice compared with sensitized male mice [14]. However, it has still to be determined which BLN cells are responsible for the female-dominant antigen-induced Th2 response.

As antigen-presenting cells that activate T cells, dendritic cells (DCs) are required for antigen-specific immune responses. DCs in the lung can engulf inhaled antigens and migrate to the BLNs to present antigen to T cells. DCs are necessary for the induction of allergic airway inflammation in murine models of asthma and are increased in the lungs of asthmatic patients [15, 16]. DC subsets in the murine lung can be separated into two categories: conventional DCs (cDCs), which express high levels of CD11c, and plasmacytoid DCs (pDCs), which express Siglec-H, Ly6C and B220 with low levels of CD11c. The cDC subtype is predominantly responsible for antigen presentation [15, 16].

We hypothesized that CD4<sup>+</sup> T cells, which primarily produce Th2 cytokines, and/or DCs, which are critical for adaptive immune responses, contribute to the sex differences in antigen-specific Th2 immune responses. To test this hypothesis, we compared the Th2 cytokine levels of T-cell receptor/CD28 (TCR/CD28)-ligated BLN cells and

purified BLN CD4<sup>+</sup> T cells between male and female mice. Furthermore, to determine which types of cells are responsible for the sex differences in antigen-induced Th2 cytokine production, we compared the Th2 cytokine levels of sex-matched or mismatched cocultures of BLN CD4<sup>+</sup> T cells and DCs.

## Materials and Methods

### Animals

Specific pathogen-free male and female C57BL/6 (B6) mice were purchased from CLEA Japan (Osaka, Japan). The animals were housed under a 12-hour light/dark cycle at a constant temperature (22  $\pm$  2°C). Sterilized food and water were available ad libitum. All the experiments described below were approved and conformed to the guidelines of the Committee of Animal Experiments at Tohoku Pharmaceutical University.

### Isolation of BLNs

B6 mice that were 6 weeks old were sensitized and were made to inhale antigen, as previously described [11]. Briefly, the mice were sensitized by intraperitoneal injections of chicken ovalbumin (OVA; grade V, Sigma Chemical Co., St. Louis, Mo., USA; 8  $\mu$ g/mouse) adsorbed with aluminum hydroxide (Wako Pure Chemical Industries, Osaka, Japan) on days 0 and 5. On day 17, the mice were challenged twice with OVA (5 mg/ml in saline) for 1 h with a 4-hour interval. Twenty-four hours after the last challenge, the BLNs were harvested from 4–6 mice and were pooled for each experiment.

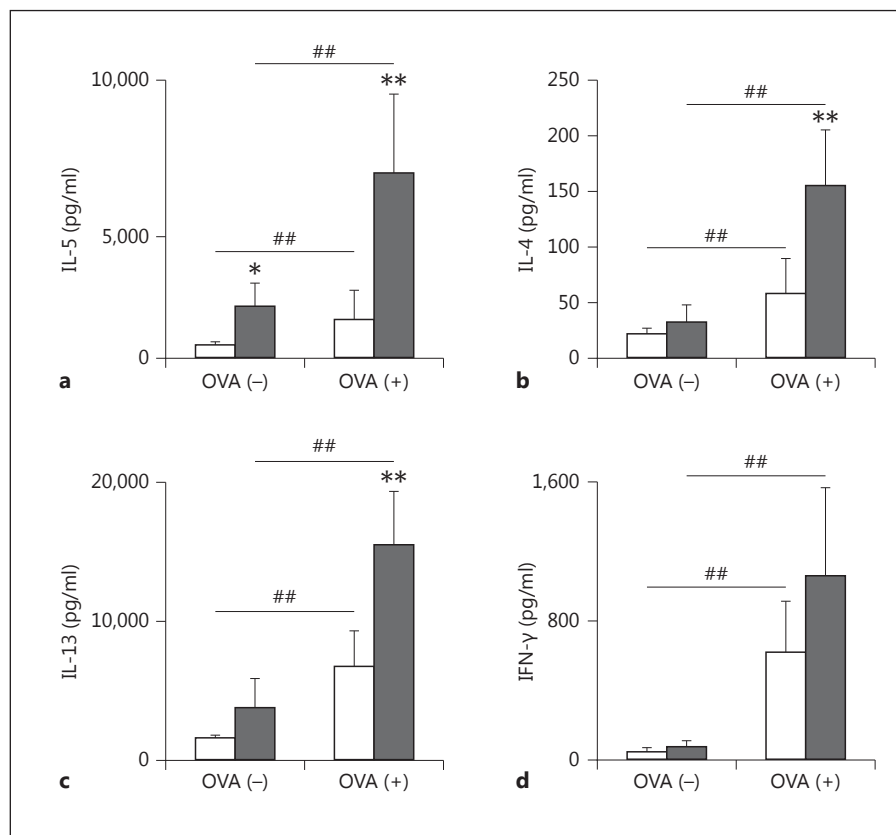
### Culture of BLN Cells

BLN cells were prepared from BLNs as previously described [14] and suspended in complete medium consisting of RPMI 1640 (Gibco BRL, Rockville, Md., USA), 10% fetal bovine serum (ICN Biomedicals, Inc., Aurora, Ohio, USA), 55  $\mu$ M 2-mercaptoethanol (Wako), 2 mg/ml NaHCO<sub>3</sub>, 50 U/ml penicillin G (MeijiSeika, Tokyo, Japan), 50  $\mu$ g/ml streptomycin sulfate (MeijiSeika) and 2 mM L-glutamine (Wako). The BLN cells (1  $\times$  10<sup>6</sup> cells/200  $\mu$ l/well) were cultured in 96-well culture plates in the presence or absence of OVA (200  $\mu$ g/ml) for 3 days. The BLN cells were also stimulated with hamster anti-mouse CD3 IgG<sub>1</sub> (5  $\mu$ g/ml; BD Biosciences Pharmingen, San Diego, Calif., USA) and hamster anti-mouse CD28 IgG<sub>2</sub> (0.1  $\mu$ g/ml; BD Biosciences, Pharmingen) antibodies for 3 days to activate the TCR and CD28, as previously described [17]. The culture supernatants were stored at –80°C for cytokine measurement using an enzyme-linked immunosorbent assay (ELISA). Because there were no significant differences in IL-4 production by female and male BLN cells between 1, 2.5, 5, 10, 20 and 50  $\mu$ g/ml of hamster anti-mouse CD3 IgG<sub>1</sub> in preliminary experiments, 5  $\mu$ g/ml of anti-CD3 was used in this study. Relevant isotype antibodies at the same concentrations were used as controls.

### Purification and Culture of CD4<sup>+</sup> T Cells and DCs from BLN Cells

CD4<sup>+</sup> T cells and DCs in BLN cells were purified by positive selection on an autoMACS Separator (Miltenyi Biotec, Bergisch Gladbach, Germany) using anti-mouse CD4 (L3T4) MicroBeads

**Fig. 1.** Cytokine production by BLN cells stimulated with antigen. BLN cells from male (open columns) and female (closed columns) mice were stimulated with or without OVA for 3 days. The concentrations of IL-5 (a), IL-4 (b), IL-13 (c) and IFN- $\gamma$  (d) in the culture supernatants were measured. Data are expressed as means  $\pm$  SD. ##  $p < 0.01$  compared with the culture without OVA; \*  $p < 0.05$  and \*\*  $p < 0.01$  compared with male mice ( $n = 4$ – $6$  for each group).



(Miltenyi Biotec) and anti-mouse CD11c MicroBeads (Miltenyi Biotec), respectively [18]. The purified CD4<sup>+</sup> T cells and DCs were suspended in complete medium. The purity and viability of both cell populations exceeded 95% after purification as assessed by flow cytometry and trypan blue exclusion, respectively. The CD4<sup>+</sup> T cells ( $0.5 \times 10^6$  cells/well) were stimulated with anti-CD3/CD28 antibodies or control antibodies as mentioned above. The CD4<sup>+</sup> T cells ( $2 \times 10^5$  cells/well) were cocultured with DCs ( $1 \times 10^5$  cells/well) in 96-well culture plates in the presence or absence of OVA (200  $\mu$ g/ml) for 3 days. The culture supernatants were stored as mentioned above.

#### Cytokine Measurement

The IL-5, IL-4, IL-13 and IFN- $\gamma$  concentrations in the supernatants were measured using ELISA kits (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's protocol. The limits of detection were 7 pg/ml for IL-5, 2 pg/ml for IL-4 and IFN- $\gamma$  and 1.5 pg/ml for IL-13.

#### Data Analysis

The data are expressed as the means  $\pm$  SD. Significant differences between 2 groups were determined using the nonparametric Mann-Whitney U test. In the coculture experiments, the Wilcoxon matched-pairs test was used. These analyses were performed using Prism4 (GraphPad Software, San Diego, Calif., USA). A  $p$  value  $< 0.05$  was considered significant.

## Results

### Sex Differences in Th2 Cytokine Production by Antigen-Stimulated BLN Cells

The concentrations of IL-5, IL-4, IL-13 and IFN- $\gamma$  in the BLN culture supernatants were significantly higher with OVA stimulation in both the female [IL-5,  $6,673.3 \pm 2,187.1$  pg/ml ( $n = 6$ ) vs.  $1,884.3 \pm 1,089.2$  pg/ml ( $n = 6$ ),  $p < 0.01$ ; IL-4,  $154.8 \pm 50.6$  pg/ml ( $n = 6$ ) vs.  $32.6 \pm 15.1$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-13,  $15,452.5 \pm 3,385.2$  pg/ml ( $n = 6$ ) vs.  $3,780.0 \pm 2,125.9$  pg/ml ( $n = 5$ ),  $p < 0.01$ ; IFN- $\gamma$ ,  $1,059.3 \pm 508.0$  pg/ml ( $n = 4$ ) vs.  $75.5 \pm 35.5$  pg/ml ( $n = 4$ ),  $p < 0.01$ ] and the male [IL-5,  $1,357.0 \pm 812.6$  pg/ml ( $n = 6$ ) vs.  $473.3 \pm 128.6$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-4,  $57.4 \pm 32.5$  pg/ml ( $n = 6$ ) vs.  $20.9 \pm 6.6$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-13,  $6,672.7 \pm 2,652.2$  pg/ml ( $n = 6$ ) vs.  $1,545.8 \pm 250.8$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IFN- $\gamma$ ,  $616.5 \pm 297.6$  pg/ml ( $n = 4$ ) vs.  $36.3 \pm 29.7$  pg/ml ( $n = 4$ ),  $p < 0.01$ ] mice. As previously reported [14], the supernatant concentrations of IL-5, IL-4 and IL-13 upon stimulation of BLN cells with OVA were significantly higher in females than in males. However, significant sex differences were only present for

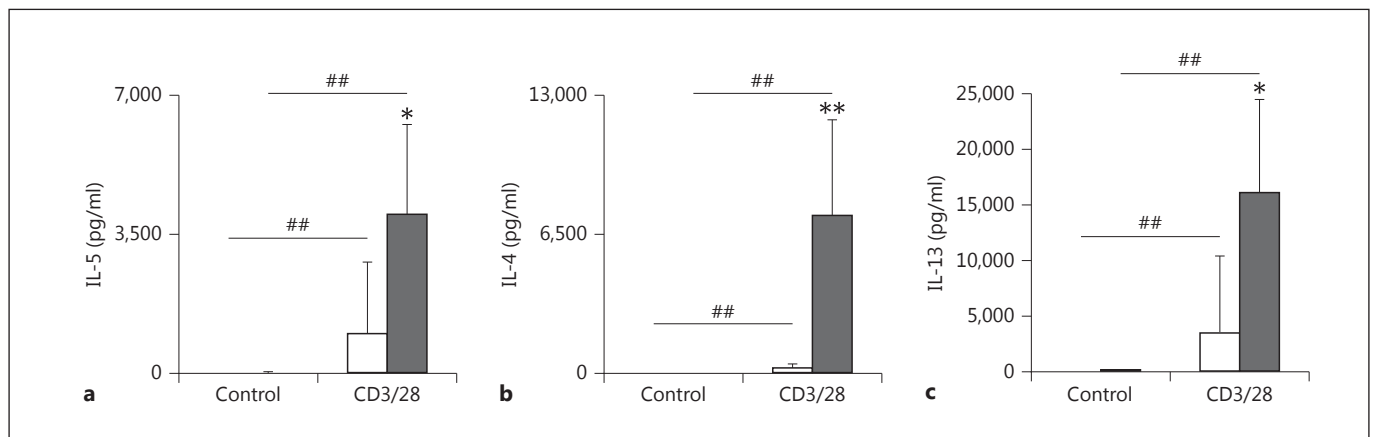


**Fig. 2.** Cytokine production by CD4<sup>+</sup> T cells stimulated with anti-CD3/CD28. Purified CD4<sup>+</sup> T cells from BLN cells of male (open columns) and female (closed columns) mice were stimulated with anti-CD3 and anti-CD28 antibodies (CD3/28) or control antibodies (control) for 3 days. The concentrations of IL-5 (**a**), IL-4 (**b**)

and IL-13 (**c**) in the culture supernatants were measured. Data are expressed as means ± SD. ##  $p < 0.01$  compared with the culture with control antibodies; \*  $p < 0.05$  and \*\*  $p < 0.01$  compared with male mice ( $n = 4-6$  for each group).

IL-5 in BLN cells without OVA. There was no significant sex difference in the IFN- $\gamma$  concentrations of BLN cells with or without OVA stimulation (fig. 1).

#### *Sex Differences in Th2 Cytokine Production by TCR/CD28-Activated CD4<sup>+</sup> T Cells and BLN Cells*

CD4<sup>+</sup> T cells are the main sources of Th2 cytokines in BLN cells upon antigen stimulation [19]. Therefore, we examined the sex differences in Th2 cytokine production by CD4<sup>+</sup> T cells after TCR/CD28 activation. IL-5, IL-4 and IL-13 levels were significantly higher in TCR/CD28-activated CD4<sup>+</sup> T cells when compared to control antibody-stimulated CD4<sup>+</sup> T cells in both female [IL-5,  $4,026.7 \pm 2,228.5$  pg/ml ( $n = 6$ ) vs.  $17.8 \pm 8.6$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-4,  $7,366.7 \pm 4,537.3$  pg/ml ( $n = 6$ ) vs.  $16.0 \pm 7.7$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-13,  $16,006.7 \pm 8,516.5$  pg/ml ( $n = 6$ ) vs.  $34.9 \pm 16.8$  pg/ml ( $n = 4$ ),  $p < 0.01$ ] and male [IL-5,  $978.1 \pm 1,834.0$  pg/ml ( $n = 5$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-4,  $250.9 \pm 205.6$  pg/ml ( $n = 6$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-13,  $3,503.2 \pm 6,880.1$  pg/ml ( $n = 6$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ ] mice. Furthermore, IL-5, IL-4 and IL-13 production by female TCR/CD28-activated CD4<sup>+</sup> T cells was significantly higher than male TCR/CD28-activated CD4<sup>+</sup> T cells (fig. 2a-c). Interestingly, in TCR/CD28-activated BLN cells, no significant differences in IL-5 or IL-13 production were observed between the sexes, while IL-4 production was significantly lower in female cells compared to male cells (fig. 3a-c).

#### *Sex Differences in Th2 Cytokine Production in the Coculture of CD4<sup>+</sup> T Cells and DCs*

As described above, sex differences in Th2 cytokine production were observed by BLN cells after stimulation with antigen but not with anti-CD3/CD28 antibodies. Antigen stimulation involves the interaction of T cells and DCs through TCR-MHC and costimulatory signals, such as CD28/CTLA4-B7-1/B7-2 and ICOS-B7RP-1, and anti-CD3/CD28 antibody stimulation cannot replace all of these interactions. Therefore, we next assessed whether the sex differences in antigen-induced Th2 cytokine production by BLN cells was attributable to the ability of the DCs to present antigen. For this purpose, we performed sex-matched and mismatched coculture experiments of CD4<sup>+</sup> T cells with DCs. The levels of IL-5, IL-4 and IL-13 were significantly higher in the coculture with OVA than in the cultures without OVA [IL-5, male/male  $233.1 \pm 248.3$  pg/ml ( $n = 8$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , male/female  $344.1 \pm 283.1$  pg/ml ( $n = 8$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/male  $137.8 \pm 112.0$  pg/ml ( $n = 7$ ) vs.  $5.0 \pm 10.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/female  $303.4 \pm 185.0$  pg/ml ( $n = 7$ ) vs.  $19.0 \pm 26.4$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-4, male/male  $189.3 \pm 132.8$  pg/ml ( $n = 9$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , male/female  $279.6 \pm 162.8$  pg/ml ( $n = 9$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/male  $82.1 \pm 74.9$  pg/ml ( $n = 8$ ) vs.  $3.0 \pm 6.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/female  $129.8 \pm 60.8$  pg/ml ( $n = 8$ ) vs.  $11.2 \pm 15.6$  pg/ml ( $n = 4$ ),  $p < 0.01$ ; IL-13, male/male  $953.3 \pm 702.2$  pg/ml ( $n = 10$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ ,

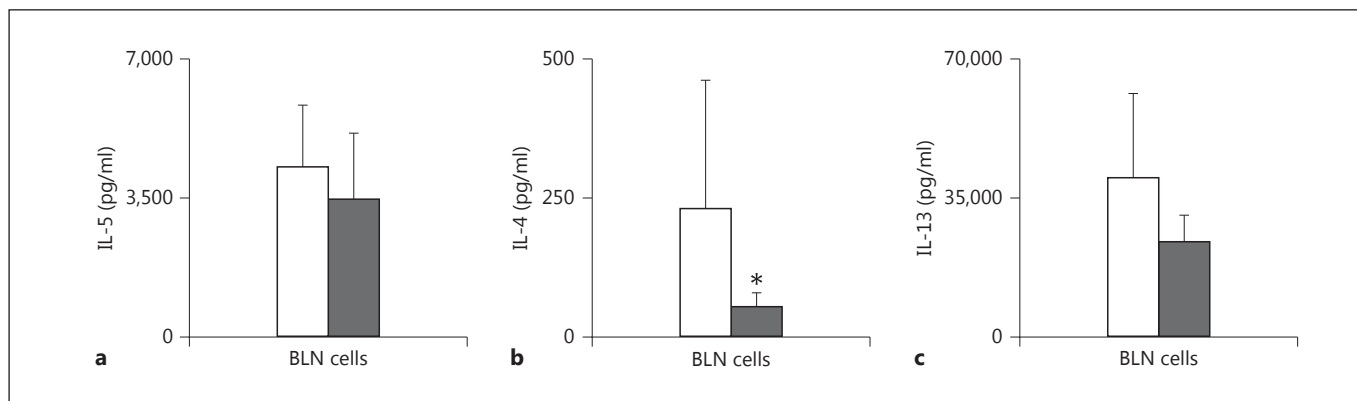


**Fig. 3.** Cytokine production by BLN cells stimulated with anti-CD3/CD28. BLN cells of male (open columns) and female (closed columns) mice were stimulated with anti-CD3 and anti-CD28 antibodies for 3 days. The concentrations of IL-5 (a), IL-4 (b) and

IL-13 (c) in the culture supernatants were measured. Data are expressed as means  $\pm$  SD. \*  $p < 0.05$  compared with male mice ( $n = 5$ – $6$  for each group).

male/female  $1,213.0 \pm 805.6$  pg/ml ( $n = 10$ ) vs.  $0.0 \pm 0.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/male  $969.1 \pm 745.2$  pg/ml ( $n = 10$ ) vs.  $27.0 \pm 54.0$  pg/ml ( $n = 4$ ),  $p < 0.01$ , female/female  $1,056.0 \pm 450.8$  pg/ml ( $n = 10$ ) vs.  $60.8 \pm 84.5$  pg/ml ( $n = 4$ ),  $p < 0.01$ . In the presence of OVA, the concentrations of IL-5 in the cocultures of male or female CD4<sup>+</sup> T cells with female DCs were significantly higher than in the cocultures with male DCs (fig. 4a). In contrast, the cocultures with female DCs did not increase the production of IL-4 and IL-13 by male or female CD4<sup>+</sup> T cells compared to male DCs (fig. 4b, c). Unexpectedly, the cocultures of male CD4<sup>+</sup> T cells with DCs from either male or female mice resulted in higher IL-4 production than female CD4<sup>+</sup> T cells (fig. 4b).

## Discussion

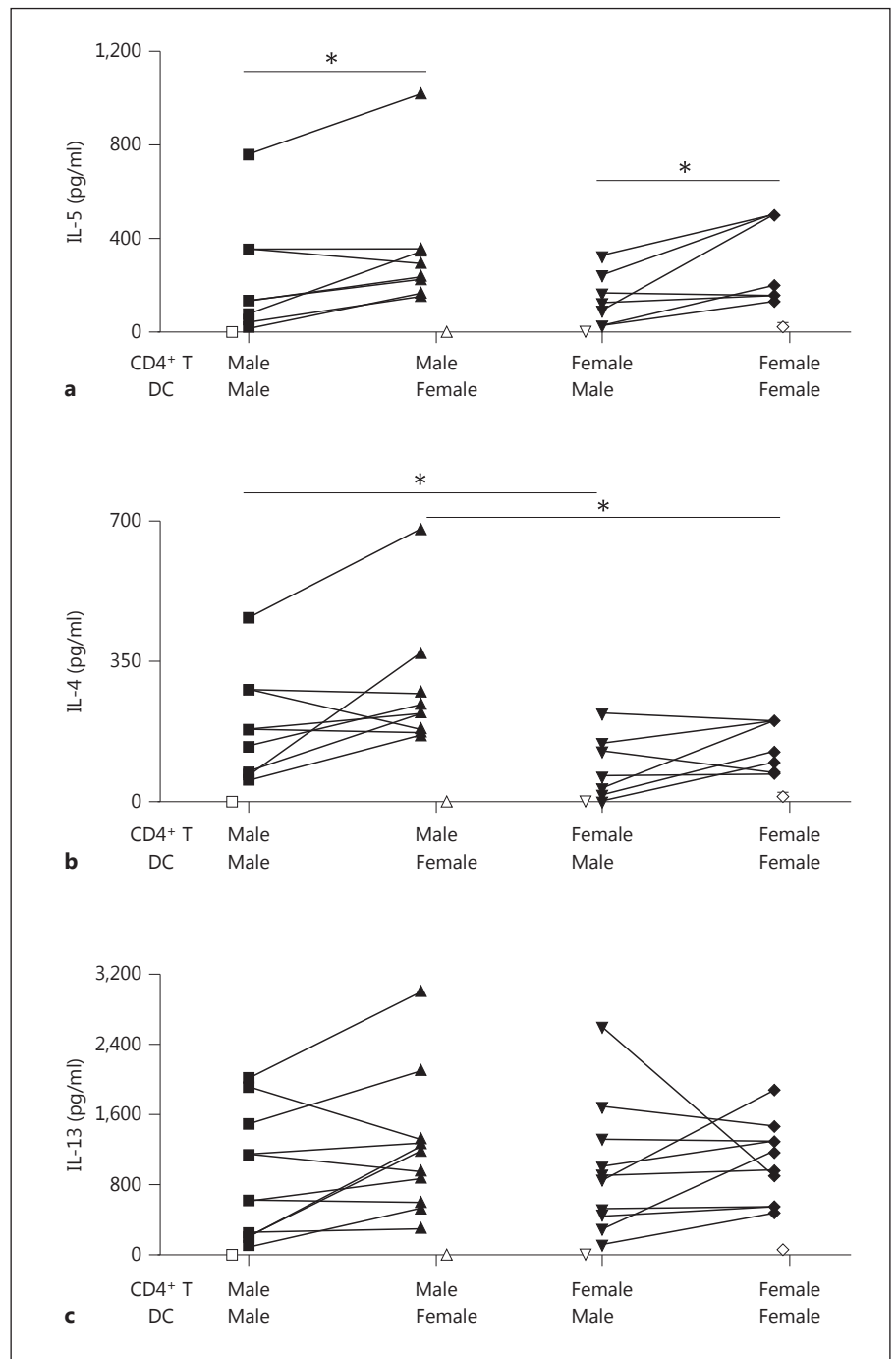
This study shows that female CD4<sup>+</sup> T cells produce greater amounts of Th2 cytokines than male CD4<sup>+</sup> T cells upon TCR/CD28 stimulation, and antigen presentation by female DCs is essential to the female-dominant production of Th2 cytokines in BLN cells, particularly IL-5.

We first examined sex differences in potential Th2 cytokine production by BLN CD4<sup>+</sup> T cells, the main sources of Th2 cytokines, upon TCR/CD28 activation. Female CD4<sup>+</sup> T cells produced higher levels of IL-5, IL-4 and IL-13 than male CD4<sup>+</sup> T cells. However, after TCR/CD28 activation of whole BLN cells, no significant sex differences were observed in IL-5 and IL-13, and IL-4 production in male BLN cells was higher than in female BLN

cells. IL-4 production by female BLN cells (fig. 3b) was markedly reduced compared to female CD4<sup>+</sup> T cells (fig. 2b), which was not observed for IL-5 or IL-13 nor for any cytokines by male cells. It is possible that the expression of IL-4, IL-5 and IL-13 may be regulated in a cytokine-specific and sex-specific fashion, as discussed below. It is likely that T cells other than CD4<sup>+</sup> T cells, such as CD8<sup>+</sup> T cells and NKT cells, may modulate the production of these Th2 cytokines by CD4<sup>+</sup> T cells. This hypothesis is supported by our previous findings that splenocyte CD8<sup>+</sup> T cells from female and male mice suppressed IL-5 production by CD4<sup>+</sup> T cells after TCR/CD28 activation [20].

BLN cells cultured with antigen exhibited sex differences, but BLN cells cultured with anti-TCR/CD28 antibodies did not, suggesting that the interaction of T cells with DCs is important for the sex-specific regulation of Th2 cytokine production. Therefore, the interaction of CD4<sup>+</sup> T cells with DCs was investigated in coculture experiments. The sex differences in IL-5 production as observed in the coculture were dependent on the sex of the DCs but not that of the CD4<sup>+</sup> T cells, although the female CD4<sup>+</sup> T cells produced more IL-5 upon TCR/CD28 stimulation. These results strongly suggest that the interaction with female DCs is responsible for the female-dominant IL-5 production in antigen-stimulated BLN cells. With regard to the effects of sex hormones on the activity of DCs, estrogen has been demonstrated to enhance differentiation, MHC II expression and T cell stimulatory capacity in vivo and in vitro. Splenic DCs isolated from estrogen-treated mice showed reduced production of Th1

**Fig. 4.** Cytokine production by CD4<sup>+</sup> T cells cocultured with DCs in the presence of antigen. Purified CD4<sup>+</sup> T cells and DCs from BLN cells of male and female mice were cocultured in the absence (open symbols) or presence (closed symbols) of OVA. The IL-5 (a), IL-4 (b) and IL-13 (c) concentrations in the culture supernatants were measured. \*  $p < 0.05$  ( $n = 4$  for each coculture without OVA and  $n = 7-10$  for each coculture with OVA).



cytokines, such as IFN- $\gamma$  and IL-12 [21]. This study is the first report indicating the divergent function of DCs in males and females under normal or diseased conditions. It is important to further investigate the differences in subsets and functions of DCs between sexes. Although we used CD11c<sup>+</sup> cells as DCs, CD11c is expressed not only

on DCs but also on several subsets of monocytes, macrophages [22], NK cells [23], T cells [24] and B cells [25]. Therefore, it cannot be ruled out that contamination by these cells might influence cytokine production.

There were no significant differences in IL-4 and IL-13 production between the sexes of DCs in the coculture ex-

periments. Moreover, IL-4 production was higher in male CD4<sup>+</sup> T cells compared to female CD4<sup>+</sup> T cells cocultured with DCs independent of the sex of the DCs. Taking these data together, we hypothesize that the interaction with DCs influences the sex differences in Th2 cytokine production. GATA-3 plays a critical role in the production of Th2 cytokines [26]. GATA-3 also regulates the production of 3 Th2 cytokines, IL-5, IL-4 and IL-13, transcriptionally and epigenetically. For instance, functional GATA-3-binding sites have been reported in both the IL-5 and IL-13 promoters, but not in the IL-4 promoter [27, 28], and the DNaseI-hypersensitive site 2 is indispensable for GATA-3-mediated IL-4 expression, but not IL-5 or IL-13, expression by the formation of active chromatin at the IL-4 locus [29]. We previously demonstrated greater levels of antigen-induced IL-5 in association with higher GATA-3 expression in BLN cells from antigen-sensitized and challenged female mice compared to cells from male mice [14]. These findings suggest that the expression of IL-5, IL-4 and IL-13 may be regulated in a cytokine-specific and sex-specific manner. IL-4 production by male CD4<sup>+</sup> T cells cocultured with male DCs was higher than female CD4<sup>+</sup> T cells cocultured with female DCs (fig. 4b), while the production by male BLN cells was lower than female BLN cells (fig. 1b). These opposite results in the sex difference of IL-4 production may be due to the effects of cells other than CD4<sup>+</sup> T cells that received an antigen signal during the BLN cell culture.

Using mouse models, we previously found that Th2 cytokines were predominantly produced in female mice, while IFN- $\gamma$  production by naïve splenocytes and sensitized BLN cells from C57BL/6 mice was similar in both sexes [14, 17]. In contrast to our findings, it has been reported that TCR/CD28 stimulation of CD4<sup>+</sup> T cells from naïve female Swiss/Jackson Laboratory (SJL) mice, an au-

toimmune-prone strain with Th1 dominance, led to higher levels of IFN- $\gamma$  when compared with male mice. A similar trend was observed upon antigen stimulation of splenocytes and lymph node cells from female experimental autoimmune encephalomyelitis-developed SJL mice when compared to male mice [10]. These findings in human and animal studies suggest that sexual dimorphism in the immune response could be influenced by genetic factors and complicated interactions between T cells and DCs, and this dimorphism may be linked to diseases.

In conclusion, female-dominant antigen-induced Th2 cytokine production in draining lymph nodes may be partly attributable to sex differences in the potential of CD4<sup>+</sup> T cells to produce Th2 cytokines and DC function. To further clarify the underlying mechanisms of sex differences in allergic immune responses, sex-biased gene regulation, which has been challenging for genetic studies on human diseases including autoimmune diseases and infections [30–32] should be investigated.

## Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 21590970 to Y.O. and No. 22590843 to I.O.) and by the Matching Fund Subsidy for Private Universities from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. This paper was checked for proper English usage by American Journal Experts (Cert. No. C971-E694-BDD4-5579-DE48).

## Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

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