Lack of lymphoid chemokines CCL19 and CCL21 enhances allergic airway inflammation in mice

Baohui Xu1,2,6, Kohji Aoyama1, Mayumi Kusumoto3, Akio Matsuzawa4, Eugene C. Butcher2, Sara A. Michie2, Takami Matsuyama5 and Toru Takeuchi1

1Department of Environmental Medicine, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
2Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5176, USA
3Department of Medicine, Kagoshima Seikyo Hospital, 5-20-10 Taniyama Chuo, Kagoshima 891-0141, Japan
4Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
5Department of Immunology, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan
6Present address: Department of Pathology, Stanford University School of Medicine, CCSR 3250, 269 Campus Drive, Stanford, CA 94305-5176, USA

Keywords: airway hypersensitivity, adhesion molecule, chicken ovalbumin, cytokines, IgE

Abstract

Lymphoid chemokines CCL19 and CCL21 are crucial for the recruitment of circulating naive T cells into lymph nodes. However, it is not completely known how they contribute to the development of allergic diseases. To determine whether the lack of CCL19 and CCL21 affects allergic airway inflammation, CCL19- and CCL21-deficient [paucity of lymph node T cells (plt/plt)] and wild-type (WT) mice were immunized intra-peritoneally and then challenged intra-nasally with chicken ovalbumin (OVA). Plt/plt mice developed more severe allergic airway inflammation characterized by increased eosinophils and lymphocytes in bronchoalveolar lavage (BAL) and profound inflammation in peribronchiolar and perivascular regions than did WT mice. CD4+α4 integrin+ and CD4+β7 integrin+ T cells were significantly increased in the BAL of OVA-immunized and OVA-challenged (OVA/OVA) plt/plt mice compared with OVA/OVA WT mice. Moreover, there were higher levels of IL-4 and IL-13 mRNAs and lower levels of IL-2 and IFN-γ mRNAs in inflamed lungs of OVA/OVA plt/plt mice compared with OVA/OVA WT mice. Plt/plt mice produced higher levels of total and OVA-specific IgE antibody. Thus, our results suggest that lack of lymphoid chemokines CCL19 and CCL21 enhances allergic airway inflammation by modulating the recruitment of CD4+ T cells into the lung, the balance between Tn1 and Tn2 cytokines and the IgE production.

Introduction

Allergic asthma is a chronic airway inflammatory disease caused by an inappropriate immune response to an inhaled antigen and is characterized by airway hyperreactivity, airway remodeling and antigen-specific IgE production (1). Although many immune cells, including dendritic cells, B cells, T cells, mast cells and eosinophils, are involved, a balance between Tn1 and Tn2 is fundamental to the development of allergic asthma. Tn2 produce IL-4, IL-5 and IL-13, which directly or indirectly contribute to recruitment and activation of principal inflammatory effector cells such as eosinophils and mast cells. In contrast, Tn1 inhibit asthma pathology by producing IFN-γ (2).

Allergic asthma attacks occur when a previously sensitized individual encounters the same or a cross-reactive allergen. Sensitization to an inhaled allergen is primarily initiated in the bronchial lymph nodes (LN) where the allergen, which is transported by airway mucosal dendritic cells, is presented to naive CD4+ and CD8+ T cells in the context of MHC II and MHC I molecules, respectively (3–6). This results in T cell activation, proliferation, differentiation and eventually allergen-specific memory T cell generation. Thus, the structural integrity of LN is critical for the priming of naive T cells by inhaled allergens.

Homeostatic migration of lymphocytes from the bloodstream into LN is one important mechanism for the maintenance of normal LN structure and function. The migration requires multiple sequential events on endothelial venules, including specific recognition of lymphocyte L-selectin by...
vascular peripheral node addressin (PNAd) and the interaction of lymphocyte chemokine receptor CCR7 with its vascular ligands (chemokines CCL19 and CCL21) (7). These two chemokines are predominantly expressed on high endothelial venules and/or stromal cells in T cell zones of LNs and Peyers patches and on lymphatic vessels (8–10). Mice homozygous for the paucity of lymph node T cells (plt/plt) lack CCL19 and CCL21 in LNs and Peyers patches and have disrupted migration of naive T cells (and partially of B cells) and dendritic cells into LNs (8, 11–14). Although the roles of these chemokines in immune responses have previously been investigated, the findings vary depending on experimental settings. For example, contact hypersensitivity to oxazolone was reduced on day 2 but enhanced on day 6 after sensitization in plt/plt mice as compared with wild-type (WT) mice (15). T cells from chicken ovalbumin (OVA)-immunized plt/plt mice produced more IL-2 to OVA stimulation in vitro (15). plt/plt mice showed a slight reduction in the initiation and maintenance of protective anti-viral memory responses but normal neutralizing anti-viral B cell response and Ig class switching (16). Blockade of CCL21 with neutralizing anti-CCL21 antibody exacerbated acute pulmonary inflammation in mice infected with Propionibacterium acnes (17). CCL21 antagonist and anti-CCL21 antibody inhibited chronic graft-versus-host disease and contact hypersensitivity, respectively, in mice (18, 19). However, it is not completely known whether the lack of CCL19 and CCL21 will affect the development of allergic airway inflammation in plt/plt mice.

Thus, this study was aimed at investigating the development of allergic airway inflammation in mice lacking lymphoid chemokines CCL19 and CCL21. plt/plt and WT mice were immunized by daily intra-peritoneal injection of OVA for 14 days followed by three consecutive intra-nasal OVA challenges on days 25, 26 and 27. We found that allergic airway inflammation was enhanced in plt/plt mice. The enhanced allergic airway inflammation in plt/plt mice was characterized by increased infiltrates of lymphocytes and eosinophils in airway lumen, airway and pulmonary vasculatures, increased total and OVA-specific IgE levels and increased Th2, but decreased Th1, cytokine mRNAs.

Methods

Mice

plt/plt mice were obtained from the Laboratory Animal Research Center, University of Tokyo, Tokyo, Japan. Ddy mice that have similar genetic background with plt/plt mice were purchased from Japan SLC, Hamamatsu, Shizuoka, Japan, and used as WT controls. Mice were housed under specific pathogen-free conditions in the Animal Facility of Kagoshima University, Kagoshima, Japan. Female plt/plt and WT mice at 8 weeks of age were used for experiments. The Institute Animal Care and Use Committee approved all experimental protocols.

Induction of allergic airway inflammation

Allergic airway inflammation was provoked according to the published protocols with some modifications (20–22). Briefly, mice were immunized by daily intra-peritoneal injection of 100 microliter of OVA (100 microgram ml⁻¹, Sigma–Aldrich, St Louis, MO, USA) in PBS from day 1 to day 14. Control mice received the equal volume of PBS. On days 25, 26 and 27, mice were challenged ina-našally with 50 microliter of OVA (1 mg ml⁻¹ in PBS) or PBS alone. Mice were sacrificed under terminal anesthesia 2 days after the last OVA or PBS challenge.

Bronchoalveolar lavage analysis

Airways were lavaged three times with 0.8 ml of PBS via a tracheal cannula. Bronchoalveolar lavage (BAL) fluid from each mouse was centrifuged at 1500 r.p.m. at 4°C for 10 min and re-suspended in PBS for total leukocyte counting. For differential leukocyte counts, we prepared a slide for each BAL sample by cytopsin and stained with Wright–Giemsa staining reagents according to the manufacturer’s instructions (Muto Chemical Co., Tokyo, Japan). The differential counts of eosinophils, lymphocytes, macrophages and neutrophils were determined blind by counting a total of 200 cells per slide at high-powered fields (×400).

Lung histology

Lungs were inflated with <1 ml of optimal cutting temperature (OCT) compound-embedding medium (Sakura Fine-technical Co. Ltd., Tokyo, Japan); PBS mixture (1:1) and embedded in OCT medium on dry ice. The lungs were cut into 6 micrometer sections and fixed with cold acetone. The sections were stained with hematoxylin and eosin (H&E). Peribronchiolar (medium to small airway) and perivascular inflammation was graded as described previously (23). To evaluate the inflammation in the lung interstitium, the nucleated cells in 10 randomly selected areas, from a 10 × 10 grid at high-powered fields (×400), were counted for each mouse. The interstitial inflammation score was expressed as the ratio of the cell number in each treatment group to that in respective control group (unimmunized and PBS challenged). The ratio that is significantly higher than theoretical value, which is set at 1, indicates interstitial inflammation in lungs.

Flow cytometric analysis

BAL cells were suspended in PBS containing 0.1% fetal bovine serum and 0.01% sodium azide, stained with the indicated mAbs on ice for 30 min and then fixed with 2% formalin–PBS. PE–Cy5-anti-CD4 and PE-anti-CD8 mAbs were obtained from BD Biosciences (San Diego, CA, USA). FITC–anti-CD4 (GK1.5), FITC–anti-L-selectin (MEL-14), FITC–anti-α4 integrin (PS/2) and FITC–anti-β2 integrin (FB504) mAbs were labeled in our laboratories. Data on stained samples were collected on a BD FACSscan flow cytometer using CellQuest software (San Jose, CA, USA) and analyzed using WinMDI free software (version 2.8, http://facs.scripps.edu/software.html).

Analysis of cytokine mRNAs in lung tissues

Total RNA was extracted from lungs and semi-quantitative reverse transcription–PCR analysis of cytokine mRNAs was performed as previously described (24, 25). The primers used were IFN-γ, sense TGAAACGTACACACTGCTATTTGG and anti-sense CGACTCTTTTTCCGCTTCTGAG; IL-2,
Development of allergic airway inflammation in plt/plt mice

Leukocytes in the BAL

In allergic asthma, infiltrates of eosinophils and lymphocytes are found in the airway lumen, walls of the airways and the lung interstitium (1). To determine whether plt/plt mice develop allergic pulmonary inflammation, we first analyzed BAL cells from unimmunized plt/plt and WT mice. There were no differences in the number of total leukocytes and cellular composition in the BAL between plt/plt and WT mice even after three intra-nasal OVA challenges (Fig. 1A). The BAL cells were mainly composed of macrophages and lymphocytes. In contrast, total cell numbers were dramatically increased in the BAL of ovalbumin-immunized and ovalbumin-challenged (referred as OVA/OVA thereafter) plt/plt and OVA/OVA WT mice (Fig. 1B). However, the total cell number was significantly higher in OVA/OVA plt/plt mice than that in OVA/OVA WT mice. Eosinophils, macrophages and lymphocytes were most common cells in the BAL of OVA/OVA plt/plt and OVA/OVA WT mice. Moreover, we observed more eosinophils and lymphocytes in the BAL from OVA/OVA plt/plt mice than from OVA/OVA WT mice. Conversely, there were fewer macrophages in the BAL from OVA/OVA plt/plt mice than from OVA/OVA WT mice. There was no difference in the numbers of neutrophils between OVA/OVA plt/plt and OVA/OVA WT mice.

Lung histology

Next, we examined H&E-stained sections of lungs from different groups. We did not find leukocytic infiltrates in the lungs of unimmunized plt/plt and WT mice that were challenged

Measurement of serum IgE

Serum total and OVA-specific IgE levels were measured using sandwich ELISA assays. Purified rat anti-mouse IgE mAb (11B11, BD Biosciences) was used as the coating antibody. Biotinylated rat anti-mouse IgE mAb (BVD6-24G2, BD Biosciences) and biotinylated OVA labeled as reported previously (26) were used to detect total and OVA-specific IgE, respectively. The formed IgE and biotinylated anti-IgE mAb complexes or OVA-specific IgE and biotinylated OVA complexes were then detected by streptavidin-conjugated horseradish peroxidase (Sigma–Aldrich) in the presence of hydrogen peroxide and diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma–Aldrich). Total serum IgE levels were calculated from the standard curve generated from mouse IgE standard (BD Biosciences) and expressed as nanogram per milliliter. OVA-specific IgE levels were calculated from the pooled standard serum generated from OVA-immunized BALB/c mice in our laboratory and were assigned the arbitrary values (units per milliliter) (26).

Statistical analysis

Data are expressed as mean value ± standard deviation. Analysis of variance was used to determine statistical significance among the groups. P < 0.05 was considered to be statistically significant.

Results

A

Unimmunized/OVA-challenged (PBS/OVA)

B

OVA-immunized/OVA-challenged (OVA/OVA)

Fig. 1. Leukocytes in the BAL of WT and plt/plt mice. BAL cells were collected from OVA/OVA or unimmunized (PBS-treated)/OVA-challenged (PBS/OVA) mice, counted and stained with Wright–Giemsa. (A) There were no differences between PBS/OVA WT and PBS/OVA plt/plt mice in the numbers of total leukocytes, lymphocytes, neutrophils or macrophages in the BAL. (B) Eosinophils, lymphocytes and macrophages were significantly increased in the BAL of OVA/OVA plt/plt and OVA/OVA WT mice as compared with PBS/OVA. The BAL of OVA/OVA plt/plt mice contained more eosinophils and lymphocytes, but fewer macrophages, than the BAL of OVA/OVA WT mice. *P < 0.05 and **P < 0.01 compared with OVA/OVA WT mice, analysis of variance; n = 6 mice in each group.
with OVA (Fig. 2A and 2B) or PBS (data not shown). Consistent with the findings in the BAL, there were significant peribronchial and perivascular leukocytic infiltrates in the lungs of OVA/OVA plt/plt and OVA/OVA WT mice (Fig. 2C and D). The inflammation was more severe in the OVA/OVA plt/plt mice, particularly in small airways and vessels than in the OVA/OVA WT mice (Figs 2E, 2F and 3). Similarly, the cell ratios in lung interstitium of OVA/OVA plt/plt and OVA/OVA WT mice were significantly higher than theoretical value (set at 1) (Fig. 3), indicating interstitial inflammation in lungs. Relative high cell ratio in lung interstitium of OVA/OVA plt/plt mice indicates more severe interstitial inflammation in OVA/OVA plt/plt mice as compared with OVA/OVA WT mice.

T cells in the BAL of OVA/OVA mice

Since CD4+ and CD8+ T cells contribute to allergic asthma (3, 27), we stained BAL lymphocytes with anti-CD4 and anti-CD8 mAbs to determine the dominant T cell subset in the BAL of OVA/OVA plt/plt and OVA/OVA WT mice. As shown in Fig. 4, the BAL of OVA/OVA plt/plt and OVA/OVA WT mice had more CD4+ T cells than CD8+ T cells. However, there were significantly more CD4+ T cells in the BAL of OVA/OVA plt/plt mice (61.4 ± 3.5%) than in the BAL of OVA/OVA WT mice (24.6 ± 6.0%). There was no difference in the proportion of CD8+ T cells in the BAL between OVA/OVA plt/plt and OVA/OVA WT mice. OVA/OVA plt/plt mouse BAL had fewer lymphocytes that were not stained with anti-CD4 or anti-CD8 mAb compared with OVA/OVA WT mice.

Adhesion molecules on the BAL T cells of OVA/OVA mice

Recognition of lymphocyte adhesion molecules by their vascular endothelial ligands is crucial for the migration of circulating lymphocytes into lymphoid and inflamed tissues. Lymphocyte L-selectin and endothelial PNAd are important for the recruitment of lymphocytes into LNs, bronchus-associated lymphoid tissues (BALTs) and chronically inflamed airways (28, 29). \( \alpha_4 \beta_1 \) integrin, which binds mainly to vascular cell adhesion molecule-1 (VCAM-1), is important for the migration of \( \alpha_4 \beta_1 \) memory T cells into BALTs (29) and

---

**Fig. 2.** Lung histology in WT and plt/plt mice. Acetone-fixed frozen sections of lungs were stained with H&E. (A and B) There was no obvious inflammation in the lungs of PBS-treated/OVA-challenged (PBS/OVA) WT (A) and PBS/OVA plt/plt (B) mice. (C and D) Significant peribronchial and perivascular inflammation was found in medium to large sizes of airways and vessels of OVA/OVA WT (C) and OVA/OVA plt/plt (D) mice. Specifically, there was more severe inflammation in the small airways and vessels in OVA/OVA plt/plt mouse BAL (F) than OVA/OVA WT (E) mice.
inflamed lung (30). αECα7 integrin, which binds to epithelial E-cadherin, plays a major role in recruitment of and retention of intra-epithelial lymphocytes (31).

To determine if the predominance of CD4+ T cells in the BAL of OVA/OVA plt/plt mice may result from the preferential recruitment of these cells from blood vessels into lungs, we analyzed the expression of L-selectin, α4 integrin and β7 integrin on BAL lymphocytes. As shown in Fig. 5(A and B), BAL lymphocytes recovered from OVA/OVA plt/plt mice contained more L-selectin+ α4 integrin+ and β7 integrin+ CD4+ T cells than those from OVA/OVA WT mice. More than 80% of the CD4+ T cells in the BAL of OVA/OVA plt/plt (84.9 ± 2.5%) and OVA/OVA WT (85.7 ± 3.3%) mice were L-selectin+ (Fig. 5A). More CD4+ T cells in the BAL of OVA/OVA plt/plt mice expressed higher levels of α4 integrin (84.1 ± 1.9%) and β7 integrin (56.2 ± 4.4%) than those in the BAL of OVA/OVA WT mice (71.8 ± 3.7% for α4 integrin+ and 28.2 ± 3.0% for β7 integrin+ ) (Fig. 5A).

In contrast, BAL CD4+ T cells from OVA/OVA plt/plt mice expressed higher levels of α4 integrin and β7 integrin than those in the BAL of OVA/OVA WT mice (Fig. 5C). In contrast, the proportions of BAL CD8+ T cells that expressed L-selectin, α4 integrin and β7 integrin were almost identical in OVA/OVA plt/plt and OVA/OVA WT mice (Fig. 6A and B). BAL CD8+ T cells from OVA/OVA plt/plt mice expressed higher levels of L-selectin than did those from OVA/OVA WT mice (Fig. 6C). There was no difference, however, in the expression levels of α4 integrin and β7 integrin on the BAL CD8+ T cells between OVA/OVA plt/plt and OVA/OVA WT mice (Fig. 6C).

T<sub>h</sub>1 and T<sub>h</sub>2 cytokine mRNAs in the lungs of OVA/OVA mice

T<sub>h</sub>2 cytokine production is a hallmark of allergic asthma (1, 2). More specifically, high levels of IL-4, IL-5 and IL-13 and low levels of IL-2 and IFN-γ are associated with a T<sub>h</sub>2-directed response to an allergen in the lung (1, 2). To determine whether the severe airway inflammation in plt/plt mice is associated with an imbalance between T<sub>h</sub>1 and T<sub>h</sub>2 cytokines, we analyzed T<sub>h</sub>1 (IL-2 and IFN-γ) and T<sub>h</sub>2 (IL-4 and IL-13) cytokine mRNAs in the lungs of plt/plt and WT mice. Very low levels of IL-2 and IFN-γ mRNAs were detected in the lungs of plt/plt and WT mice after OVA immunization (or PBS-challenged), whereas IL-4 and IL-13 mRNAs were undetectable (data not shown). In contrast, OVA challenge significantly enhanced the expression of T<sub>h</sub>1 and T<sub>h</sub>2 cytokine mRNAs in the lungs of plt/plt and WT mice (Fig. 7). However, lungs from OVA/OVA plt/plt mice expressed significantly higher levels of IL-4 and IL-13 mRNAs and lower levels of IL-2 and IFN-γ mRNAs than did lungs from OVA/OVA WT mice.

IgE production

IgE is important in the development of allergic asthma (1, 2). To determine whether plt/plt mice have a high IgE response to allergen immunization, we measured total and OVA-specific IgE levels in the sera of plt/plt and WT mice that were immunized with OVA or unimmunized. Although unimmunized plt/plt mice had slightly higher total IgE levels than unimmunized WT mice, total IgE was undetectable in the sera from plt/plt mice (Fig. 8A). OVA-specific IgE was increased in the sera from plt/plt mice (Fig. 8B). OVA immunization significantly increased total and OVA-specific IgE levels in plt/plt and WT mice. However, total and antigen-specific IgE levels were significantly
We observed the enhanced allergic airway inflammation in plt/plt mice, which lack the lymphoid chemokines CCL19 and CCL21, as compared with WT mice. Specially, the OVA/OVA plt/plt mice had significantly more eosinophils and lymphocytes in the BAL (Figs 1) and more severe peribronchiolar, perivascular and interstitial infiltrates in the lungs than did OVA/OVA WT mice (Figs 2 and 3). The BAL of OVA/OVA plt/plt mice contained more CD4+ T cells than did OVA/OVA WT mice (Fig. 4). Most BAL CD4+ T cells in either OVA/OVA plt/plt or OVA/OVA WT mice were L-selectin+; thus, these cells have a memory/effector phenotype, as previously reported in asthma patients and mouse models of asthma (32–34). Furthermore, BAL CD4+, but not CD8+, T cells in OVA/OVA plt/plt mice expressed the higher levels of α4 integrin and β7 integrin than those in OVA/OVA WT mice (Figs 5 and 6).

α4 integrin forms the α4β1 and α4β7 heterodimers with β1 and β7 integrins, respectively. α4β1 integrin mainly binds to VCAM-1 and recruits α4β1+ memory T cells and eosinophils into inflamed lungs, whereas α4β7 integrin binds to mucosal addressin cell adhesion molecule-1 on endothelia and recruits α4β7+ lymphocytes into inflamed gut (30, 35). αEβ7 integrin binds to epithelial E-cadherin and recruits αEβ7+ lymphocytes into epithelial layers of mucosal tissues (31). However, α4β7 integrin is rarely expressed on lung lymphocytes (32, 36, 37). Thus, the relatively high levels of α4 and β7 integrins on BAL CD4+ T cells from OVA/OVA plt/plt mice suggest that more α4β1+ and αEβ7+ memory CD4+ T cells were recruited into inflamed lungs of plt/plt mice.

There is impaired migration of naive T cells from the bloodstream into LNs of plt/plt mice and L-selectin-deficient mice. In contrast to plt/plt mice, L-selectin-deficient mice develop allergic airway inflammation as well as do WT mice in response to OVA immunization and challenge (38). Thus, CCL19 and CCL21 in LNs and L-selectin on lymphocytes may play distinct roles in the development of allergic airway inflammation.

Binding of CCL19 or CCL21 to CCR7 on naive lymphocytes causes activation of a tyrosine kinase which in turn leads to conformational changes in β2 integrin and α4...
integrin on the lymphocyte surface (39, 40). Thus, a4β1 integrin binds to intercellular cell adhesion molecule (ICAM)-1 and ICAM-2, while aLβ2 integrin binds to VCAM-1. In the present study, most CD4+ T cells in the BAL of OVA/OVA plt/plt mice had an activated/memory phenotype (Fig. 5). Therefore, recruitment of a4 integrinhigh CD4+ T cells into the BAL of OVA/OVA plt/plt mice does not depend on CCR7 signaling-induced integrin activation.

We found increased Th2 cytokine (IL-4 and IL-13) mRNAs and decreased Th1 cytokine (IL-2 and IFN-γ) mRNAs in the lungs of OVA/OVA plt/plt mice compared with OVA/OVA WT mice (Fig. 7). Our findings are consistent with the differential roles of Th1,2 cytokines in the development of allergic asthma (2). For example, IL-4 and IL-13 are essential for the polarization and expansion of Th1,2 and for the production of downstream cytokines such as eotaxin which is crucial for the full development of eosinophilia (41–43). IL-4 can up-regulate the expression of endothelial ICAM-1 and VCAM-1, which play critical roles in recruiting eosinophils and a4β1+ memory T cells into inflamed lungs (30, 43, 44). In contrast, IFN-γ produced by Th1 inhibits eosinophil recruitment and mucus production (45, 46). Local expression of IFN-γ inhibits airway hyperresponsiveness and antigen-specific IgE secretion (47). Thus, the increased Th2 and decreased Th1 cytokines in the lungs of OVA/OVA plt/plt mice favor the development of enhanced allergic airway inflammation in plt/plt mice.

We showed that plt/plt mice produce more total and OVA-specific IgE than WT mice in response to OVA immunization (Fig. 8). These results are consistent with the enhanced Th2 cytokine production in OVA/OVA plt/plt mice (Fig. 7) and the well-known regulatory roles of IL-4 and IL-13 in IgE synthesis (48). Elevated levels of total IgE have also been found in CCR7-deficient mice (49). IgE contributes to allergic asthma mainly by modulating the effects of mast cells (50–52). Binding of IgE to its high-affinity receptor, FcεRI, on mast cells increases receptor levels and thus effector mediator release in response to specific antigens. In addition, the binding of specific antigen to FcεRI-expressing cells increases the recruitment of CD4+ T cells into the airway in trinitrophenyl-specific IgE transgenic mice (53). Thus, high
IgE levels in OVA/OVA plt/plt mice may enhance allergic airway inflammation by promoting the mast cell function and the recruitment of CD4+ T cells into airways. α7β1 integrin recruits mast cell progenitors into small intestine, whereas α4 integrin and VCAM-1 recruit mast cell progenitors into inflamed lung (54, 55). To address whether enhanced airway inflammation in plt/plt mice is associated with the recruitment of mast cells into the lungs, we stained mast cells on the longitudinal left lung frozen sections of OVA/OVA plt/plt and OVA/OVA WT mice using acidic toluidine blue. Mast cells per lung section were comparable in OVA/OVA plt/plt and OVA/OVA WT mice (2.0 ± 2.0) and OVA/OVA WT (2.2 ± 3.3) mice (n = 6 mice in each group). Thus, enhanced allergic airway inflammation in plt/plt mice does not accompany increased recruitment of mast cells into the lungs.

Yamashita et al. (56) recently reported the development of allergic airway inflammation in plt/plt mice. They focused on the disease’s resolution phase and observed the enhanced airway inflammation in OVA-immunized plt/plt mice on days 21, 28 and 42 after several aerosol OVA challenges. However, they did not examine airway inflammation on day 1–3 after OVA challenge, the time points at which airway inflammation reach the peak in most published mouse models of asthma. We and Grinnan et al. (57) showed the increased numbers of eosinophils and CD4+ T cells in the BAL of OVA-immunized plt/plt mice 24 or 48 h after the last OVA challenge. Thus, our and Grinnan’s (57) studies compliment the data from Yamashita et al. (56), further suggesting that allergic airway inflammation is enhanced in plt/plt mice. However, neither Yamashita et al. (56) nor Grinnan et al. (57) found significant differences in total and OVA-specific IgE levels between OVA-immunized plt/plt and OVA-immunized WT mice. This is contrast to our findings that OVA immunization induced more IgE production in plt/plt mice than WT mice. The different IgE response in these studies may be due to differences in immunization protocols and sampling time points.

Finally, CCR7, the receptor for CCL19 and CCL21, is expressed on B cells, naive T cells, central memory T cells and dendritic cells. Campbell et al. (33) showed that nearly half of CD4+ or CD8+ T cells recovered from the BAL of asthma patients expressed CCR7. Conversely, Syed et al. (58) reported down-regulation of CCR7 on peripheral blood CD4+ T cells isolated from asthma patients. Bromley et al. (59) recently reported that most CD4+CD44high T cells in the BAL and lung of OVA/OVA mice did not express CCR7. In the present study, we did not examine CCR7 on the BAL.
T cells of OVA/OVA plt/plt and OVA/OVA WT mice. However, Grinnan et al. (57) found more CCR7-expressing CD4+ and CD8+ T cells in the lungs of OVA/OVA plt/plt mice than those in OVA/OVA WT mice. Given the important role of CCR7 in lymphocyte exit from peripheral tissues (59, 60) and the reduced expression of CCL21 on lymphatic endothelia in plt/plt mice (8, 11, 61), increased CD4+ T cells in the BAL and lung of OVA/OVA plt/plt mice observed by us and Grinnan et al. (57) may in part result from reduced lymphocyte exit from inflamed lung via afferent lymphatic vessels.

Based on our findings and those in two previous studies, (56, 57) we propose that enhanced allergic airway inflammation in plt/plt mice is mainly caused by increased recruitment of lymphocytes and eosinophils into the lungs, increased Tc2 and decreased Tc1 cytokines and high IgE production by B mice (8, 11, 61), increased CD4+ plt/plt in OVA/OVA WT mice. Given the important role of CCR7 in the lung and of OVA/OVA plt/plt mice. However, the reduced CCL21 on lymphatic vessels in plt/plt mice may delay or impair lymphocyte exit from inflamed lung which in turn augments airway inflammation. CCL19 and CCL21 are absent in stromal cells and high endothelial cells but not dendritic cells in plt/plt mice (9). The number of dendritic cells was almost equal in the inflamed lung of OVA/OVA plt/plt and OVA/OVA WT mice (57). Therefore, it is unlikely that lung dendritic cells make major contributions to enhanced allergic airway inflammation in plt/plt mice.

Acknowledgements
We thank Chiko Yuma for her administrative and technical assistance during this work. This work was supported by the grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, (C2-15590515 and C2-13670339) given to B.X.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>bronchoalveolar lavage</td>
</tr>
<tr>
<td>BALT</td>
<td>bronchus-associated lymphoid tissue</td>
</tr>
<tr>
<td>GAPDH</td>
<td>glyceraldehydes-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>hematoxylin and eosin</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular cell adhesion molecule</td>
</tr>
<tr>
<td>LN</td>
<td>lymph node</td>
</tr>
<tr>
<td>OCT</td>
<td>optimal cutting temperature</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
<tr>
<td>OVA/OVA</td>
<td>ovalbumin-immunized and ovalbumin-challenged</td>
</tr>
<tr>
<td>plt/plt</td>
<td>paucity of lymph node T cell</td>
</tr>
<tr>
<td>PNAd</td>
<td>peripheral node addressin</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
</tbody>
</table>

References
784 Development of allergic airway inflammation in plt/plt mice
27 Miyahara, N., Swanson, B. J., Takeda, K. et al. 2004. Effector CD8+ T cells mediate inflammation and airway hyperresponsive-