

Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model

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Background: Dendritic cells are the most powerful of the antigen-presenting cells and are known to play important roles in sensitization and inflammation in allergen-specific asthma. Various cytokines and chemokines are involved in the maturation and activation of dendritic cells. Among them is CC chemokine ligand (CCL)21, a key chemokine in the entry of naive T cells and antigen-stimulated dendritic cells into the T-cell zones of secondary lymphoid organs, which is a critical process in antigen-specific T-cell activation.

Objective: We studied the role of CCL21 in airway inflammation in asthma by using BALB/c-plt/plt (plt) mice, which possess genetic defects in expression of both CCL21 and CCL19.

Methods: Plt and control BALB/c mice were immunized with ovalbumin and alum 4 times and thereafter were subjected to a 2-week regimen of ovalbumin inhalation.

Results: In plt mice, ovalbumin-specific IgE response was delayed compared with control BALB/c mice, but they had the same level of response after final immunization. Although airway inflammation and response to acetylcholine were significantly reduced compared with BALB/c mice, significant eosinophilic inflammation and hyperresponsiveness were also observed in plt mice after 2 weeks of inhalation. Four weeks after cessation of inhalation, airway inflammation and hyperresponsiveness in plt mice were greater than in BALB/c mice. At the time of resolution of airway inflammation, IL-10 production was enhanced in BALB/c mice but not in plt mice.

Conclusion: The chemokines CCL21 and CCL19 were critical for resolution of airway inflammation.

Clinical implications: The findings about the chemokines for induction and resolution of inflammation are key to establishing a new strategy for asthma immunotherapy. (J Allergy Clin Immunol 2006;117:1040-6.)

Key words: Asthma, airway hyperresponsiveness, CCL21, dendritic cells, airway inflammation

In allergic asthma, immune response to inhaled antigen is skewed to the T_H2-type response.¹⁻⁷ For the recognition of antigen, dendritic cells (DCs) work as professional antigen-presenting cells (APCs). Administration of antigen pulsed DCs induces allergic inflammation.^{8,9} In addition, DC maturation is accelerated in the asthmatic airway.¹⁰ Immature DCs possess the ability to take up and process antigens but have low antigen-presenting ability.¹¹ After taking up antigen, DCs become mature, expressing CD86, CD80, CD40, and CC chemokine receptor (CCR).^{12,13} Mature DCs migrate to lymph nodes through the interaction of CCR7 and CC chemokine ligand (CCL)21 (secondary lymphoid tissue chemokine), where they effectively present antigen to naive T cells. CCL21 has potent activity in attracting naive and mature T cells, B cells, and DCs.^{14,15} CCL21 was expressed in high endothelial venules in the peribronchial area and perivascular lymphatics in the lung. Another ligand of CCR7 is CCL19 (Epstein-Barr ligand chemokine), which also promotes efficient interaction of T cells and DCs at the site of lymph nodes. The process of antigen presentation has been clearly shown at the skin.⁹ Antigen that penetrates the skin is taken up by DCs. Subsequently, these professional APCs enter afferent lymph vessels, a process promoted by CCL21. Antigen-laden APCs then induce the activation, expansion, and differentiation of antigen-specific T cells. These processes are efficiently performed only at skin-draining lymph nodes. Although CCL21 is also expressed in peribronchial areas and perivascular lymphatics in the lung,¹⁶ the role of CCL21 and CCL19 in the asthmatic airway has not been clarified.

CCL21 is encoded by 2 genes, SCYA21a and SCYA21b, which encode CCL21-ser and CCL21-leu

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Abbreviations used

APC: Antigen-presenting cell
BALF: Bronchoalveolar lavage fluid
CCL: CC chemokine ligand
CCR: CC chemokine receptor
DC: Dendritic cell
PAS: Periodic acid-Schiff
Penh: Enhanced pause
plt: BALB/c-plt/plt

protein, respectively. CCL21-leu (secondary lymphoid tissue) is absent in mice with a paucity of lymph node T cells (BALB/c-plt/plt [plt] mice), which possess a defect in homing of naive T cells in lymph nodes, resulting in impaired secondary lymphoid organs.¹⁷⁻¹⁹ Plt mice are also deficient in CCL19.¹⁸ To test the role of CCL21 and CCL19 in antigen-specific asthma, we investigated the ability of plt mice to exhibit allergen-specific airway inflammation. We found that airway inflammation and airway response to acetylcholine were significantly reduced in plt mice compared with BALB/c mice. However, significant eosinophilic inflammation and hyperresponsiveness were also observed in plt mice after 2 weeks of inhalation. Interestingly, after cessation of inhalation for 4 weeks, airway inflammation and airway hyperresponsiveness of plt mice were greater than those of BALB/c, suggesting that in the resolution phase of airway inflammation, CCL21 and CCL19 play a critical role.

METHODS

Sensitization

BALB/c mice were purchased from Charles River Japan (Kanagawa, Japan). Plt mice were bred in the animal facilities of Musashino and Teikyo University School of Medicine under Specific Pathogen-Free conditions. Care and use of the animals followed the guidelines of the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The mice were initially immunized intraperitoneally 4 times with 10 μ g ovalbumin + 2 mg aluminum hydroxide gel (on days 0, 28, 35, and 49) as previously reported with slight modifications.²⁰ After immunization, animals were subjected to inhalation of ovalbumin 3 mL/min every other day for 2 weeks (day 50 to day 63 from the first immunization) using a micromist nebulizer (DeVilbiss, Somerset, Pa). Mean particle diameter was 3.0 μ m. In some mice, recombinant macrophage inflammatory protein 3 β /CCL19 (Epstein-Barr ligand chemokine; 5 μ g/mouse), purchased from R&D Systems (Minneapolis, Minn), was administered by using an osmotic pump (Alzet Minipump; Durect Corp, Cupertino, Calif), which was implanted subcutaneously in the anterior back region. Four to 5 mice were used per treatment group in each experiment. Untreated mice were also used as controls.

Assessment of airway responsiveness

The assessment of airway responsiveness was undertaken 24 hours after the last ovalbumin inhalation, as previously reported.²⁰ We used barometric whole-body plethysmography (Buxco Electronics, Inc, Troy, NY) as described by Hamelmann et al.²¹ Data are expressed as enhanced pause (Penh). Airway responsiveness was determined by calculating the ratio of the Penh to control Penh (%Penh). In some mice, invasive measurements were undertaken as previously

reported.²² Briefly, the anesthetized mice were tracheostomized and injected with pancuronium bromide. The animals were connected to a Harvard ventilator with 0.3 mL tidal volume and a respiratory frequency of 120/min. Next, they were placed in the whole-body plethysmograph (Buxco Electronics) to measure airway resistance.

Measurements of serum ovalbumin-specific IgE and IgG_{2a}

To measure ovalbumin-specific IgE, we coated the plate with ovalbumin using the AlaSTAT system (Diagnostics Products, Los Angeles, Calif) with some modification as previously reported.²⁰ After incubation with samples diluted 10-fold, horseradish peroxidase-labeled rat antimurine IgE specific antibody, purchased from Morinaga Co, Ltd (Kanagawa, Japan), was added to the plate as the second antibody. Color was developed and measured at 450 OD.

For ovalbumin-specific IgG_{2a}, plates were coated with 50 μ g/mL ovalbumin in 50 mmol/L carbonate buffer, pH 9.0. After blocking with SuperBlock (Pierce, Rockford, Ind), samples diluted 2000-fold were added in the plates. Antibodies were detected by peroxidase-labeled antimouse IgG_{2a} (1:500; Invitrogen, Carlsbad, Calif). The color was developed with TMB+ (Dako Corp, Glodtrup, Denmark) and stopped by 1N H₂SO₄. The antibody titers were calculated by comparison with internal standards.

Bronchoalveolar lavage fluid cell analysis and histologic examination

After measurement of pulmonary function, each mouse was exsanguinated. Bronchoalveolar lavage fluid (BALF) was obtained from selected mice by incubating and washing the lung with 1 mL saline until the recovered volume was 5 mL. BALF was centrifuged at 1500 rpm for 10 minutes at 4°C. Pellets were dissolved in 1 mL PBS, and the number of the cells was counted. Cytospin specimen was obtained by rotating at 640 rpm for 2 minutes. Then, the cells were stained with Diff Quik (International Reagents Corp, Osaka, Japan), and the cell fractions were examined on microscope.

The lungs were fully inflated using 10 cm H₂O pressure and fixed with 10% formaldehyde for hematoxylin-eosin and periodic acid-Schiff (PAS) staining.

RNA extraction and quantification of mRNAs

Lungs were frozen in liquid nitrogen immediately after isolation and were used for RNA extraction. Lung tissue was homogenized at 4°C, and total RNA was extracted by using ISOGEN, a modified acid guanidium-phenol-chloroform method (Nippon Gene Co, Ltd, Tokyo, Japan). RNA was treated with 10U DNase (Qiagen, Hilden, Germany) following the manufacturer's instructions. The purity of the RNA was established by spectrophotometer using a DNA purity calculating program (Hitachi Seisakusho, Tokyo, Japan). The absorbance OD 260/280 ratio was 1.8. After the amount of total RNA was measured, cDNA synthesis was performed with 0.125 μ mol/L oligo-dT (Takara Biochemicals, Tokyo, Japan) as previously described.²⁰ The levels of mRNA were examined by real-time PCR using the Light Cycler-Fast Start DNA Master SYBR GreenI kit (Roche Diagnostics, Mannheim, Germany). In this system, double-stranded DNA is labeled with SYBR Green I and then detected. Quantification was performed on the basis of the standard curve obtained by using serial dilution of specific PCR products. Results are shown as ratios of the level of mRNAs standardized by the level of β -actin mRNA. The primers used were as follows: β -actin, 5'-CCTGTATGC-CTCTGGTCGTA-3' 5'-CCATCTCCTGCTCGAAGTCT-3' 260 bp; IL-13, 5'-GATATGAAGTTGAAGAGGAGAG-3' 5'-GGCAAA-GAAGTAACAAAAGG-3' 208 bp; IFN- γ , 5'-TCTTGGATAT-CTGGAGGAAC-3' 5'-GACCTCAAACCTGGCAATAC-3' 215 bp.

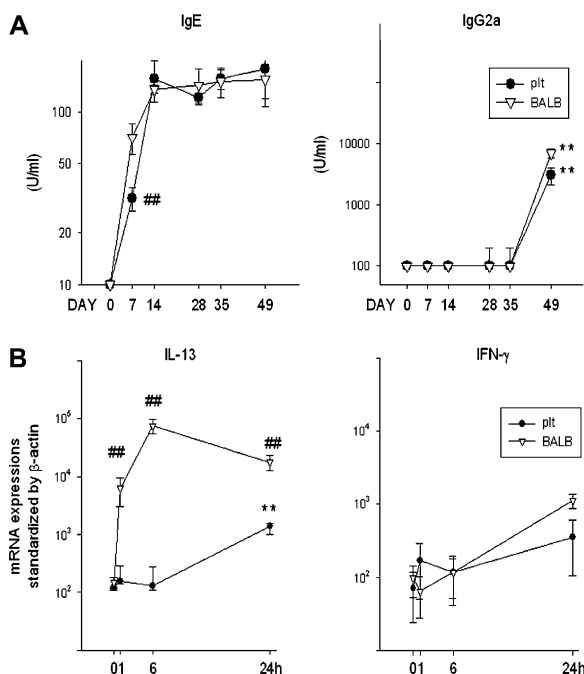


FIG 1. Time course of ovalbumin-specific IgE and IgG_{2a} response (A) and mRNA expressions of IL-13 and IFN- γ (B). Each bar represents the mean $\pm$ SEM of values obtained from 5 different mice. Five mice per group were examined. Similar results were obtained from 4 other experiments. ## P < .01 BALB/c vs plt. ** P < .01 indicated time vs baseline.

Quantification of IL-10 production

Lung cells were extracted from some of the mice in which airway responsiveness was measured and used for cell culture. Lungs were cut into small pieces and incubated at 37°C for 1 hour in 150 U/mL collagenase in RPMI. Then, lung pieces were homogenized in 1% penicillin/streptomycin (Gibco Invitrogen, Carlsbad, Calif)/RPMI 1640 using cell strainers with 70- μ m pores (BD Falcon, Franklin Lakes, NJ) and centrifuged at 1500 rpm for 10 minutes at 4°C. Cells were then washed twice, and CD3⁺ cells were separated by using the Dynabead mouse pan T-cell system following the manufacturer's instructions (DynaBiot ASA, Oslo, Norway). CD3⁺ cells with about 5% adherent cells were then suspended in 1% penicillin/streptomycin/10% FCS in RPMI 1640 and cultured with or without ovalbumin (100 μ g/mL) in 24-well plates (Corning, Inc, Corning, NY) at 1×10^6 cells/well at 37°C. After 2 days of culture, the supernatant was collected, and the levels of IL-10 were evaluated by the ELISA Quantising kit (R&D Systems).

Flow cytometry

Data were collected by using FACSaria (BD Biosciences, San Jose, Calif). A total of 1×10^6 cells from lung, spleen, blood, and lymph node were stained with indicated antibodies. Antibodies used were antimouse CD16/CD32 (Fc block), phycoerythrin-Cy7-conjugated rat antimouse CD25 antibody, CD4-Alexa 647 (BD Biosciences), and phycoerythrin-labeled CCL19-Fc chimera antibody (Santa Cruz Biotechnology Inc, Santa Cruz, Calif). Cells were first treated with antimouse CD16/CD32 (Fc block) for 10 minutes. Then staining antibodies were added for 30 minutes at 4°C. All plots show log10 fluorescence.

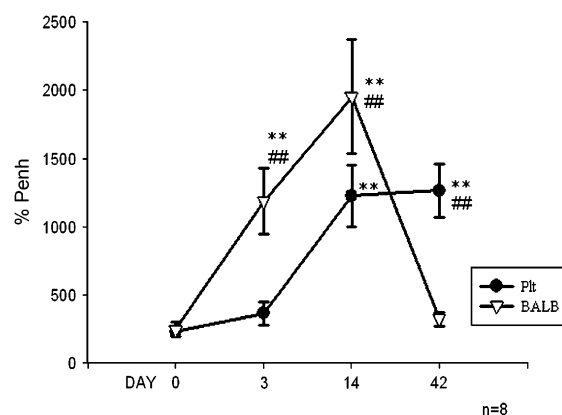


FIG 2. Time course of airway response to acetylcholine. Airway response to 100 mg/mL acetylcholine was expressed as % Penh. Each bar represents the mean $\pm$ SEM of values obtained from 6 different mice. Similar results were obtained from 3 other experiments. ** P < .01 indicated day vs day 0. ## P < .01 BALB/c vs plt mice.

Statistics

For comparisons of multiple parameters, we used ANOVA with Bonferroni correction. The level of statistical significance was set at P < .05. Data are expressed as means $\pm$ SEMs.

RESULTS

Ovalbumin-specific IgE and IgG_{2a} synthesis in plt mice

To elucidate the functional role of CCL21 and CCL19 in IgE synthesis, we determined the kinetics of IgE synthesis with immunization of ovalbumin and alum in plt mice and BALB/c mice. Examination of the ovalbumin-specific IgE level on day 7 after the first immunization revealed a significantly lower IgE level in plt mice compared with BALB/c mice (Fig 1, A; P < .01). On day 14, the difference was no longer evident, a tendency that was continued after the final immunization (Fig 1, A). These data suggested that induction of IgE was delayed but that the response was similar to the IgG response reported previously.²³ When we compared IgG_{2a} synthesis, there was significant synthesis on day 49 but no difference between plt and BALB/c mice (Fig 1, A).

Next, we examined IL-13 and IFN- γ mRNA expression of the lung after ovalbumin inhalation. One hour after ovalbumin inhalation, significant IL-13 mRNA was detected in BALB/c mice but not plt mice (Fig 1, B). After 24 hours, IL-13 mRNA expression was detected in plt mice but significantly lower compared with BALB/c mice (Fig 1, B). IFN- γ expression was not different between BALB/c and plt mice. These data suggested that plt mice expressed significant TH₂ response but lower compared with BALB/c mice.

Airway hyperresponsiveness and inflammation in plt mice

To determine airway response, we first measured airway resistance after 100 mg/mL acetylcholine

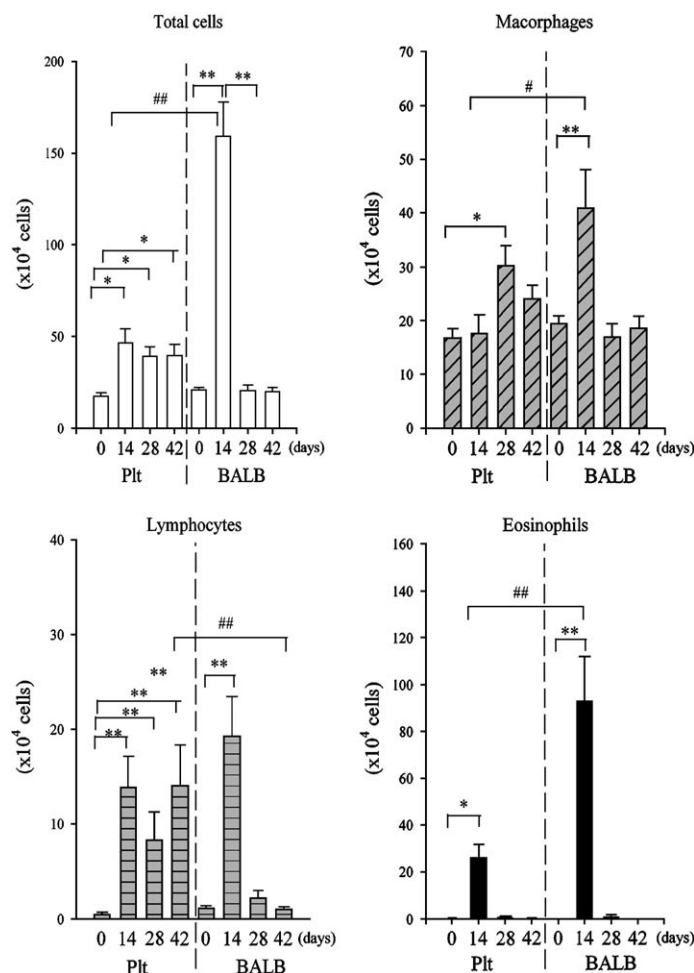


FIG 3. BALF cell analysis. Data shown are means $\pm$ SEMs of 8 mice per group. * $P < .05$; ** $P < .01$ indicated day vs day 0. # $P < .05$; ## $P < .01$ BALB/c vs plt mice.

inhalation (Fig 2). We followed a sensitization system as previously reported.²⁰ Mice were analyzed 24 hours after the last ovalbumin inhalation at the times indicated in Fig 2. Before ovalbumin inhalation, no increase in airway resistance was observed after acetylcholine inhalation compared with saline inhalation. On day 3, a significant increase in airway response was observed in BALB/c mice but not in plt mice. On day 14, a further increase in airway response was observed in BALB/c mice. On day 14, plt mice also exhibited a significant increase in airway response compared with untreated mice; however, the response was significantly lower than in BALB/c mice. On day 42 (4 weeks after cessation of ovalbumin inhalation on day 14), airway response was decreased in BALB/c mice but not in plt mice. To confirm the results, airway resistance after inhalation of 2.5 mg/mL acetylcholine was also examined by using an invasive measurement on day 42. Airway resistance of plt mice and BALB/c mice was 4.33 ± 0.25 versus 2.94 ± 0.33 cm H₂O/L/s, respectively ($n = 5$ each group; $P < .05$).

To determine airway inflammation, BALF cells were also analyzed in BALB/c mice (Fig 3). On day 14,

significant eosinophilia was observed in BALF. Increase in eosinophil and lymphocytes resolved on day 28 (2 weeks after cessation of exposure to ovalbumin). Although the level was lower, plt mice exhibited significant increase in the number of eosinophils on day 14 (Fig 3). In contrast to BALB/c, increases in lymphocytes remained present until day 42 (4 weeks after cessation of exposure to ovalbumin).

Histologic analysis revealed less airway inflammation at day 14 in plt mice than in BALB/c mice (Fig 4, A). PAS staining revealed that mucus-producing cells significantly appeared both in plt and BALB/c mice. However, the number of mucus-producing cells was small in plt mice as compared with BALB/c mice (Fig 4, B, on day 14). In BALB/c mice, airway inflammation resolved on day 28, but plt mice exhibited significant airway inflammation on day 28 (Fig 4, A). These data indicated that airway inflammation was delayed but that significant responses occurred in plt mice compared with BALB/c mice, as is the case with antibody response. It was also noted that resolution of airway inflammation and airway responsiveness was delayed in plt mice.

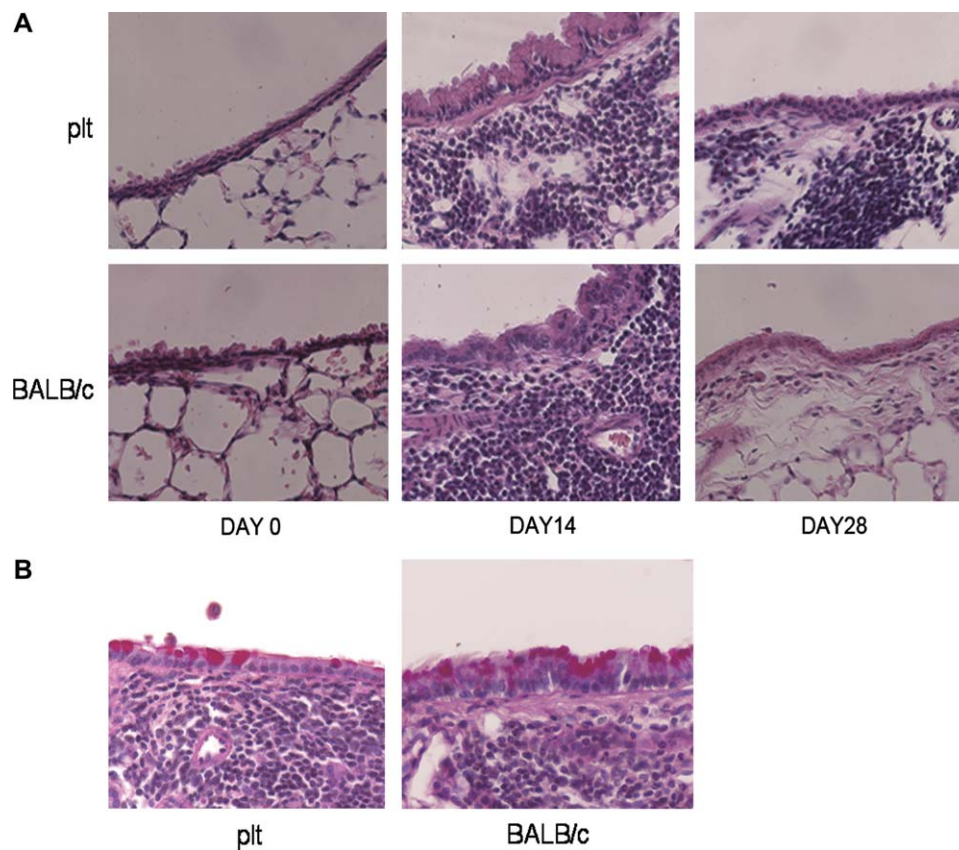


FIG 4. Histologic analysis was performed by hematoxylin-eosin staining (**A**) and PAS staining (**B**) in BALB/c and plt mice at the indicated time. Figures are representative data of histologic examination of 5 mice. Original magnification $\times 200$.

Analysis of CCR7 and CD25 expression

To clarify the mechanisms of delay in resolution of airway inflammation, we performed flow-cytometric analysis to assess CCR7, receptor for CCL19 and CCL21, on T cells, because it has been reported that CCR7 expression is critical for T-cell exit from peripheral tissues.^{24,25} CCR7⁺ cells were present in the CD4⁺ cells of lung after ovalbumin inhalation both in BALB/c (Fig 5, A) and plt mice (Fig 5, B). When we examined after cessation of ovalbumin inhalation in plt mice, there were CD4⁺CCR7⁺ cells, but the majority were CD4⁺CCR7⁻ cells (Fig 5, B).

Regulatory T cells, which are known to suppress airway inflammation, express CCR7 and are activated by pulmonary DCs at the secondary lymph nodes.^{26,27} Therefore, we next examined the expression of CD25 on CD4⁺ cells. CD4⁺CD25⁺ cells appeared in the BALF after ovalbumin inhalation. Seven days after cessation of ovalbumin inhalation, the number of CD4⁺CD25⁺ cells in the BALF was significantly smaller in plt mice than that in BALB/c mice (Fig 5, C). CD4⁺CD25⁺ cells marginally existed in mediastinal lymph node of plt mice (Fig 5, C). There were no differences in the number of CD4⁺CD25⁺ cells in the blood and spleen between plt and BALB/c mice (Fig 5, C). Although the number of CD4⁺CD25⁺ cells in the BALF was decreased, a significant number of CD4⁺CD25⁺ cells was found in the BALF of plt mice.

Role of CCL19 in resolution of airway inflammation

To investigate the mechanisms of delayed resolution of allergic inflammation in plt mice, we added CCL19, another ligand of CCR7, for which recombinant protein is available. After 14 days of ovalbumin exposure, we added CCL19 (5 μ g/mouse by osmotic pump) continuously. For BALB/c mice, saline was added instead of CCL19. After 1 week of administration, lung lymphocytes were separated and were cultured for 2 days, after which the supernatants were harvested. We measured IL-10 because this protein contributes to the resolution of allergic inflammation. As shown in Fig 6, A (in the BALF), and Fig 6, B (in the cultured supernatants), IL-10 production in BALB/c mice was significantly greater than in plt mice. The treatment with CCL19 significantly enhanced IL-10 production in plt mice (Fig 6, B), suggesting that the defect in CCL19 and CCL21 impairs IL-10 production in lung cells. To assess the effect of CCL19 administration, we also analyzed BALF cells and airway response after 1 week of CCL19 administration. Although lymphocytes in CCL19-treated plt mice did not reach the same level in BALB/c mice, CCL19-treated plt mice exhibited a significantly lower number of lymphocytes in the BALF and Penh level after 100 mg acetylcholine inhalation compared with nontreated plt mice (Fig 6, C and D).

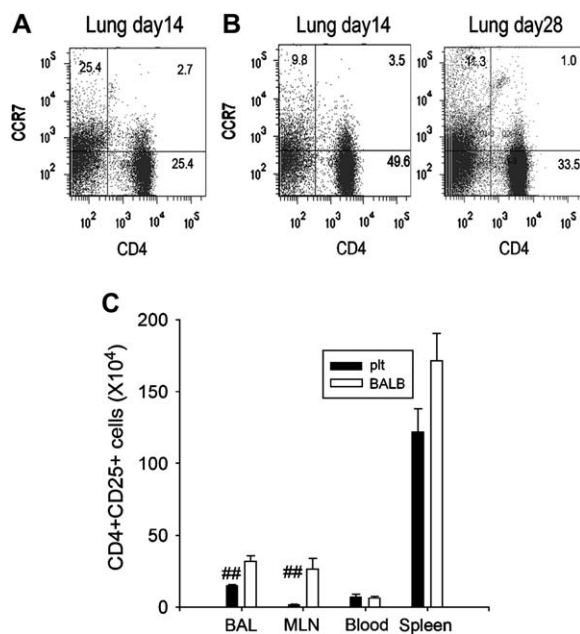


FIG 5. Flow-cytometric analysis of BALF cells, lung, secondary lymph node, blood, and spleen. **A**, CCR7 expression in BALB/c mice. **B**, CCR7 expression in plt mice. **C**, Number of CD4⁺CD25⁺ T cells on day 21 (1 week after cessation of ovalbumin inhalation). BAL, Bronchoalveolar lavage; MLN, mediastinal lymph node.

DISCUSSION

In the current study, we showed that CCL19 and CCL21 are important but not critical for sensitization and induction of allergic inflammation. In plt mice, which are genetically defective with regard to CCL19 and CCL21,¹⁷ the ovalbumin-specific IgE response was delayed, although the same level of response was noted as in BALB/c mice after the final immunization. Although airway inflammation and airway response to acetylcholine were significantly reduced in plt mice compared with BALB/c mice, significant eosinophilic inflammation and hyperresponsiveness were also observed in plt mice after 2 weeks of inhalation. However, 4 weeks after cessation of inhalation, airway inflammation and airway hyperresponsiveness in plt mice were greater than in BALB/c. At the time of resolution of airway inflammation, IL-10 production was enhanced in BALB/c but not in plt mice.

Enhanced T-cell immune response was observed in plt mice when examined by contact sensitization.²³ CCL21 is present in the luminal surface of venules in peripheral lymph nodes of wild-type mice but not plt/plt.²³ High endothelial venules in plt mice did not express CCL21 and did not support T-cell sticking, resulting in a marked reduction of T-cell homing to peripheral lymph nodes.²³ After immunization of plt mice, T cells and DCs located in the superficial cortex of lymph nodes and in splenic bridging channels.²³ It has been reported that airway inflammation can be induced without secondary lymph nodes.²⁸ In that study, lymphotoxin α/β knockout mice were used for impairment of secondary lymph nodes.

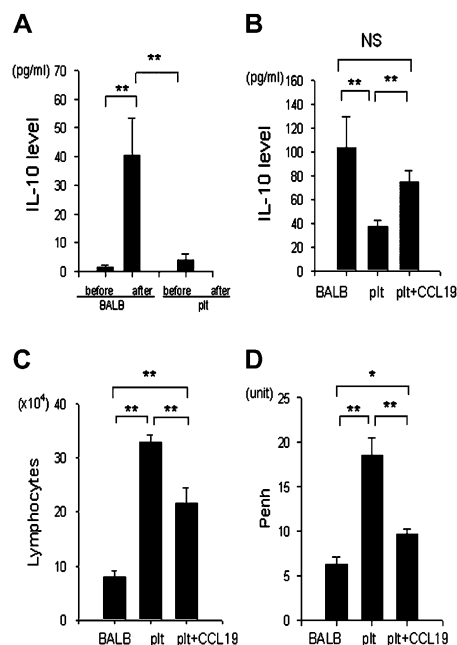


FIG 6. IL-10 production after cessation of allergen inhalation and effect of CCL19 administration. **A**, IL-10 level in the BALF before and after ovalbumin inhalation. **B**, IL-10 level in the cultured cells. Data shown are means $\pm$ SEMs of triplicate examination from 4 mice per group. The effect of CCL19 administration on airway inflammation (**C**) and on airway response (**D**). * $P < .05$; ** $P < .01$. NS, Not significant.

Consistent with previous reports, our data showed that significant airway inflammation in a lesser magnitude was induced without secondary lymph nodes.

Blockade of CCL21 by neutralizing antibody exacerbates lung T_H1-dominant inflammation induced by propionibacterium acnes, which is thought to possess linkage to sarcoidosis.¹⁶ Immune response in CCR7 receptor knockout mice was also reported.¹² CCR7 is the receptor for CCL19 and CCL21, and its knockout mice possess impairment of secondary lymph nodes similar to plt mice. However, the impairment of contact sensitivity is severely suppressed compared with plt mice. Antibody response to subcutaneous immunization with complete Freund adjuvant is also depressed in the first 10 days and reaches a level similar to that in wild-type mice after 20 days. Although the immunization protocol and antigens are different, our data with plt mice exhibited similar kinetics.

Plt mice exhibited impaired resolution of airway inflammation. Recently, it has been reported that CCR7 plays a critical role in the lymphocyte exit from peripheral tissue and entry into afferent lymphatics.^{24,25} As shown in Fig 5, both CCR7⁺ and CCR7⁻ CD4 T cells existed in the lung of both plt and BALB/c mice. Even at the resolution phase, both CCR7⁺ and CCR7⁻ cells exist in the lung of plt mice, suggesting that we could not ascribe delayed resolution of inflammation only to impairment of T-cell exit in plt mice. Because IL-10 regulates airway inflammation,²⁹⁻³¹ our data on impairment of IL-10 production in plt mice after cessation of ovalbumin exposure might be

critical in elucidating factors for resolution of airway inflammation. Furthermore, administration of CCL19 resulted in recovery of impaired IL-10 production, suggesting that the defect in IL-10 production was directly related to genetic defects of plt mice. Natural recovery from autoimmune encephalomyelitis has been ascribed to IL-10 producing CD4⁺CD25⁺ regulatory T cells,³² which are known to suppress airway inflammation and produce IL-10 or TGF- β .^{6,7,26,30,33,34} Plt mice also demonstrate defective DC localization,¹⁹ which is a regulator of adaptive immune response as well as a critical inducer.³⁵ Pulmonary DCs also activate regulatory T cells at the secondary lymph nodes.²⁷ Although we could not show phenotypical defects in CD4⁺CD25⁺ cells in plt mice, we identify functional impairment of induction of IL-10 production in plt mice, which might be ascribed to functional impairment of regulatory T cells or DCs. Another possible mechanism of delayed resolution is a defect in apoptosis in plt mice, which had been reported previously in T_H1-dominant inflammation.³⁶

REFERENCES

- Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996;183:109-17.
- Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
- Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
- Vogel G. Interleukin-13's key role in asthma shown. *Science* 1998;282:2168.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
- Chinen J, Shearer WT. Basic and clinical immunology. *J Allergy Clin Immunol* 2005;116:411-8.
- Chinen J, Shearer WT. Advances in asthma, allergy and immunology series 2004: basic and clinical immunology. *J Allergy Clin Immunol* 2004;114:398-405.
- Lambrecht BN, Peleman RA, Bullock GR, Pauwels RA. Sensitization to inhaled antigen by intratracheal instillation of dendritic cells. *Clin Exp Allergy* 2000;30:214-24.
- Lambrecht BN. Allergen uptake and presentation by dendritic cells. *Curr Opin Allergy Clin Immunol* 2001;1:51-9.
- Vermaelen KY, Pauwels RA. Accelerated airway dendritic cell maturation, trafficking and elimination in a mouse model of asthma. *Am J Respir Cell Mol Biol* 2003;29:405-9.
- Palucka K, Banchereau J. How dendritic cells and microbes interact to elicit or subvert protective immune responses. *Curr Opin Immunol* 2002;14:420-31.
- Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 1999;99:23-33.
- Nakano H, Yanagita M, Gunn MD. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J Exp Med* 2001;194:1171-8.
- Cyster JG. Chemokines and cell migration in secondary lymphoid organs. *Science* 1999;286:2098-102.
- Sallusto F, Lanzavecchia A. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol Rev* 2000;177:134-40.
- Itakura M, Tokuda A, Kimura H, Nagai S, Yoneyama H, Onai N, et al. Blockade of secondary lymphoid tissue chemokine exacerbates propionibacterium acnes-induced acute lung inflammation. *J Immunol* 2001;166:2071-9.
- Nakano H, Mori S, Yonekawa H, Nariuchi H, Matsuzawa A, Kakiuchi T. A novel mutant gene involved in T-lymphocyte-specific homing into peripheral lymphoid organs on mouse chromosome 4. *Blood* 1998;91:2886-95.
- Nakano H, Gunn MD. Gene duplications at the chemokine locus on mouse chromosome 4: multiple strain-specific haplotypes and the deletion of secondary lymphoid-organ chemokine and EBI-1 ligand chemokine genes in the plt mutation. *J Immunol* 2001;166:361-9.
- Gunn MD, Kyuwa S, Tam C, Kakiuchi T, Matsuzawa A, Williams LT, et al. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J Exp Med* 1999;189:451-60.
- Yamashita N, Tashimo H, Ishida H, Kaneko F, Nakano J, Kato H, et al. Attenuation of airway hyperresponsiveness in a murine asthma model by neutralization of granulocyte-macrophage colony-stimulating factor (GM-CSF). *Cell Immunol* 2002;219:92-7.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766-75.
- Yamashita N, Sekine K, Miyasaka T, Kawashima R, Nakajima Y, Nakano J, et al. Platelet-derived growth factor is involved in the augmentation of airway responsiveness through remodeling of airways in diesel exhaust particulate-treated mice. *J Allergy Clin Immunol* 2001;107:135-42.
- Mori S, Nakano H, Aritomi K, Wang CR, Gunn MD, Kakiuchi T. Mice lacking expression of the chemokines CCL21-ser and CCL19 (plt mice) demonstrate delayed but enhanced T cell immune responses. *J Exp Med* 2001;193:207-18.
- Bromley SK, Thomas SY, Luster AD. Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. *Nat Immunol* 2005;6:895-901.
- Debes GF, Arnold CN, Young AJ, Krautwald S, Lipp M, Hay JB, et al. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. *Nat Immunol* 2005;6:889-94.
- Huehn J, Siegmund K, Lehmann JC, Siewert C, Haubold U, Feuerer M, et al. Developmental stage, phenotype, and migration distinguish naive and effector/memory-like CD4⁺ regulatory T cells. *J Exp Med* 2004;199:303-13.
- Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001;2:725-31.
- Gajewska BU, Alvarez D, Vidric M, Goncharova S, Stampfli MR, Coyle AJ, et al. Generation of experimental allergic airways inflammation in the absence of draining lymph nodes. *J Clin Invest* 2001;108:577-83.
- Stampfli MR, Cwiartka M, Gajewska BU, Alvarez D, Ritz SA, Inman MD, et al. Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. *Am J Respir Cell Mol Biol* 1999;21:586-96.
- Umetsu DT, Akbari O, DeKruyff RH. Regulatory T cells control the development of allergic disease and asthma. *J Allergy Clin Immunol* 2003;112:480-7; quiz 8.
- Zemann B, Schwaerzler C, Griot-Wenk M, Neftzger M, Mayer P, Schneider H, et al. Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-beta expression and prevents allergy in adult life. *J Allergy Clin Immunol* 2003;111:1069-75.
- McGeachy MJ, Stephens LA, Anderton SM. Natural recovery and protection from autoimmune encephalomyelitis: contribution of CD4⁺CD25⁺ regulatory cells within the central nervous system. *J Immunol* 2005;175:3025-32.
- McHugh RS, Shevach EM. The role of suppressor T cells in regulation of immune responses. *J Allergy Clin Immunol* 2002;110:693-702.
- Johnston B, Kim CH, Soler D, Emoto M, Butcher EC. Differential chemokine responses and homing patterns of murine TCR alpha beta NKT cell subsets. *J Immunol* 2003;171:2960-9.
- Morel PA, Feili-Hariri M, Coates PT, Thomson AW. Dendritic cells, T cell tolerance and therapy of adverse immune reactions. *Clin Exp Immunol* 2003;133:1-10.
- Sanchez-Sanchez N, Riolo-Blanco L, de la Rosa G, Puig-Kroger A, Garcia-Bordas J, Martin D, et al. Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. *Blood* 2004;104:619-25.