

Enhanced allergen-induced airway inflammation in paucity of lymph node T cell (*plt*) mutant mice

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Background: Dendritic cells and lymphocytes play a central role in allergic asthma. Chemokines for these cells include the CCR7 agonists secondary lymphoid chemokine/CCL21 and EBV-induced lymphoid chemokine/CCL19, but their role in allergic asthma is poorly understood.

Objective: We sought to determine the effects of abrogation of lymphoid tissue expression of CCR7 agonists on allergic airway responses.

Methods: Paucity of lymphocyte T cell (*plt*) mutant mice, deficient in EBV-induced lymphoid chemokine/CCL19 and the lymphoid form of secondary lymphoid chemokine/CCL21, were evaluated in an established ovalbumin (OVA)-induced asthma model (*plt*-OVA group) and compared with similarly immunized +/+ BALB/c mice (+/+OVA group).

Results: APTI responses to methacholine increased similarly in OVA-challenged *plt* and +/+ mice. However, airway inflammation was strikingly enhanced in *plt*-OVA mutants over +/+OVA mice and included increased numbers of eosinophils, CD4 and B cells, neutrophils, and total leukocytes in bronchoalveolar lavage fluid and inflammatory cell cuffing around pulmonary arterioles. Enhanced airway inflammation was accompanied by an increase in lung T_H2 activity, with increased levels of IL-4 and monocyte-derived chemoattractant/CCL22.

Conclusions: Induction of allergic asthma in mutant mice with impaired CCR7 responses results in characteristics that resemble severe asthma in human subjects, including severe bronchial lymphocytosis, eosinophilia, and neutrophilia, but not in enhancement in airway hyperreactivity.

Clinical implications: Disruption of chemokines responsible for trafficking of antigen-processing cells and lymphocytes to the

draining lymph nodes might lead to enhanced allergic airway responses. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, chemokines, CCR7, dendritic cells, EBV-induced lymphoid chemokine/CCL19, eosinophils, lymphocytes, mice, monocyte-derived chemoattractant/CCL22, secondary lymphoid chemokine/CCL21

Allergic asthma is a disease characterized by reversible airflow obstruction, airway inflammation, and airway hyperresponsiveness.¹ A large body of evidence supports a key role for dendritic cells²⁻⁵ and lymphocytes and T_H2 cytokines⁶⁻⁸ in the pathogenesis of allergic asthma. Central to the role of the dendritic cell and lymphocyte is the ability to colocalize, which is likely regulated by coordinated expression of chemokines and adhesion molecules. Immature dendritic cells migrate not only to liver- and activation-related chemokine/CCL20 but also to macrophage inflammatory protein 1 α /CCL3 and RANTES/CCL5,⁹ mediated by chemokine receptors, including CCR1, CCR2, CCR5, and CCR6.^{10,11} However, on exposure to maturational stimuli, including LPS, TNF- α , and CD40 ligand, dendritic cells lose responsiveness to these chemokines¹⁰ and, by emergence of CCR7 receptors, emigrate to secondary lymphoid chemokine (SLC/CCL21) and EBV-induced lymphoid chemokine (ELC/CCL19),^{9,12} which are highly expressed in the T-cell zones of draining lymph nodes. Acquisition of CCR7 expression by mature dendritic cells and central memory T cells results in development of responsiveness to chemokines, which direct these cells away from the periphery for interaction in T-cell zones of lymph nodes.^{13,14}

Paucity of lymph node T cell (*plt*) mice are null mutants for the lymphoid (serine) forms of SLC/CCL21^{15,16} and ELC/CCL19-atg,^{17,18} but the nonlymphoid (leucine) form of SLC/CCL21 is intact. There is decreased homing of dendritic cells and T lymphocytes to lymph nodes in *plt* mice.^{16,19-21} Despite the preservation of SLC/CCL21-leucine on lymphatic vessels in nonlymphoid tissue,¹⁸ dendritic cell migration to draining lymph nodes after contact sensitization is markedly attenuated in *plt* mice.²¹ In addition, the reduced numbers of dendritic cells that do migrate to *plt* lymph nodes only reach the subcapsular area and do not migrate normally out into the T-cell zones.²¹

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Abbreviations used

APTI:	Airway pressure time index
BAL:	Bronchoalveolar lavage
ELC/CCL19:	EBV-induced lymphoid chemokine
HRP:	Horseradish peroxidase
I-A:	Major histocompatibility complex class II Ia antigen
MCP:	Monocyte chemoattractant protein
MDC/CCL22:	Monocyte-derived chemoattractant
OVA:	Ovalbumin
Penh:	Enhanced pause
<i>plt</i> :	Paucity of lymph node T cell
SLC/CCL21:	Secondary lymphoid chemokine
TLN:	Thoracic lymph node
WT:	Wild-type

The role of SLC/CCL21 or ELC/CCL19 in allergic airway responses is poorly understood. The purpose of the present study was to determine the effects of abrogation of lymphoid tissue CCR7 agonists in allergic asthma by using *plt* mice. We show that allergen-challenged *plt* mutant mice had similar development of airway hyperresponsiveness and, surprisingly, tremendous enhancement of airway inflammation.

METHODS

Animals

The *plt* mutation was back-crossed onto a BALB/c background for at least 10 generations, and all studies were performed in female mice. Wild-type (WT) control mice were obtained from Jackson Laboratory (Bar Harbor, Maine). All experiments and protocols were approved by the Animal Care and Use Committee at the University of Virginia and conducted according to "The guide for the care and use of laboratory animals."²² Mice were deeply anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (8 mg/kg) before euthanasia.

Immunization protocols

Plt and WT mice were immunized and challenged, as previously described,²³ by means of intraperitoneal injection of 25 μ g of ovalbumin (OVA; Fraction V; Sigma Chemical Company, St Louis, Mo) with 2 mg of alum on days 0 and 7, followed by aeroallergen challenge by means of nebulization of 1% OVA in saline every other day from days 14 through 28 (*plt*-OVA/OVA or +/+OVA/OVA groups). Control *plt* and +/+ mice were treated with intraperitoneal saline with 2 mg of alum on days 0 and 7, followed by nebulization of saline every other day on days 14 to 28 (*plt*-SAL/SAL or +/+SAL/SAL groups). All mice were studied within 24 hours of the last challenge. Additional +/+ and *plt* control groups were primed with intraperitoneal OVA with alum but then treated with nebulized saline from days 14 through 28 (+/+OVA/SAL or *plt*-OVA/SAL groups, respectively).

Airway hyperresponsiveness

Airway hyperreactivity was evaluated by assessment of the airway pressure time index (APTI).²⁴ For measurement of APTI, animals were ventilated through a 19-gauge metal tracheal cannula with a tidal volume of 0.2 mL, a rate of 120 breaths/min, and a positive end-expiratory pressure of 2 to 3 cm H₂O. After administration of

0.5 mg of intraperitoneal decamethonium bromide, baseline airway pressure was measured for 5 minutes before and after intravenous injection of 100 μ g/kg methacholine through an inferior vena cava catheter, with calculation of APTI as previously described.²⁴

Data analysis

APTI was analyzed by using the SAS GLM procedure (SAS Institute, Cary, NC).²⁵ All other data were analyzed by using the Kruskal-Wallis test. When a significant treatment effect was identified for a variable at a *P* value of less than .05, the SAS GLM procedure was performed on the rank order of each variable. Specific differences between groups were determined by using *t* tests corrected by the false discovery rate procedure.²⁶ In the text, tables, and figures data are represented as the mean $\pm$ SEM.

Additional methods

We have previously reported methods for measurement of enhanced pause (Penh), bronchoalveolar lavage (BAL), lung morphometry, ribonuclease protection assay,²³ RT-PCR,²⁷ flow cytometry,²⁷ lymphocyte antigen stimulation,²³ and ELISAs for measurement of IgE and IgG1.²⁸ Further details on these methods and methods for intracellular cytokine staining are outlined in the "Methods" section in the Online Repository at www.jacionline.org.

RESULTS

APTI response to intravenous methacholine increased significantly (*P* < .05) in both *plt*-OVA/OVA (1179 $\pm$ 196 cm H₂O sec, *n* = 8) and +/+OVA/OVA mice (1027 $\pm$ 158 cm H₂O sec, *n* = 12) compared with that seen in their respective *plt*-SAL/SAL (482 $\pm$ 86 cm H₂O sec, *n* = 8) and +/+SAL/SAL (577 $\pm$ 55 cm H₂O sec, *n* = 15) control animals. However, APTI responses between *plt*-OVA/OVA mice and +/+OVA/OVA mice were comparable. In contrast, the Penh responses to inhaled methacholine not only increased in both *plt*-OVA/OVA and +/+OVA/OVA mice but were also significantly enhanced in *plt*-OVA/OVA mice over +/+OVA/OVA mice (see Table E1 in the Online Repository at www.jacionline.org).

BAL eosinophils, lymphocytes, and neutrophils and the total number of BAL leukocytes were increased in +/+OVA/OVA mice compared with +/+SAL/SAL control animals (Fig 1, A). However, there was marked enhancement in airway inflammation in *plt*-OVA/OVA mice, with significant increases in BAL eosinophil, lymphocyte, neutrophil, and total leukocyte numbers over those seen in +/+OVA/OVA mice. Analysis of BAL lymphocyte subsets by means of flow cytometry revealed that the number of CD4, CD8, and B cells increased in +/+OVA/OVA mice and in *plt*-OVA/OVA mutants compared with that seen in their respective saline control animals (Fig 1, B). Moreover, the numbers of BAL CD4 and B cells were significantly greater in *plt*-OVA/OVA mutants compared with those in +/+OVA/OVA mice.

The primary lung histologic change in +/+OVA/OVA mice consisted of significant inflammatory cuffing around the pulmonary arterioles (Fig 2, A and B). However, after the administration of identical OVA priming and challenge, *plt* mice had significantly greater periarteriol

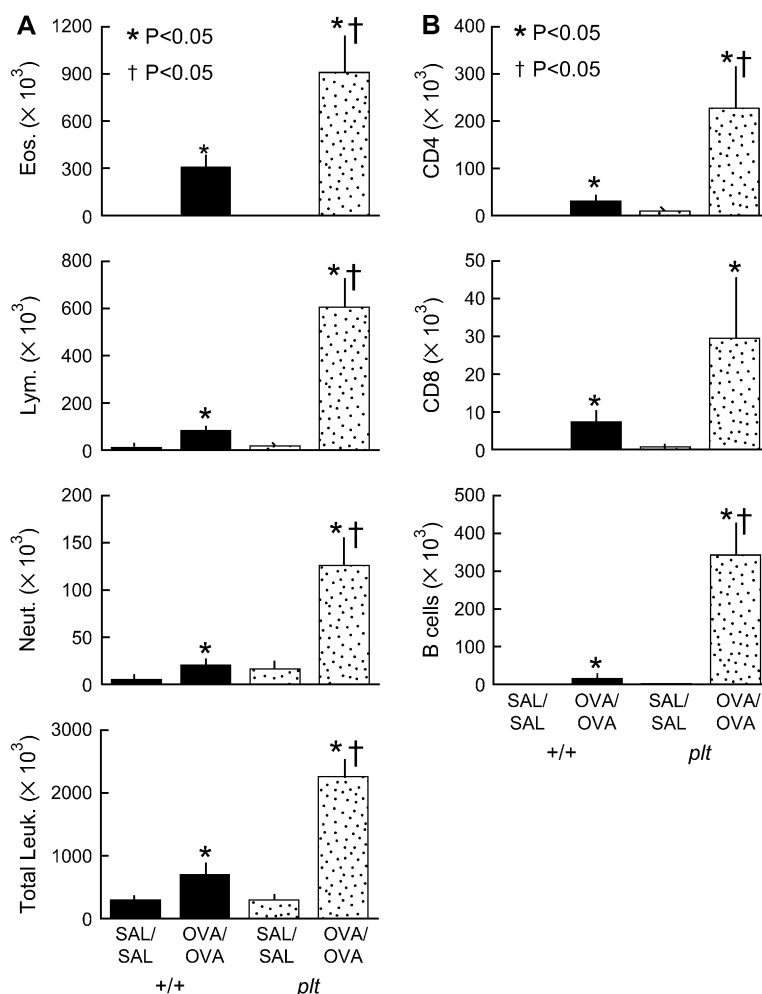


FIG 1. Enhanced BAL leukocytosis in *plt*-OVA/OVA mice. **A**, BAL leukocyte numbers in +/+SAL/SAL ($n = 24$), +/+OVA/OVA ($n = 28$), *plt*-SAL/SAL ($n = 10$), and *plt*-OVA/OVA ($n = 12$) mice. **B**, BAL lymphocyte subsets, as determined by using flow cytometry, in +/+SAL/SAL ($n = 3$), +/+OVA/OVA ($n = 3$), *plt*-SAL/SAL ($n = 3$), and *plt*-OVA/OVA ($n = 2$) mice. *Significantly different from respective genotype saline control: $P < .05$. †Significantly different compared with comparably treated +/+ group: $P < .05$. *Eos.*, Eosinophils; *Lym.*, lymphocytes; *Neut.*, neutrophils; *Leuk.*, leukocytes.

inflammatory cuffing compared with that seen in +/+OVA/OVA mice. This difference was unrelated to differences in the diameter of the pulmonary arterioles evaluated, which were comparable in *plt*-OVA/OVA, *plt*-SAL/SAL, +/+OVA/OVA, and +/+SAL/SAL mice at 76 ± 10 , 73 ± 12 , 67 ± 5 , and 75 ± 13 μm , respectively. Despite enhanced periarteriolar inflammation, the degree of goblet cell hyperplasia with OVA challenge in +/+ mice and *plt*-OVA/OVA mutants was comparable (Fig 2, C).

Separate groups of OVA-alum-primed mice were treated with nebulized saline rather than allergen to determine whether the airway inflammatory responses were a generalized systemic response to peritoneal allergen priming. Compared with +/+SAL/SAL and *plt*-SAL/SAL mice, APTI, total and leukocyte subset numbers, periarteriolar inflammatory cell cuffing, and numbers of periodic acid-Schiff-positive bronchial mucosal cells were unchanged in +/+OVA/SAL and *plt*-OVA/SAL mice (data not shown).

Measurement of lung cytokine and chemokine protein concentrations by means of ELISA revealed that both lung IL-4 and monocyte-derived chemoattractant (MDC/CCL22) levels increased significantly in +/+OVA/OVA and *plt*-OVA/OVA mice compared with those seen in their respective saline control animals. However, lung levels of both IL-4 and MDC/CCL22 in the *plt*-OVA/OVA mice were significantly greater ($P < .05$) than in +/+OVA/OVA mice (see Table E2 in the Online Repository at www.jacionline.org). Lung monocyte chemoattractant protein (MCP)-1/CCL2, IL-5, IFN- γ , and SLC/CCL21 levels were unchanged, but thoracic lymph node (TLN) SLC/CCL21 levels were markedly decreased in the *plt* mutants (see Table E2 in the Online Repository).

Lung mRNA expression of SLC/CCL21 (Fig 3, A and C) was reduced in *plt*-OVA/OVA mutants. Expression of ELC/CCL19 was undetectable in the lungs of both +/+ and *plt* mice. However, there was an increase in lung

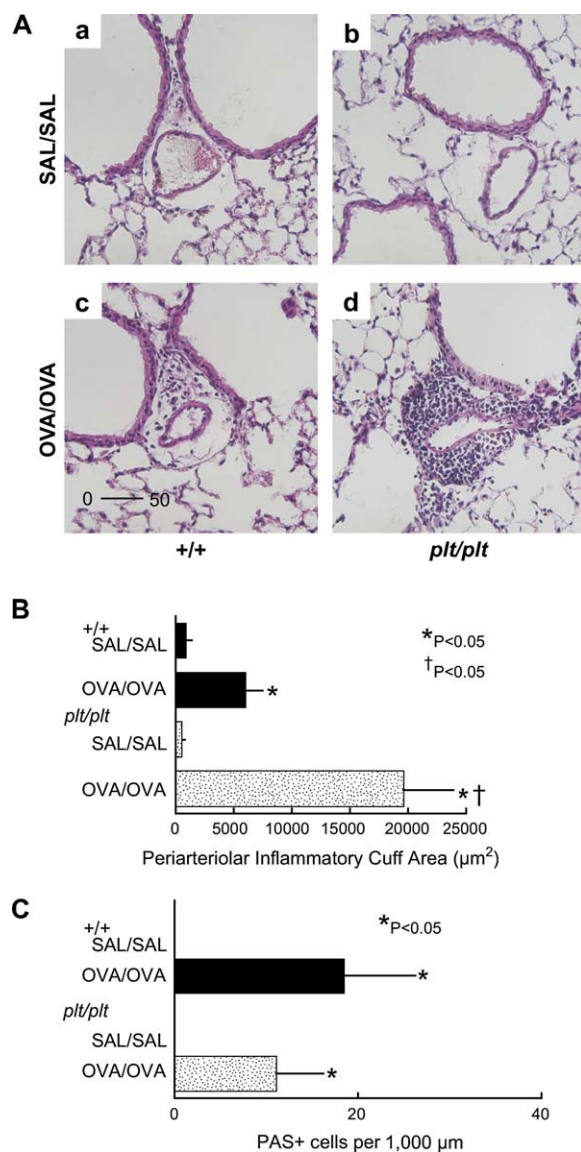


FIG 2. Vascular cuffing in *plt*-OVA/OVA mice. **A**, Magnification $\times 400$, hematoxylin and eosin. Bar = 50 μ m. **B**, Vascular cuffing in *+/+* SAL/SAL (n = 9), *+/+* OVA/OVA (n = 12), *plt*-SAL/SAL (n = 5), and *plt*-OVA/OVA (n = 5) mice. **C**, Goblet cell hyperplasia in *+/+* SAL/SAL (n = 6) and *+/+* OVA/OVA (n = 6), *plt*-SAL/SAL (n = 4), and *plt*-OVA/OVA (n = 5) mice. *Significantly different from respective genotype saline control (* $P < .05$) or comparably treated *+/+* group († $P < .05$).

expression of MCP-2/CCL8 in *plt* mutants over *+/+* mice. Lung expression of other chemokines appeared to be comparable in *+/+* and *plt* mice. Increased lung expression of MCP-2/CCL8 in *plt*-OVA/OVA mice was confirmed by means of RT-PCR, with an increase ($P < .05$) in the percentage relative expression over β -actin of $5.5\% \pm 2.5\%$ in *plt*-OVA/OVA mice (n = 3) compared with $0.1\% \pm 0.02\%$ in *plt*-SAL/SAL mice (n = 3). In contrast, MCP-2/CCL8 expression was unchanged at $1.1\% \pm 1.0$ and $1.9\% \pm 0.4\%$ in *+/+* SAL/SAL (n = 3) and *+/+* OVA/OVA (n = 3) mice, respectively. Lung MCP-1/

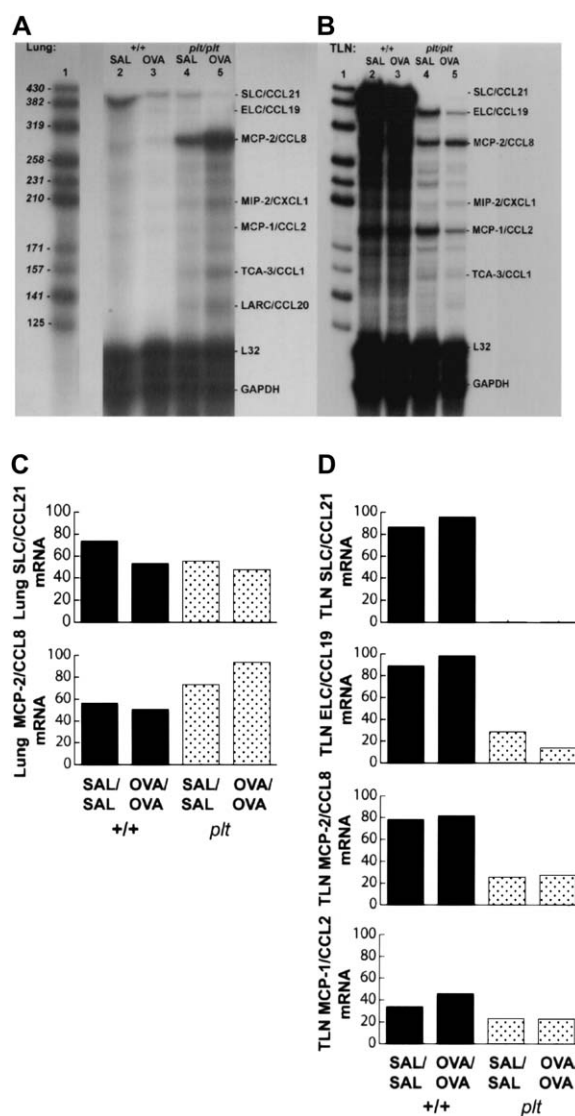


FIG 3. Lung and TLN chemokine expression. Lung (**A**) and TLN (**B**) chemokine expression by ribonuclease protection assay in *+/+* SAL/SAL (lane 2), *+/+* OVA/OVA (lane 3), *plt*-SAL/SAL (lane 4), and *plt*-OVA/OVA (lane 5) mice compared with size markers (lane 1). **C** and **D**, Densitometry of selected hybridized bands as a percentage of L32. TCA-3, T-cell activation protein 3; LARC, liver and activation-related chemokine; L32, ribosomal protein L32; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

CCL2 and SLC/CCL21 levels, as determined by means of RT-PCR, were unchanged with OVA challenge in *+/+* and *plt* mice (data not shown). Although TLN SLC/CCL21 was strongly expressed in *+/+* mice, it was undetectable in the *plt* mutants (Fig 3, B and D). TLN ELC/CCL19 expression was evident in *plt* mutants, but it was substantially decreased compared with that seen in *+/+* mice. In addition, there was decreased TLN expression of MCP-2/CCL8 and MCP-1/CCL2 in the *plt* mice.

The total numbers of leukocytes harvested by means of positive selection from pooled lungs from 4 mice in each

TABLE I. Lung dendritic cell and T-cell subsets ($\times 10^3$ /mouse)

	+/+SAL/SAL	+/+OVA/OVA	<i>plt</i>-SAL/SAL	<i>plt</i>-OVA/OVA
I-A ⁺ CD11c ⁺	2640	5990	4190	6100
[no. CCR7 ⁺]	[1340]	[2100]	[1600]	[1806]
CD11c ⁺ CD103 ⁺	459	391	635	341
[no. CCR7 ⁺]	[28]	[59]	[0]	[17]
I-A ⁺ PDCA ⁺ B220 ⁺	183	182	550	182
[no. CCR7 ⁺]	[96]*	[46]*	[263]*	[104]*
F4/80 ⁺ I-A ⁻	149	6180	335	2137
[no. CCR7 ⁺]	[17]	[130]	[61]	[75]
F4/80 ⁺ I-A ^{moderate}	2297	3190	4220	4330
[no. CCR7 ⁺]	[489]	[918]	[1402]	[992]
CD4 [no. CCR7 ⁺]	4530 $\pm$ 2540 (2) [960]	6184 $\pm$ 2825 (2) [1150]	10,615 $\pm$ 5785 (2) [10,400]	11,365 $\pm$ 3635 (2) [6150]
CD8 [no. CCR7 ⁺]	2317 $\pm$ 877 (2) [240]	3315 $\pm$ 545 (2) [214]	4740 $\pm$ 2030 (2) [1717]	5170 $\pm$ 500 (2) [1157]

Numbers in parentheses indicate number of observations, and numbers in brackets indicate number of cells expressing CCR7, as determined by means of flow cytometry.

*Number of I-A⁺PDCA⁺CCR7⁺ cells.

group by using anti-CD11c anti-murine plasmacytoid dendritic cell antigen (PDCA)-1 magnetic beads were 37×10^6 and 85×10^6 cells per 4 mice in +/+SAL/SAL and +/+OVA/OVA mice, respectively, and 66×10^6 and 77×10^6 cells per 4 mice in *plt*-SAL/SAL and *plt*-OVA/OVA mice, respectively. The number of CD11c⁺ (major histocompatibility complex class II Ia antigen positive [I-A⁺]) cells in *plt*-SAL/SAL mice were higher than the number observed in +/+SAL/SAL mice but were similar in *plt*-OVA/OVA and +/+OVA/OVA mice (Table I). The novel lung epithelial dendritic cell subset (CD11c⁺CD103⁺)²⁷ appeared to decrease in number with OVA challenge in both +/+ and *plt* mice (Table I). The numbers of lung plasmacytoid dendritic cells (I-A⁺PDCA⁺B220⁺) were comparable between OVA-challenged and saline control +/+ mice but were increased in *plt*-SAL/SAL mice. In regard to CCR7 expression by lung leukocyte population, the number (Table I, brackets) of total lung dendritic cells (I-A⁺CD11c⁺) expressing CCR7 appeared to increase comparably with OVA challenge in both genotypes. However, there was a decrease in both the fraction (Fig 4, B) and number of CD11c⁺CD103⁺ CCR7⁺ epithelial dendritic cells in the lungs of *plt* mutants, regardless of treatment. There was a higher fraction (Fig 4, C) and number (Table I) of lung plasmacytoid dendritic cells expressing CCR7 in *plt*-SAL/SAL mutants, and both fraction and number of lung plasmacytoid dendritic cells decreased with OVA challenge in both +/+ and *plt* mice. A small fraction of eosinophils (F4/80⁺I-A⁻) expressed CCR7 (Table I). There was substantial CCR7 expression in the lung macrophage (F4/80⁺I-A^{moderate}) population (Table I), with higher numbers of CCR7⁺ macrophages in *plt*-SAL/SAL mutants compared with in +/+SAL/SAL mice. There appeared to be both a substantial increase in the number of CD4 and CD8 cells (Table I) and fraction of CD4 and CD8 cells expressing CCR7 (Fig 4, D and E and Table I) in *plt* mutants compared with in +/+ mice.

In the draining TLNs the numbers of total and CCR7⁺ myeloid (I-A⁺CD11b⁺), epithelial (CD11c⁺CD103⁺), and plasmacytoid (I-A⁺PDCA⁺B220⁺) dendritic cells and CD4 and CD8 cells appeared to be greater in *plt*-OVA/OVA mice than in all other groups (see Table E3 in the Online Repository at www.jacionline.org).

In vitro antigen stimulation of TLN lymphocyte cocultures revealed that OVA-stimulated IL-4, IL-5, and IFN- γ release was greater in *plt*-OVA/OVA mutants compared with that seen in +/+OVA/OVA mice (Fig 5). On the basis of intracellular cytokine staining, there were increased numbers of CD4 cells producing IL-4, IL-5, and IFN- γ in TLN cells from *plt*-OVA/OVA mice (see Table E4 in the Online Repository at www.jacionline.org).

Plasma OVA-specific IgE levels increased significantly ($P < .05$), from 168 ± 63 units in +/+SAL/SAL mice ($n = 17$) to 256 ± 50 units in OVA-challenged +/+ mice ($n = 19$). However, OVA-specific IgE levels in *plt*-OVA/OVA mice ($n = 12$) of 505 ± 231 units versus 61 ± 21 units in *plt*-SAL/SAL mice ($n = 8$) did not achieve statistical significance. Plasma IgG1 levels increased ($P < .05$) in +/+OVA/OVA and *plt*-OVA/OVA mice to 304 ± 50 units ($n = 6$) and 279 ± 61 units ($n = 5$) compared with levels in their respective +/+SAL/SAL (0.1 ± 0.1 units, $n = 6$) and *plt*-SAL/SAL (0.2 ± 0.1 units, $n = 5$) control animals. However, plasma IgG1 levels in +/+OVA/OVA and *plt*-OVA/OVA mice were comparable.

DISCUSSION

Induction of allergic asthma in *plt* mutant mice led to enhanced airway and lung perivascular inflammation, but the increase in airway hyperreactivity, as determined on the basis of APTI, in OVA-challenged *plt* mice was not enhanced to greater than that seen in allergen-challenged WT mice. Compared with +/+OVA mice, the

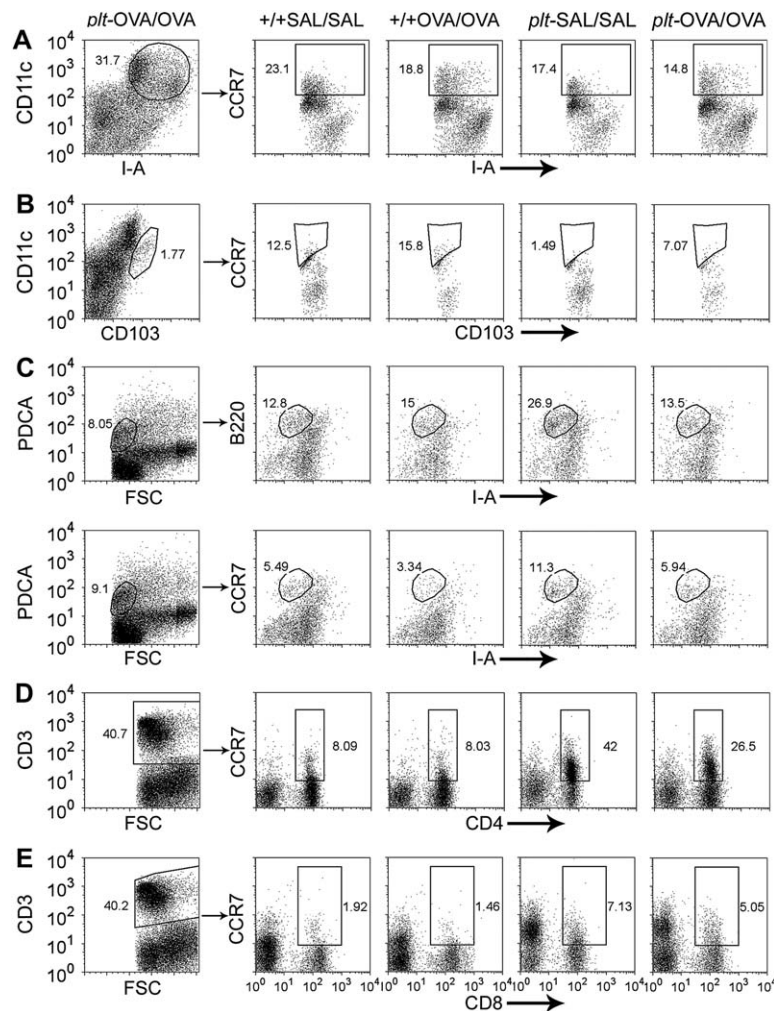


FIG 4. CCR7 expression in lung dendritic cells and CD3 cells. Flow cytometry depicting CCR7 expression in the lung: **A**, total dendritic cells (I-A⁺ CD11c⁺); **B**, epithelial dendritic cells (CD11c⁺ CD103⁺); **C**, plasmacytoid dendritic cells (I-A⁺ PDCA⁺ B220⁺); **D**, CD4 cells; **E**, CD8 cells.

heightened inflammatory responses in the *plt*-OVA mutants were accompanied by increased lung protein levels of the T_H2 cytokines IL-4 and MDC/CCL22, increased lung mRNA expression of MCP-2/CCL8, and diminished TLN mRNA expression of MCP-1/CCL2, MCP-2/CCL8, ELC/CCL19, and SLC/CCL21 in *plt*-OVA mice. A T_H2 phenotype was present in TLN lymphocytes from OVA-sensitized *plt* mice, with increased production of IL-4 and IL-5 in response to antigen stimulation.

A number of explanations are possible for enhanced airway inflammatory responses in the *plt*-OVA/OVA mice. First, it is likely that enhanced T_H2 cytokines contributed to an increase in airway inflammation.⁶⁻⁸

Second, upregulation of lung MCP-2/CCL8 mRNA needs to be considered in view of its chemotactic activity for eosinophils,²⁹⁻³¹ monocytes,^{32,33} and T cells, including CD4 and CD8 cells.^{30,34} In addition, MCP-2/CCL8 might induce leukotriene C₄ release from IL-3-pretreated basophils.³⁰

Third, mature dendritic cells express MDC/CCL22, which might enable them to attract antigen-specific T cells for antigen presentation.³⁵ There is differential expression of the putative MDC/CCL22 receptor CCR4¹² on T_H1 and T_H2 cells, with enhanced CCR4 expression on T_H2 cells.³⁶ Significantly greater levels of MDC in the lungs of *plt*-OVA mice compared with those in OVA-challenged WT mice could have led to enhancement of antigen presentation to memory T cells or to greater emigration of T_H2 cells into the lungs. However, previous studies in animal models of allergic airways disease with attenuation of MDC or CCR4 are conflicting, either inhibiting³⁷ or having no effect^{38,39} on allergic airway responses.

Finally and most important, it is possible that airway responses in the *plt* mutants were heightened because of diminished egress of CCR7-expressing dendritic cells and T cells from the airways of the *plt* mice, with the consequence of *in situ* amplification of airway inflammation. This possibility is supported by the finding of an increased

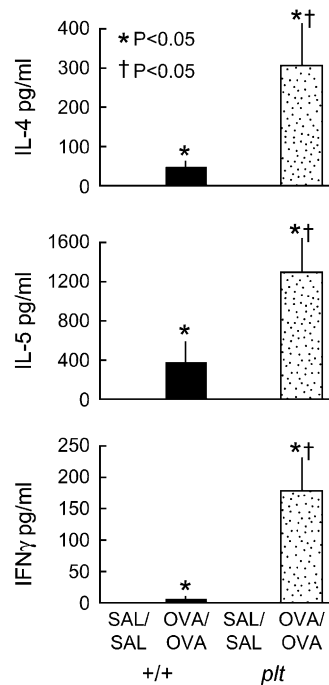


FIG 5. Lymphocyte antigen stimulation of TLN cells. Change in cytokine production in lymphocyte and irradiated splenocyte cocultures treated with 250 μ g/mL OVA versus 0 μ g/mL OVA in +/+SAL/SAL ($n = 3$), +/+OVA/OVA ($n = 7$), *plt*-SAL/SAL ($n = 3$), and *plt*-OVA/OVA ($n = 6$) mice. *Significantly different from respective genotype saline control (* $P < .05$) or comparably treated +/+ group († $P < .05$).

number of CCR7-expressing CD4 and CD8 cells in the lungs of OVA-challenged *plt* mice. Surprisingly, there were enhanced numbers of all dendritic cell subsets and CD4 and CD8 cells in the TLNs of OVA-challenged *plt* mice, suggesting that alternate mechanisms in addition to CCR7 direct these cells to draining lymphoid tissue. Nevertheless, the most striking abnormality, retention of CCR7-positive CD4 and CD8 cells in the lungs of *plt* mutants, supports the notion that inflammatory responses were enhanced because of impairment of egress of the cells from the lung.

Altered kinetics of lymphocyte and dendritic cell migration to the draining lymph nodes and spleen in *plt* mutants is known.¹⁶ T- and B-cell accumulation in the draining lymph nodes of *plt* mice 4 days after subcutaneous immunization was absent compared with that seen in WT mice but increased progressively by day 20. At 20 days after sensitization, although the number of CD8 cells reached the level in WT mice, the number of CD4 cells, B cells, and dendritic cells in draining lymph nodes never reached the numbers in intact mice.

Although we observed an increase in lung IL-4 levels in *plt*-OVA/OVA mice, there was no evidence of altered Ig isotype switching compared with that seen in +/+ mice because there was a comparable increase in IgG1 levels between *plt* and +/+ mice challenged with OVA. Impaired humeral responses in CCR7^{-/-} mutant mice⁴⁰ suggests that CCR7 is important in B-cell help in lymph

nodes. The reason that humeral responses were intact in the *plt* mutant is unclear.

In summary, the role of SLC/CCL21 and ELC/CCL19 in allergic asthma is incompletely understood. In a murine model of allergic asthma, there was profound enhancement in airway inflammation and T_H2 activity in *plt* mice, null mutants for CCR7-agonists CCL21-serine and CCL19. Further studies need to be performed to identify specific mechanisms for amplification of airway inflammatory responses in the *plt* mutants. These observations underscore the importance of leukocyte trafficking in the occurrence and severity of allergic asthma.

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