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Kazuyuki Nakagome, Mitsuru Imamura, Hirokazu Okada,
Kimito Kawahata, Tsutomu Inoue, Kumiko Hashimoto,
Hiroaki Harada, Takehiro Higashi, Rie Takagi, Kazuhisa
Nakano, Koichi Hagiwara, Minoru Kanazawa, Makoto Dohi,
Makoto Nagata and Sho Matsushita

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Dopamine D1-Like Receptor Antagonist Attenuates Th17-Mediated Immune Response and Ovalbumin Antigen-Induced Neutrophilic Airway Inflammation

Kazuyuki Nakagome,^{*,†} Mitsuru Imamura,[‡] Hirokazu Okada,[§] Kimito Kawahata,[‡] Tsutomu Inoue,[§] Kumiko Hashimoto,^{†,¶} Hiroaki Harada,[‡] Takehiro Higashi,^{†,¶} Rie Takagi,^{†,¶} Kazuhisa Nakano,[¶] Koichi Hagiwara,^{*} Minoru Kanazawa,^{*} Makoto Dohi,[‡] Makoto Nagata,^{*,†} and Sho Matsushita^{†,¶}

Allergic airway inflammation is generally considered a Th2-type immune response. Recent studies, however, demonstrated that Th17-type immune responses also play important roles in this process, especially in the pathogenesis of neutrophilic airway inflammation, a hallmark of severe asthma. We previously reported that dendritic cells release dopamine to naive CD4⁺ T cells in Ag-specific cell–cell interaction, in turn inducing Th17 differentiation through dopamine D1-like receptor (D1-like-R). D1-like-R antagonist attenuates Th17-mediated diseases such as experimental autoimmune encephalomyelitis and autoimmune diabetes. However, the effect of antagonizing D1-like-R on Th17-mediated airway inflammation has yet to be studied. In this study, we examined whether D1-like-R antagonist suppresses OVA-induced neutrophilic airway inflammation in OVA TCR-transgenic DO11.10 mice and then elucidated the mechanism of action. DO11.10 mice were nebulized with OVA or PBS, and some mice received D1-like-R antagonist orally before OVA nebulization. D1-like-R antagonist significantly suppressed OVA-induced neutrophilic airway inflammation in DO11.10 mice. It also inhibited the production of IL-17 and infiltration of Th17 cells in the lung. Further, D1-like-R antagonist suppressed the production of IL-23 by lung CD11c⁺ APCs. In contrast, D1-like-R antagonist did not increase Foxp3⁺ regulatory T cells in the lung. D1-like-R antagonist neither suppressed nonspecific LPS-induced neutrophilic airway inflammation nor OVA-induced eosinophilic airway inflammation. These results indicate that D1-like-R antagonist could suppress Th17-mediated neutrophilic airway inflammation, raising the possibility that antagonizing D1-like-R serves as a promising new strategy for treating neutrophil-dominant severe asthma. *The Journal of Immunology*, 2011, 186: 000–000.

Allergic inflammation in the airway is generally considered a Th2-type response-mediated process (1). However, recent studies demonstrated that Th1- and Th17-type immune responses also play some roles in this process (2–8). For example, passive transfer of OVA-specific Th1 cells exacerbated OVA-induced airway eosinophilia (2, 3). Further, passive transfer of OVA-specific Th17 cells induced steroid-resistant airway inflammation and airway hyperresponsiveness (4). Among the Th17-type immune responses, IL-17 is thought to play an important role in the development of severe asthma as it induces neutrophilic inflammation (5), a hallmark of severe asthma (6).

Barczyk et al. (7) reported that an elevated sputa IL-17 concentration correlates with clinical severity of asthma. Al-Ramli et al. (8) reported that IL-17 airway expression increased only in severe asthmatics. Therefore, inhibition of Th17 cells or IL-17 could be a reasonable control strategy for severe asthma.

The initiation of Ag-induced immune response is under the control of dendritic cells (DCs), the most potent of all APCs (9). Ag presentation from APCs to naive T cells induced the expression of selective sets of cytokines, thus affecting Th differentiation such as Th1, Th2, or Th17 (10, 11). Furthermore, DCs play an essential role in not only the development but also the maintenance of Ag-induced immune response in the airway (12, 13). We previously demonstrated that dopamine synthesized and released by DCs is involved in Th17 differentiation (14, 15). Therefore, modulation of DC or APC functions including dopamine release might affect the development of Th17-mediated neutrophilic airway inflammation. There are five types of dopamine receptors, termed D1–D5 (16). D1 and D5 form the D1-like group, which leads to an increase in intracellular cAMP formation, whereas D2, D3, and D4 form the D2-like group, which leads to a decrease in intracellular cAMP formation (16). We also reported that antagonizing dopamine D1-like receptor (D1-like-R) could attenuate Th17-mediated (or Th17-involved) immune disease such as experimental autoimmune encephalomyelitis (EAE) (14), autoimmune diabetes (17), and nephrotoxic serum nephritis (18). However, whether antagonism of D1-like-R can suppress Th17-type response in the airway has yet to be studied.

In OVA TCR-transgenic DO11.10 mice or OTII mice, OVA nebulization alone could induce IL-17–dependent neutrophil-

^{*}Department of Respiratory Medicine, Saitama Medical University, Saitama 350-0495, Japan; [†]Allergy Center, Saitama Medical University, Saitama 350-0495, Japan; [‡]Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan; [§]Department of Nephrology, Saitama Medical University, Saitama 350-0495, Japan; and [¶]Department of Allergy and Immunology, Saitama Medical University, Saitama 350-0495, Japan

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Address correspondence and reprint requests to Prof. Sho Matsushita, Department of Allergy and Immunology, Saitama Medical University, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan. E-mail address: shomat@saitama-med.ac.jp

Abbreviations used in this article: BAL, bronchoalveolar lavage; DC, dendritic cell; D1-like-R, dopamine D1-like receptor; EAE, experimental autoimmune encephalomyelitis; Ly, lymphocytes; M, macrophages; Neu, neutrophils; SA, physiologic saline; Treg, regulatory T cell.

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dominant airway inflammation without an increase in serum OVA-specific IgE (19–22). Although it is unknown if the mechanism of neutrophilic airway inflammation in OVA-challenged DO11.10 mice is similar to that in severe asthma, this model is thought to be useful for the investigation of the pathogenesis of Ag-induced Th17-mediated neutrophilic airway inflammation.

The purpose of this study was to examine whether D1-like-R antagonist SCH23390 suppresses OVA Ag-induced neutrophilic airway inflammation in OVA TCR-transgenic DO11.10 mice, and then to elucidate the mechanism of action. D1-like-R antagonist significantly suppressed neutrophilic airway inflammation and Th17-mediated immune responses in the lung. Furthermore, D1-like-R antagonist suppressed the production of IL-23 by lung CD11c⁺ APCs. These results indicate that antagonizing D1-like-R could suppress Th17-mediated neutrophilic airway inflammation.

Materials and Methods

Mice

BALB/c mice were obtained from Charles River Japan (Kanagawa, Japan). OVA TCR-transgenic DO11.10 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All animal experiments were approved by and performed in compliance with the institutional animal care and use committee guidelines.

Induction of airway inflammation and administration of D1-like-R antagonist

Six-week-old female DO11.10 mice were challenged with an aerosolized solution of 3% OVA (Sigma-Aldrich, St. Louis, MO) or PBS for 10 min from days –2 to 0. From weeks –6 to 0, some mice received one of the D1-like-R antagonists SCH23390 (0.3 mg/kg; Sigma-Aldrich), SKF83566 (0.3 mg/kg; Tocris Bioscience, Bristol, U.K.), or LE300 (0.3 mg/kg; Tocris Bioscience) in PBS (50 μ l) or PBS alone orally three times a week as previously reported (14, 17, 18). The mice were analyzed on day 1. To induce nonspecific neutrophilic airway inflammation, BALB/c mice received an intratracheal injection of 1 μ g LPS (Sigma-Aldrich) dissolved in 50 μ l physiologic saline (SA) on day 0. To generate OVA-induced eosinophilic airway inflammation, BALB/c mice were sensitized with an i.p. injection of 2 μ g OVA plus 2 mg aluminum hydroxide or SA on days –20 and –9, and the mice were challenged with an aerosolized solution of 3% OVA or PBS for 10 min from day –2 to day 0 (23–26). From weeks –6 to 0, some mice received SCH23390 (0.3 mg/kg) in PBS (50 μ l) or PBS alone orally three times a week. The mice were analyzed on day 1.

Bronchoalveolar lavage fluid analyses

Bronchoalveolar lavage (BAL) fluid analyses were performed as previously reported (23–28). The mice were anesthetized by i.p. injection of sodium pentobarbital (50 mg/kg). Then, the lungs were lavaged four times with PBS (0.5 ml each). Approximately 1.6 ml of the instilled PBS was consistently recovered with gentle handling. The cell suspension was centrifuged at $150 \times g$ for 10 min at 4°C. The cells were resuspended into 1 ml PBS, and the total cell numbers were counted with a hemocytometer. Cytospin samples were prepared by centrifuging the suspensions at 300 rpm for 10 min. On the basis of the findings with May–Grünwald–Giemsa stain, cell differentials were counted with at least 300 leukocytes in each sample. The cell types were judged according to standard hemocytologic procedures as neutrophils, eosinophils, lymphocytes, or macrophages.

Histological examination

Histological examination was performed as previously reported (23–28). After perfusion with PBS, the right lungs were resected, fixed with 10% neutralized buffered formalin (Wako, Osaka, Japan), and embedded in paraffin. Three-micrometer-thick sections were stained with H&E.

Measurement of concentration of SCH23390

Plasma concentration of SCH23390 was determined by HPLC as previously reported (29). Thus, one-third volume of acetonitrile was added to plasma samples, vibrated, and centrifuged. The supernatant was loaded onto a 4.5×250 mm C18 reversed-phase HPLC column (Shiseido, Tokyo, Japan) equilibrated with 25% acetonitrile containing 0.06% trifluoroacetic acid. The column was eluted at a flow rate of 1.0 ml/min at room temperature using an isocratic mode, with the A282 continuously monitored.

Measurement of cytokine concentrations in the lung

The left lungs were cut out and homogenized in 1.0 ml PBS containing 0.5% Triton X-100 and complete protease inhibitor mixture (Roche Applied Science, Mannheim, Germany) (25). The lung homogenates were then cleared of debris and cells by centrifugation at $10,000 \times g$ for 10 min. Cytokine concentrations in the lung homogenates were measured using ELISA kits (IL-17 and IL-22; R&D Systems, Minneapolis, MN).

Immunohistochemistry

Immunohistochemistry was performed as previously reported (17). Briefly, the tissue was deparaffinized and rehydrated with decreasing concentrations of ethyl alcohol. The slides were autoclaved (121°C for 15 min) with Ag Unmasking Solution (Vector Laboratories, Burlingame, CA) for Ag retrieval. The Ab used for immunohistochemistry was anti-IL-23R (LifeSpan, Seattle, WA), which was diluted 30-fold with 1% BSA in PBS as the blocking buffer, and the sections were incubated with the primary Ab for 12 h at 37°C. The reaction was amplified using tyramide signal amplification kit and labeled with Alexa Fluor 555 (Molecular Probes, Carlsbad, CA). The negative control sections were treated as described earlier, but rabbit IgG was used for the primary Ab. TO-PRO-3 (Molecular Probes) was used to stain the nuclei. A confocal laser scanning microscope (FV1000; Olympus, Tokyo, Japan) was used for data acquisition.

Intracellular staining

Lung cells were obtained according to previously reported methods (23, 25, 26). For detection of IL-17⁺ CD4⁺ T cells, lung cells were stimulated with lymphocyte activator mixture (PMA/ionomycin/brefeldin A; BD Pharmingen, San Diego, CA) for 5 h and stained with FITC anti-CD3 mAb (BD Pharmingen) and PE–Cy5 anti-CD4 mAb (BioLegend, San Diego, CA) for 30 min. Then, the cells were incubated with intracellular fixation buffer (eBioscience, San Diego, CA) for 20 min. After washing with permeabilization buffer (eBioscience), the cells were stained with PE anti-IL-17 mAb (eBioscience) for 30 min. For detection of Foxp3⁺ CD4⁺ T cells, lung cells were stained with PE–Cy5 anti-CD4 mAb for 30 min. Then, the cells were incubated with the fixation/permeabilization solution (eBioscience) for 2 h at 4°C. After washing with permeabilization buffer, the cells were stained with PE anti-Foxp3 mAb (eBioscience) for 30 min. For detection of IL-23⁺ CD11c⁺ APCs, lung cells were stimulated with brefeldin A (GolgiPlug; BD Pharmingen) and LPS (1 μ g/ml) plus anti-CD40 Ab (5 μ g/ml; eBioscience) for 6 h, and then stained with FITC anti-CD11c mAb (BD Pharmingen) for 30 min. Preliminary studies suggested that IL-23–producing cells were not detected when we used LPS only for stimulation, and that incubation with brefeldin A and LPS/anti-CD40 Ab for 6 h increased IL-23–producing cells more than that for 3 h. After incubation with intracellular fixation buffer, the cells were incubated with IL-23R Fc chimera (R&D Systems). After washing with permeabilization buffer, the cells were incubated with PE anti-human IgG (eBioscience) to detect IL-23–producing cells. Then, stained cells were analyzed by flow cytometry (EPICS XL System II; Coulter). For analyses of IL-17⁺ CD4⁺ T cells, the cells were gated according to FITC–CD3 parameters.

Cytokine production by lung CD11c APCs

Lung CD11c⁺ APCs were prepared using magnetic beads as previously reported (23, 25, 26). CD11c⁺ cells (1.25×10^5 cells/well) were incubated with LPS (1 μ g/ml) or LPS plus anti-CD40 Ab (5 μ g/ml) for 24 h, and then cytokine concentrations in the supernatants were measured using an ELISA kit (R&D Systems).

Statistics

Values are expressed as mean $\pm$ SEM. Statistical analyses were performed with a one-way ANOVA followed, when differences were significant, by appropriate post hoc tests using the Tukey–Kramer test. To analyze differences between the two groups, we used Student *t* test. All *p* values <0.05 were considered statistically significant.

Results

D1-like-R antagonist suppresses OVA-induced neutrophilic airway inflammation in DO11.10 mice

We first elucidated the effect of D1-like-R antagonist SCH23390 on OVA-induced neutrophilic airway inflammation in OVA TCR-transgenic DO11.10 mice (Fig. 1). DO11.10 mice were challenged with nebulized OVA or PBS, and administration of

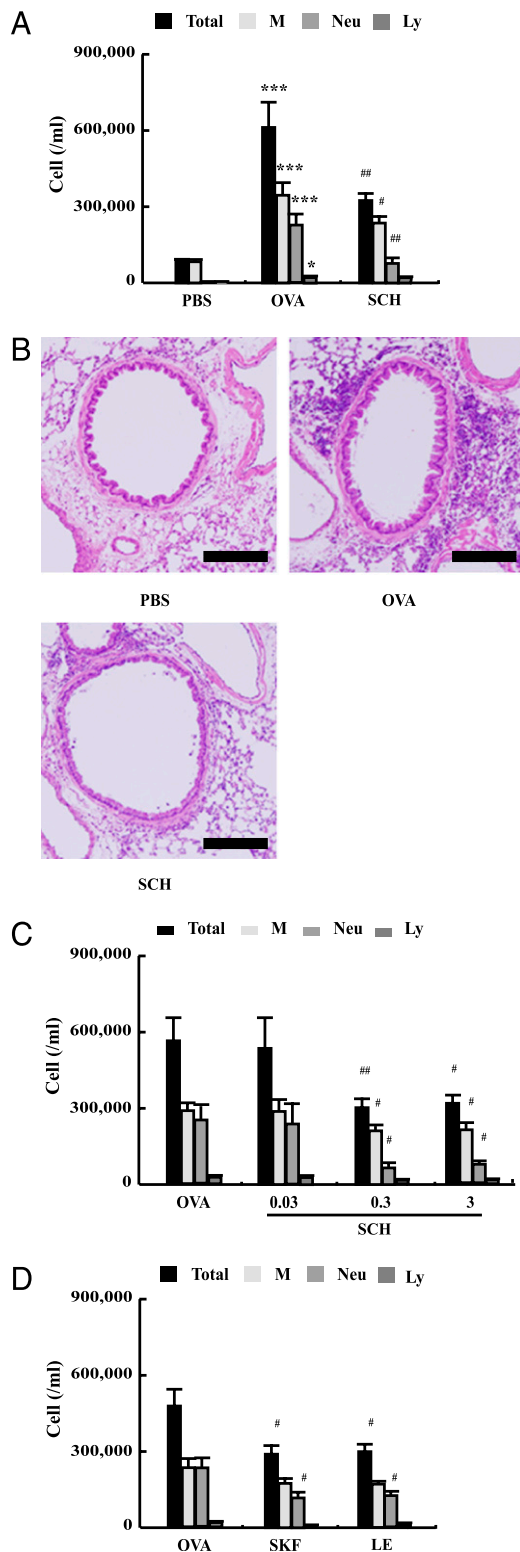


FIGURE 1. D1-like-R antagonist suppresses neutrophilic airway inflammation in DO11.10 mice. **A** and **B**, SCH23390 suppresses OVA-induced neutrophilic airway inflammation. DO11.10 mice were nebulized with OVA or PBS from days -2 to 0 . Some mice received SCH23390 (0.3 mg/kg) in PBS (50 μ l) or PBS alone orally three times a week from weeks -6 to 0 . On day 1 , the mice were analyzed. **A**, SCH23390 reduces the neutrophil count in BAL fluid. BAL fluid analyses were performed ($n = 8$). Leukocytes were identified by morphologic criteria. $*p < 0.05$, $***p < 0.001$ (compared with the value of PBS); $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (compared with the value of OVA). **B**, Histological findings. Lungs were excised and subjected to H&E staining. Scale bars, 200 μ m. **C**, Dose-response

SCH23390 was performed starting 6 wk before OVA nebulization. Actual plasma concentration of SCH23390 when given orally in the concentration and regimen we used was 5.4 ± 2.2 μ M, although that without SCH23390 was below the detection limit. OVA nebulization markedly increased the number of neutrophils as well as macrophages in BAL fluid in DO11.10 mice (Fig. 1A). SCH23390 treatment strongly suppressed the infiltration of neutrophils and slightly suppressed airway macrophages (Fig. 1A). OVA nebulization also increased the number of lymphocytes in DO11.10 mice; however, SCH23390 did not suppress lymphocyte infiltration (Fig. 1A [$\times 10^3$ /ml]: PBS: M, 111 ± 10 , Neu, 3 ± 1 , Ly, 4 ± 1 ; OVA: M, 360 ± 51 , Neu, 229 ± 47 , Ly, 25 ± 8 ; SCH: M, 232 ± 17 , Neu, 78 ± 12 , Ly, 14 ± 2). The histology of OVA-challenged DO11.10 mice showed prominent neutrophil infiltration into the peribronchial area (Fig. 1B). In mice that received SCH23390, infiltration declined (Fig. 1B). Next, we examined the dose-response relationship between SCH23390 and neutrophilic airway inflammation. Administration of 0.03 mg/kg SCH23390 three times a week did not suppress neutrophilic airway inflammation, whereas 0.3 or 3 mg/kg SCH23390 suppressed it (Fig. 1C [$\times 10^3$ /ml]: OVA: macrophages (M), 292 ± 23 , neutrophils (Neu), 263 ± 49 , lymphocytes (Ly), 31 ± 9 ; SCH (0.03): M, 290 ± 33 , Neu, 235 ± 68 , Ly, 33 ± 7 ; SCH (0.3): M, 209 ± 13 , Neu, 75 ± 17 , Ly, 14 ± 3 ; SCH (3): M, 210 ± 19 , Neu, 84 ± 8 , Ly, 18 ± 2). Thus, 0.3 mg/kg SCH23390 was used throughout the study. Furthermore, other D1-like-R antagonists such as SKF83566 or LE300 suppressed OVA-induced neutrophilic airway inflammation, although the suppression by SKF83566 or LE300 seemed to be weaker than that by SCH23390 (Fig. 1D [$\times 10^3$ /ml]: OVA: M, 227 ± 34 , Neu, 230 ± 40 , Ly, 18 ± 4 ; SKF: M, 173 ± 15 , Neu, 107 ± 20 , Ly, 7 ± 1 ; LE: M, 170 ± 7 , Neu, 123 ± 12 , Ly, 12 ± 3). These results indicated that antagonizing D1-like-R suppressed OVA Ag-induced neutrophilic airway inflammation in DO11.10 mice.

D1-like-R antagonist SCH23390 suppresses Th17-mediated immune response in the lung

Next we examined the effect of SCH23390 on Th17-mediated immune response in the lung. We first examined whether SCH23390 could suppress the production of IL-17 in the lung. Because the concentrations of IL-17 in BAL fluid did not increase in OVA-challenged DO11.10 mice in our system (data not shown), we measured the concentrations in the lung. The concentrations of IL-17 increased in the lungs of OVA-challenged DO11.10 mice, and SCH23390 treatment suppressed them (Fig. 2A [pg/ml]: PBS: 114 ± 8 ; OVA: 206 ± 14 ; SCH: 163 ± 7). Similar results were obtained when we measured the concentrations of IL-22, another Th17-type cytokine (data not shown). In contrast, concentrations of IL-5 or IFN- γ slightly increased in the lungs of OVA-challenged DO11.10 mice, and SCH23390 treatment did not

relationship between SCH23390 and neutrophilic airway inflammation. DO11.10 mice were nebulized with OVA from days -2 to 0 . Some mice received SCH23390 (0.03 , 0.3 , or 3 mg/kg) in PBS (50 μ l) orally three times a week from weeks -6 to 0 . On day 1 , the mice were analyzed. BAL fluid analyses were performed ($n = 8$). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ (compared with the value of OVA). **D**, Other D1-like-R antagonists such as SKF83566 or LE300 also suppress OVA-induced neutrophilic airway inflammation. DO11.10 mice were nebulized with OVA from days -2 to 0 . Some mice received SKF83566 (0.3 mg/kg) or LE300 (0.3 mg/kg) in PBS (50 μ l) orally three times a week from weeks -6 to 0 . On day 1 , the mice were analyzed. BAL fluid analyses were performed ($n = 8$). $^{\#}p < 0.05$ (compared with the value of OVA).

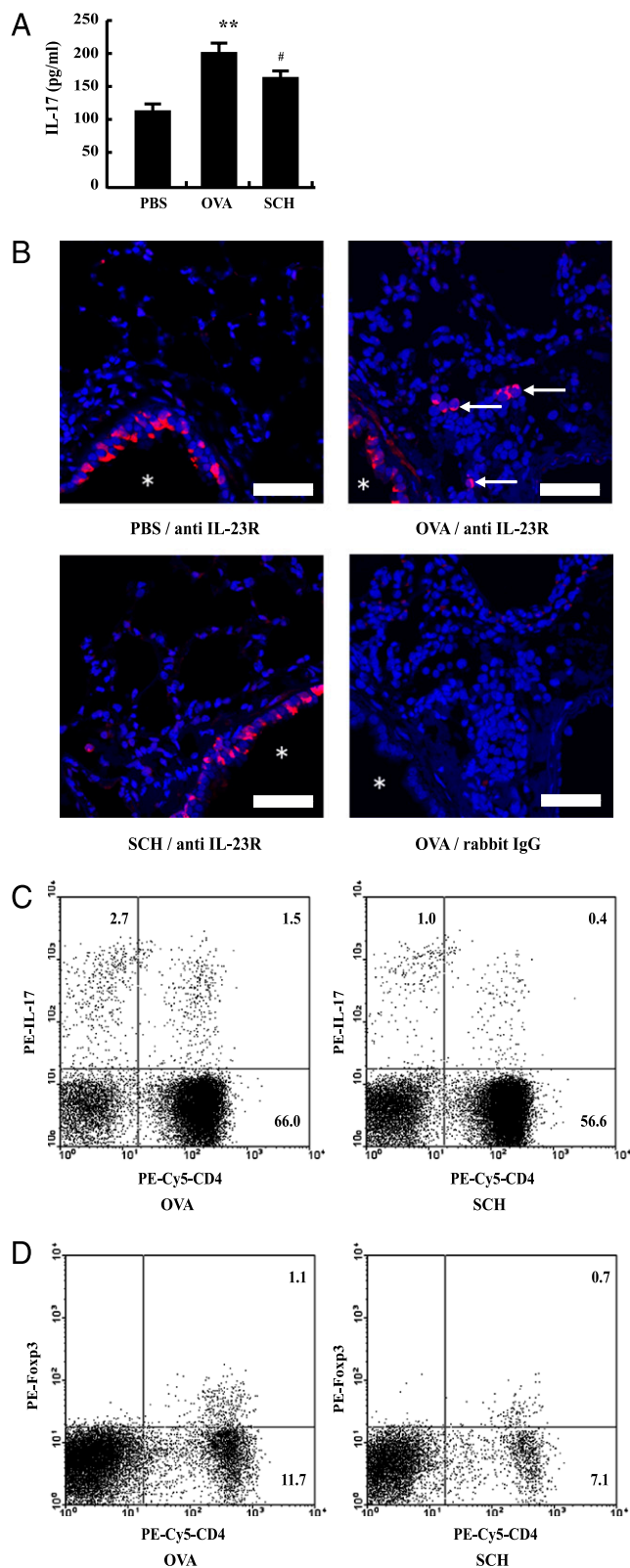


FIGURE 2. D1-like-R antagonist SCH23390 suppresses the Th17-mediated immune response in the lung. DO11.10 mice were treated as described for Fig. 1. **A**, IL-17 concentrations in the lung. Lung homogenate was assayed for concentrations of IL-17 by ELISA ($n = 8$). ** $p < 0.01$ (compared with the value of PBS), # $p < 0.05$ (compared with the value of OVA). **B**, Immunohistochemistry for IL-23R. Lungs were excised, and staining for IL-23R was performed (red). TO-PRO-3 was used to stain the nuclei (blue). Negative control sections were treated with rabbit IgG instead of primary Ab. Representative images are shown. Asterisk (*) indicates bronchus. IL-23R⁺ mononuclear cells were clearly detected

affect the concentrations (data not shown). We then examined using immunohistochemistry of IL-23R, a specific Th17 marker (30), whether SCH23390 could suppress the infiltration of Th17 cells into the lung. IL-23R was expressed on bronchial epithelial cells of PBS-challenged mice, OVA-challenged mice, and OVA-challenged SCH23390-treated mice (Fig. 2B). In OVA-challenged DO11.10 mice, IL-23R⁺ mononuclear cells obviously increased in the lung (Fig. 2B). SCH23390 treatment suppressed their increase (Fig. 2B), suggesting the inhibition of Th17 cell infiltration into the lung. We also confirmed by intracellular staining that administration of SCH23390 suppressed the number of IL-17-producing CD4⁺ T cells (Th17 cells) in the lung (Fig. 2C). We finally examined the possibility that this suppression was mediated through the production of regulatory T cells (Tregs) in vivo. However, SCH23390 treatment did not increase the expression of Foxp3, a transcription factor specifically expressed in Tregs (31, 32), in CD4⁺ T cells in the lung (Fig. 2D), suggesting that antagonizing D1-like R would not increase Foxp3⁺ Tregs in our experimental system. These results suggested that antagonizing D1-like-R could suppress the development of Th17-mediated immune response in the lungs of OVA-challenged DO11.10 mice.

D1-like-R antagonist SCH23390 suppresses IL-23 production by lung CD11c⁺ APCs

To elucidate the mechanism for suppression, we examined the effect of SCH23390 on lung APC functions. SCH23390 slightly suppressed the MHC alloreactivity or expression of MHC class II in lung CD11c⁺ APCs, although this was not significant (data not shown). SCH23390 suppressed the production of IL-23, one of the important cytokines used to differentiate and promote the Th17 response (33), by lung CD11c⁺ APCs in response to LPS (Fig. 3A [pg/ml]: OVA: 44 ± 3 ; SCH: 36 ± 2). In contrast, SCH23390 did not suppress the production of IL-10 or IL-12p70 by lung CD11c⁺ APCs (Fig. 3B, 3C). As the increase in IL-23 production by lung CD11c⁺ APCs in response to LPS was not very strong, other stimulation would be required to produce IL-23 more. We therefore used LPS plus anti-CD40 Ab for stimulation and examined the effect of SCH23390 on the production of IL-23. SCH23390 treatment significantly suppressed IL-23 production by lung CD11c⁺ APCs in response to LPS/anti-CD40 Ab (Fig. 3D [pg/ml]: OVA: 236 ± 11 ; SCH: 118 ± 8). We further confirmed by intracellular cytokine staining that SCH23390 suppressed the number of IL-23-producing CD11c⁺ or CD11c^{high} APCs in the lung (Fig. 3E). These results demonstrate that antagonizing D1-like-R could suppress the production of IL-23 by lung CD11c⁺ APCs, thus suppress the development of Th17-mediated immune response in the lung.

D1-like-R antagonist SCH23390 neither suppresses nonspecific neutrophilic airway inflammation nor OVA-induced eosinophilic airway inflammation

Finally, we examined whether SCH23390 would suppress non-specific neutrophilic airway inflammation. BALB/c mice received intratracheal LPS or SA, and administration of SCH23390 was performed starting 6 wk before LPS administration. LPS administration (1 μ g) markedly increased the number of neutrophils in

(white arrows). Scale bars, 50 μ m. **C**, Intracellular staining for IL-17⁺ CD4⁺ T cells. Lung cells were obtained, and IL-17⁺ CD4⁺ T cells were detected by flow cytometry. The cells were gated according to FITC-CD3 parameters. Representative results are shown. **D**, Intracellular staining for Foxp3⁺ CD4⁺ T cells. Lung cells were obtained, and Foxp3⁺ CD4⁺ T cells were detected by flow cytometry. Representative results are shown.

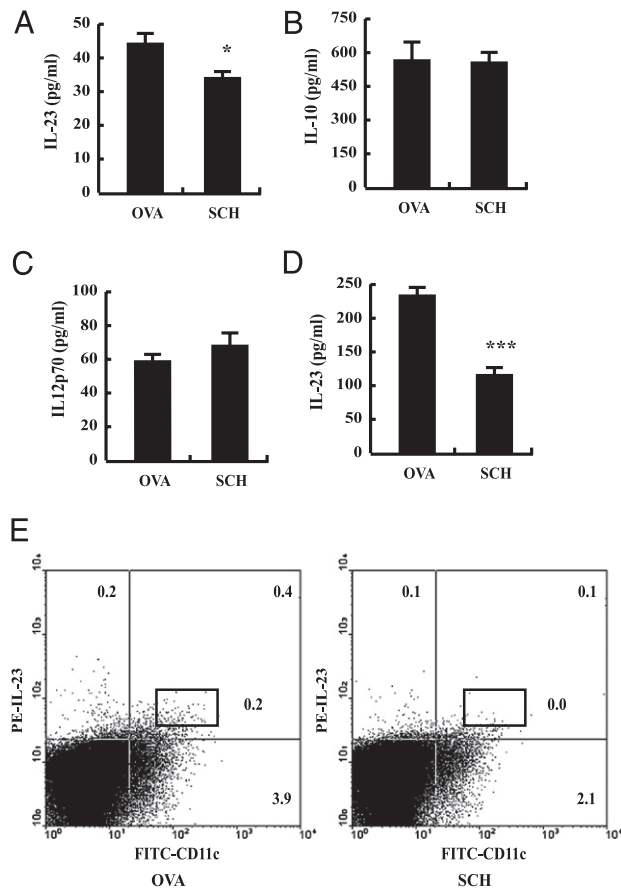


FIGURE 3. D1-like-R antagonist SCH23390 suppresses the production of IL-23 by lung CD11c⁺ APCs. DO11.10 mice were treated as described for Fig. 1. *A–C*, Cytokine production by lung CD11c⁺ APCs in response to LPS. Lung CD11c⁺ cells were positively selected and cultured (1.25×10^5 cells/well) with LPS (1 μ g/ml). After 24 h, concentrations of IL-23 (*A*), IL-10 (*B*), and IL-12p70 (*C*) in the supernatants were assayed ($n = 8$). * $p < 0.05$ (compared with the value of OVA). *D*, IL-23 production by lung CD11c⁺ APCs in response to LPS/anti-CD40 Ab. Lung CD11c⁺ cells were positively selected and cultured (1.25×10^5 cells/well) with LPS (1 μ g/ml) plus anti-CD40 Ab (5 μ g/ml). After 24 h, IL-23 concentrations in the supernatants were assayed ($n = 8$). *** $p < 0.001$ (compared with the value of OVA). *E*, Intracellular staining for IL-23⁺ CD11c⁺ APCs. Lung cells were obtained, and IL-23⁺ CD11c⁺ or CD11c^{high} cells were detected by flow cytometry. Representative results are shown.

BAL fluid (Fig. 4A). However, SCH23390 treatment did not suppress LPS (1 μ g)-induced neutrophilic infiltration into the airway (Fig. 4A). Similar results were obtained when mice received 0.1 μ g LPS and milder airway neutrophilia was induced (data not shown). We next examined whether SCH23390 would suppress OVA-induced eosinophilic airway inflammation. BALB/c mice were sensitized with OVA or SA and nebulized with OVA or PBS for 3 d. Administration of SCH23390 was performed starting 6 wk before OVA nebulization. OVA sensitization and nebulization increased the number of eosinophils in BAL fluid (Fig. 4B). However, SCH23390 treatment did not suppress OVA-induced eosinophilic airway inflammation (Fig. 4B). These findings suggested that antagonizing D1-like-R neither suppressed nonspecific neutrophilic airway inflammation nor Ag-induced Th2-mediated eosinophilic airway inflammation.

Discussion

The results of the current study clearly demonstrate that D1-like-R antagonist such as SCH23390 attenuates OVA Ag-induced neu-

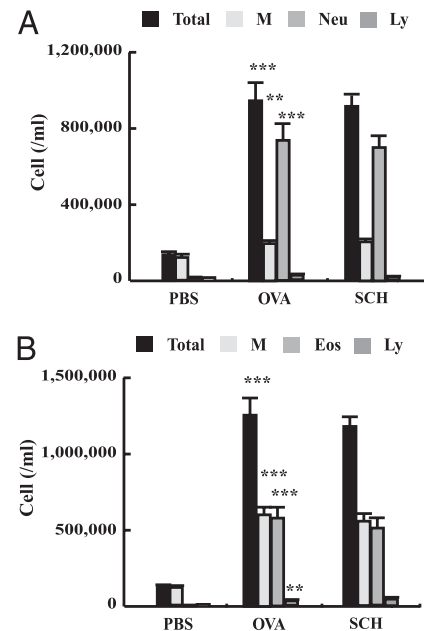


FIGURE 4. D1-like-R antagonist SCH23390 neither suppresses non-specific neutrophilic airway inflammation nor OVA-induced eosinophilic airway inflammation. *A*, SCH23390 does not suppress LPS-induced neutrophilic airway inflammation. BALB/c mice received an intratracheal injection of 1 μ g LPS in SA on day 0. From weeks –6 to 0, some mice received SCH23390 (0.3 mg/kg) in PBS (50 μ l) or PBS alone orally three times a week. The mice were analyzed on day 1. BAL fluid analyses were performed ($n = 8$). ** $p < 0.01$, *** $p < 0.001$ (compared with the value of PBS). *B*, SCH23390 does not suppress OVA-induced eosinophilic airway inflammation. BALB/c mice were sensitized with an i.p. injection of 2 μ g OVA plus 2 mg aluminum hydroxide or SA on days –20 and –9, and the mice were challenged with an aerosolized solution of 3% OVA or PBS for 10 min from day –2 to day 0. From weeks –6 to 0, some mice received SCH23390 (0.3 mg/kg) in PBS (50 μ l) or PBS alone orally three times a week. The mice were analyzed on day 1. BAL fluid analyses were performed ($n = 8$). ** $p < 0.01$, *** $p < 0.001$ (compared with the value of PBS).

trophilic airway inflammation in DO11.10 mice. It also inhibited the production of Th17-type cytokines and infiltration of IL-23R⁺ Th17 cells in the lung. Moreover, it suppressed the production of IL-23 by lung CD11c⁺ APCs in response to LPS or LPS/anti-CD40 Ab. These results indicate a possible, novel role of dopamine as a regulator of Th17-mediated immune response and neutrophilic inflammation in the airway.

Increasing evidence suggests that in OVA TCR-transgenic DO11.10 mice (BALB/c) or OTII mice (C57BL/6), OVA nebulization alone (without systemic sensitization) could induce airway inflammation (19–22). Like severe asthma, inflammation in OVA-challenged DO11.10 mice or OTII mice is characterized by a predominant influx of neutrophils rather than eosinophils without an increase in serum IgE. Nakae et al. (22) reported that this airway response is OVA Ag specific, as other Ags such as keyhole limpet hemocyanin could not induce neutrophilic inflammation. They also demonstrated that deletion of IL-17 gene diminished the neutrophilic airway inflammation (22), indicating that this inflammation is considered a Th17-type response-mediated process. Although it is unknown if the mechanism of neutrophilic airway inflammation in OVA-challenged DO11.10 mice is similar to that in severe asthma, this model is thought to be useful for the investigation of the pathogenesis of Ag-induced Th17-mediated neutrophilic airway inflammation.

The effect of dopamine and its agonists on immune responses including proliferation and cytokine production is still controversial (34–41). Dopamine is synthesized in immune cells such as T cells, B cells, peritoneal macrophages, and DCs (15, 34, 35, 42). Several reports suggested that dopamine would play an immunosuppressive role (34–37). For example, Bergquist et al. (34) reported that dopamine suppressed lymphocyte proliferation and IFN- γ production. Josefsson et al. (35) reported that L-DOPA and dopamine suppressed the Con A- or LPS-induced proliferation and cytokine production of lymphocytes. Ghosh et al. (36) reported that dopamine suppressed anti-CD3-mediated proliferation and cytokine production of activated T cells that was associated with the suppression of Lck and Fyn expression. Saha et al. (37) reported that dopamine inhibited IL-2-induced proliferation and cytotoxicity of CD4⁺ and CD8⁺ T cells through D1-like-R. In contrast, some reports suggested that dopamine could increase the proliferation of immunocytes (38, 39). Carr et al. (38) reported that L-DOPA increased Con A-stimulated splenocyte proliferation. Tsao et al. (39) reported that in vivo or in vitro administration of D1-like-R agonist enhanced the splenocyte proliferation stimulated by LPS or Con A. These findings suggested that the effect of dopamine and its agonist on immune responses including proliferation might depend on various factors such as stimulation protocol, incubation time, concentration of dopamine, and so on. However, the effect of dopamine on Ag-induced immune response or anti-CD3/CD28-mediated response has not been fully clarified.

As for cytokine production, several studies suggested dopamine would suppress the production of IFN- γ (34, 38). For example, Bergquist et al. (34) reported that dopamine suppressed IFN- γ production as described earlier. Carr et al. (38) reported that L-DOPA and dopamine suppressed the number of IFN- γ -, but not IL-4-, producing cells in spleen cells. Although some studies suggested that dopamine suppressed overall cytokine production (35, 36), the effect on the production of other cytokines than IFN- γ is still controversial. Besser et al. (40) reported that dopamine increased TNF- α and IL-10 production by human T cells. Further, the effect of dopamine on the production of IL-17 or on the differentiation of Th17 cells has not been fully clarified.

Recent evidence has established that dopamine receptor expresses in immune cells (14, 41, 43–46). Several reports suggested that PBLs express D1- and D2-like-R, and D5 receptor is the dominant receptor of D1-like-R in lymphocytes (41, 43–45). McKenna et al. (43) reported that B cells and NK cells had higher and more consistent expression, and neutrophils and eosinophils had moderate expression, although T cells and monocytes had low expression. They also reported that D3 and D5 receptors were found in most individuals, whereas D2 and D4 receptors had more variable expression. As for T cells, Kipnis et al. (46) reported that CD4⁺CD25⁺ Tregs expressed significantly more D1-like-R compared with that of CD4⁺CD25[−] effector T cells. Further, D1 receptor is the dominant receptor in naive CD4⁺CD45RA⁺ T cells (14). Therefore, D1-like-R antagonist might modify the development of Tregs or Th differentiation. As for DCs, D1-like-R is expressed in immature DCs (14), suggesting that D1-like-R antagonist could modify DC functions.

We previously demonstrated that dopamine synthesized and stored in DCs is released upon the interaction with CD4⁺ T cells and increases the formation of cAMP in naive CD4⁺ T cells, thereby polarizing Th17 differentiation through D1-like-R (14, 15). In vitro study demonstrated D2-like-R antagonist polarized Th17 differentiation, whereas D1-like-R antagonist inhibited Th17 differentiation (14). Further, dopamine increased the expression of retinoic acid orphan receptor γ t and the production of IL-6 and IL-

17 when CD4⁺CD45RA⁺ naive T cells were stimulated with anti-CD3/CD28 Abs (K. Nakano, K. Saito, K. Yamaoka, S. Matsushita, and Y. Tanaka, unpublished observations). D1-like-R antagonist suppressed their increase, suggesting that D1-like-R antagonist would suppress Th17 differentiation of the existing cells. Moreover, an in vivo study showed D1-like-R antagonist attenuates Th17-mediated immune disease such as EAE (14), autoimmune diabetes in NOD mice (17), and crescent formation of nephrotoxic serum nephritis (18). These findings suggested that dopamine played an important role in the development of Th17-mediated immune disease and that D1-like-R or D2-like-R could be an important therapeutic target for controlling Th17-mediated neutrophilic airway inflammation.

In this study, D1-like-R antagonist suppressed neutrophilic airway inflammation in OVA-challenged DO11.10 mice (Fig. 1). It also inhibited the production of IL-17 in the lung (Fig. 2A). As IL-17 is also produced by cells other than Th17 cells, we examined whether D1-like-R antagonist reduced the infiltration of Th17 cells into the lung by immunohistochemistry. D1-like-R antagonist inhibited the infiltration of IL-23R⁺ Th17 cells (Fig. 2B), suggesting that it indeed suppressed Th17-mediated immune response in the lung. We also confirmed by intracellular cytokine staining that D1-like-R antagonist suppressed the number of IL-17-producing CD4⁺ T cells (Th17 cells) in the lung (Fig. 2C). In this study, D1-like-R antagonist did not suppress nonspecific neutrophilic airway inflammation induced by intratracheal LPS injection (Fig. 4A). Further, D1-like-R antagonist did not suppress OVA-induced Th2-mediated eosinophilic airway inflammation, which was induced by OVA sensitization and nebulization (Fig. 4B). Therefore, antagonizing D1-like-R could suppress specific Th17 immune response-mediated inflammation in the lung.

D1-like-R antagonist significantly suppressed the production of IL-23—one very important cytokine used to differentiate and accelerate Th17-mediated immune response (33)—by lung CD11c⁺ APCs (Fig. 3A, 3D). We also confirmed by intracellular cytokine staining that D1-like-R antagonist inhibited the number of IL-23-producing CD11c⁺ or CD11c^{high} APCs in the lung (Fig. 3E). We previously reported that treatment of proteolipid protein peptide-pulsed DCs with D1-like-R antagonist SCH23390 directly prevents the development of EAE induced by adoptive transfer of DCs (14). These findings suggest that modulation of DC function including IL-23 production would be one major mechanism of Th17 suppression by a D1-like-R antagonist. Further, this raised the possibility that D1-like-R antagonist would decrease cAMP formation and subsequently decrease dopamine synthesis in DCs, thus inhibiting Th17 differentiation.

Other mechanisms such as direct action on T cells might be involved in this Th17 immune suppression. In this study, we found that D1-like-R antagonist would not increase Foxp3⁺ Tregs in the lung in DO11.10 mice (Fig. 2D). However, Kipnis et al. (46) reported that dopamine reduced the suppressive activity by CD4⁺CD25⁺ Tregs, and D1-like-R antagonist SCH23390 abolished their suppression. Therefore, increase in suppressive activity of Tregs might be one mechanism of Th17 suppression by D1-like-R antagonist. Furthermore, the effect on other cells such as epithelial cells (47–49) should also be examined.

D2-like-R agonist such as pramipexole hydrochloride attenuates Th17-mediated immune responses and thus the development of EAE in a similar way to D1-like-R antagonists (14). Preliminary study suggested that pramipexole hydrochloride also suppressed OVA-induced neutrophilic airway inflammation in DO11.10 mice (data not shown). Pramipexole hydrochloride is known to be effective for treating Parkinson's disease (50) and is prescribed to patients. Although whether D2-like-R agonist indeed suppresses

Th17 responses in patients with Parkinson's disease has not been fully clarified, our results suggested that D2-like-R agonist might be useful for controlling other Th17-mediated disease such as neutrophil-dominant severe asthma.

The extent of dopamine receptor expression is different among diseases (51–55). For example, expression of D3 receptor is up-regulated in schizophrenia (51, 52). Expression of D2-like-R decreased in Alzheimer's disease (53). Furthermore, Barbanti et al. (54) reported that expression of D1-like-R and D2-like-R on PBLs was upregulated in Parkinson's disease. However, the expression of dopamine receptor in asthma and the effect of asthma severity on the expression of dopamine receptor were not investigated. Thus, the effect of D1-like-R antagonist on airway inflammation might depend on the asthma severity.

In this study, we measured actual plasma concentration of SCH23390. Concentration of SCH23390 when given orally in the concentration and regimen we used was $5.4 \pm 2.2 \mu\text{M}$. To date, the pharmacokinetics of SCH23390 has not been fully clarified. For example, Hietala et al. (56) reported that s.c. administration of SCH23390 into the rat resulted in rapid absorption and caused peak concentration in plasma after 5 min and in brain after 15 min. An estimated elimination half-life was ~ 30 min in plasma and 40–60 min in brain. They also suggested that SCH23390 still remained in the brain at least after 4 h from administration, although there were no measurable levels in plasma after 2 or 4 h. Further, Monsma et al. (57) reported that SCH23390 binds to the receptor with high sp. act. (400 fmol/mg protein) and an affinity (0.3 nM). Therefore, SCH23390 might remain for a longer time in the tissue including the immune system and work on D1-like-R-expressed cells even if it was rapidly eliminated in plasma. Although effective plasma concentration of SCH23390 in mice has not been fully elucidated, these findings and our data suggested that SCH23390 could work on the tissue including the immune system in DO11.10 mice.

In conclusion, the current study demonstrates a novel role of dopamine as an immune regulator of Th17-mediated response in the lung. D1-like-R antagonist suppressed Th17-mediated immune response and neutrophilic inflammation in the lung, at least partly by inhibiting the production of IL-23 by lung APCs. Therefore, antagonizing D1-like-R could be a very effective strategy for regulating Th17-mediated neutrophilic airway inflammation and thus control neutrophil-dominant severe asthma.

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References

- Bochner, B. S., B. J. Udem, and L. M. Lichtenstein. 1994. Immunological aspects of allergic asthma. *Annu. Rev. Immunol.* 12: 295–335.
- Randolph, D. A., R. Stephens, C. J. Carruthers, and D. D. Chaplin. 1999. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J. Clin. Invest.* 104: 1021–1029.
- Randolph, D. A., C. J. Carruthers, S. J. Szabo, K. M. Murphy, and D. D. Chaplin. 1999. Modulation of airway inflammation by passive transfer of allergen-specific Th1 and Th2 cells in a mouse model of asthma. *J. Immunol.* 162: 2375–2383.
- McKinley, L., J. F. Alcorn, A. Peterson, R. B. Dupont, S. Kapadia, A. Logar, A. Henry, C. G. Irvin, J. D. Piganelli, A. Ray, and J. K. Kolls. 2008. Th17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J. Immunol.* 181: 4089–4097.
- Lindén, A. 2001. Role of interleukin-17 and the neutrophil in asthma. *Int. Arch. Allergy Immunol.* 126: 179–184.

- European Network for Understanding Mechanisms of Severe Asthma. 2003. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur. Respir. J.* 22: 470–477.
- Barczyk, A., W. Pierzchala, and E. Sozańska. 2003. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir. Med.* 97: 726–733.
- Al-Ramli, W., D. Préfontaine, F. Chouiali, J. G. Martin, R. Olivenstein, C. Lemièrre, and Q. Hamid. 2009. Th17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J. Allergy Clin. Immunol.* 123: 1185–1187.
- Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245–252.
- Murphy, K. M., W. Ouyang, J. D. Farrar, J. Yang, S. Ranganath, H. Asnagli, M. Afkarian, and T. L. Murphy. 2000. Signaling and transcription in T helper development. *Annu. Rev. Immunol.* 18: 451–494.
- Weaver, C. T., R. D. Hatton, P. R. Mangan, and L. E. Harrington. 2007. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.* 25: 821–852.
- Lambrecht, B. N., and H. Hammad. 2003. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* 3: 994–1003.
- van Rijt, L. S., S. Jung, A. Kleinjan, N. Vos, M. Willart, C. Duez, H. C. Hoogsteden, and B. N. Lambrecht. 2005. In vivo depletion of lung CD11c⁺ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.* 201: 981–991.
- Nakano, K., T. Higashi, K. Hashimoto, R. Takagi, Y. Tanaka, and S. Matsushita. 2008. Antagonizing dopamine D1-like receptor inhibits Th17 cell differentiation: preventive and therapeutic effects on experimental autoimmune encephalomyelitis. *Biochem. Biophys. Res. Commun.* 373: 286–291.
- Nakano, K., T. Higashi, R. Takagi, K. Hashimoto, Y. Tanaka, and S. Matsushita. 2009. Dopamine released by dendritic cells polarizes Th2 differentiation. *Int. Immunol.* 21: 645–654.
- Missale, C., S. R. Nash, S. W. Robinson, M. Jaber, and M. G. Caron. 1998. Dopamine receptors: from structure to function. *Physiol. Rev.* 78: 189–225.
- Hashimoto, K., T. Inoue, T. Higashi, S. Takei, T. Awata, S. Katayama, R. Takagi, H. Okada, and S. Matsushita. 2009. Dopamine D1-like receptor antagonist, SCH23390, exhibits a preventive effect on diabetes mellitus that occurs naturally in NOD mice. *Biochem. Biophys. Res. Commun.* 383: 460–463.
- Okada, H., T. Inoue, K. Hashimoto, H. Suzuki, and S. Matsushita. 2009. D1-like receptor antagonist inhibits IL-17 expression and attenuates crescent formation in nephrotoxic serum nephritis. *Am. J. Nephrol.* 30: 274–279.
- Knott, P. G., P. R. Gater, and C. P. Bertrand. 2000. Airway inflammation driven by antigen-specific resident lung CD4(+) T cells in alpha-beta-T cell receptor transgenic mice. *Am. J. Respir. Crit. Care Med.* 161: 1340–1348.
- Wilder, J. A., D. D. Collie, D. E. Bice, Y. Tesfagzi, C. R. Lyons, and M. F. Lipscomb. 2001. Ovalbumin aerosols induce airway hyperactivity in naive DO11.10 T cell receptor transgenic mice without pulmonary eosinophilia or OVA-specific antibody. *J. Leukoc. Biol.* 69: 538–547.
- Nakae, S., Y. Komiyama, A. Nambu, K. Sudo, M. Iwase, I. Homma, K. Sekikawa, M. Asano, and Y. Iwakura. 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17: 375–387.
- Nakae, S., H. Suto, G. J. Berry, and S. J. Galli. 2007. Mast cell-derived TNF can promote Th17 cell-dependent neutrophil recruitment in ovalbumin-challenged OTH mice. *Blood* 109: 3640–3648.
- Nakagome, K., M. Dohi, K. Okunishi, Y. Komagata, K. Nagatani, R. Tanaka, J. Miyazaki, and K. Yamamoto. 2005. In vivo IL-10 gene delivery suppresses airway eosinophilia and hyperactivity by down-regulating APC functions and migration without impairing the antigen-specific systemic immune response in a mouse model of allergic airway inflammation. *J. Immunol.* 174: 6955–6966.
- Nakagome, K., M. Dohi, K. Okunishi, R. Tanaka, T. Kouro, M. R. Kano, K. Miyazono, J. Miyazaki, K. Takatsu, and K. Yamamoto. 2007. IL-5-induced hypereosinophilia suppresses the antigen-induced immune response via a TGF- β -dependent mechanism. *J. Immunol.* 179: 284–294.
- Nakagome, K., K. Okunishi, M. Imamura, H. Harada, T. Matsumoto, R. Tanaka, J. Miyazaki, K. Yamamoto, and M. Dohi. 2009. IFN- γ attenuates antigen-induced overall immune response in the airway as a Th1-type immune regulatory cytokine. *J. Immunol.* 183: 209–220.
- Okunishi, K., M. Dohi, K. Nakagome, R. Tanaka, S. Mizuno, K. Matsumoto, J. Miyazaki, T. Nakamura, and K. Yamamoto. 2005. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. *J. Immunol.* 175: 4745–4753.
- Nakagome, K., M. Dohi, K. Okunishi, R. Tanaka, J. Miyazaki, and K. Yamamoto. 2006. In vivo IL-10 gene delivery attenuates bleomycin induced pulmonary fibrosis by inhibiting the production and activation of TGF- β in the lung. *Thorax* 61: 886–894.
- To, Y., M. Dohi, R. Tanaka, A. Sato, K. Nakagome, and K. Yamamoto. 2001. Early interleukin 4-dependent response can induce airway hyperactivity before development of airway inflammation in a mouse model of asthma. *Lab. Invest.* 81: 1385–1396.
- Hyllbrant, B., N. Tyrefors, K. E. Markides, and B. Långström. 1999. On the use of liquid chromatography with radio- and ultraviolet absorbance detection coupled to mass spectrometry for improved sensitivity and selectivity in determination of specific radioactivity of radiopharmaceuticals. *J. Pharm. Biomed. Anal.* 20: 493–501.
- Takagi, R., T. Higashi, K. Hashimoto, K. Nakano, Y. Mizuno, Y. Okazaki, and S. Matsushita. 2008. B cell chemoattractant CXCL13 is preferentially expressed by human Th17 cell clones. *J. Immunol.* 181: 186–189.
- Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057–1061.

32. Zou, W. 2006. Regulatory T cells, tumour immunity and immunotherapy. *Nat. Rev. Immunol.* 6: 295–307.
33. Aggarwal, S., N. Ghilardi, M. H. Xie, F. J. de Sauvage, and A. L. Gurney. 2003. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* 278: 1910–1914.
34. Bergquist, J., A. Tarkowski, R. Ekman, and A. Ewing. 1994. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. *Proc. Natl. Acad. Sci. USA* 91: 12912–12916.
35. Josefsson, E., J. Bergquist, R. Ekman, and A. Tarkowski. 1996. Catecholamines are synthesized by mouse lymphocytes and regulate function of these cells by induction of apoptosis. *Immunology* 88: 140–146.
36. Ghosh, M. C., A. C. Mondal, S. Basu, S. Banerjee, J. Majumder D. Bhattacharya, and P. S. Dasgupta. 2003. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. *Int. Immunopharmacol.* 3: 1019–1026.
37. Saha, B., A. C. Mondal, S. Basu, and P. S. Dasgupta. 2001. Circulating dopamine level, in lung carcinoma patients, inhibits proliferation and cytotoxicity of CD4+ and CD8+ T cells by D1 dopamine receptors: an in vitro analysis. *Int. Immunopharmacol.* 1: 1363–1374.
38. Carr, L., A. Tucker, and R. Fernandez-Botran. 2003. In vivo administration of L-dopa or dopamine decreases the number of splenic IFN γ -producing cells. *J. Neuroimmunol.* 137: 87–93.
39. Tsao, C. W., Y. S. Lin, and J. T. Cheng. 1997. Effect of dopamine on immune cell proliferation in mice. *Life Sci.* 61: 361–371.
40. Besser, M. J., Y. Ganor, and M. Levite. 2005. Dopamine by itself activates either D2, D3 or D1/D5 dopaminergic receptors in normal human T-cells and triggers the selective secretion of either IL-10, TNF α or both. *J. Neuroimmunol.* 169: 161–171.
41. Meredith, E. J., A. Chamba, M. J. Holder, N. M. Barnes, and J. Gordon. 2005. Close encounters of the monoamine kind: immune cells betray their nervous disposition. *Immunology* 115: 289–295.
42. Bergquist, J., A. Tarkowski, A. Ewing, and R. Ekman. 1998. Catecholaminergic suppression of immunocompetent cells. *Immunol. Today* 19: 562–567.
43. McKenna, F., P. J. McLaughlin, B. J. Lewis, G. C. Sibbring, J. A. Cummmerson, D. Bowen-Jones, and R. J. Moots. 2002. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. *J. Neuroimmunol.* 132: 34–40.
44. Ricci, A., E. Bronzetti, F. Mignini, S. K. Tayebati, D. Zaccheo, and F. Amenta. 1999. Dopamine D1-like receptor subtypes in human peripheral blood lymphocytes. *J. Neuroimmunol.* 96: 234–240.
45. Ricci, A., and F. Amenta. 1994. Dopamine D5 receptors in human peripheral blood lymphocytes: a radioligand binding study. *J. Neuroimmunol.* 53: 1–7.
46. Kipnis, J., M. Cardon, H. Avidan, G. M. Lewitus, S. Mordehay, A. Rolls, Y. Shani, and M. Schwartz. 2004. Dopamine, through the extracellular signal-regulated kinase pathway, downregulates CD4⁺CD25⁺ regulatory T-cell activity: implications for neurodegeneration. *J. Neurosci.* 24: 6133–6143.
47. Lin, Y. S., Q. Gu, and L. Y. Lee. 2003. Activation of dopamine D2-like receptors attenuates pulmonary C-fiber hypersensitivity in rats. *Am. J. Respir. Crit. Care Med.* 167: 1096–1101.
48. Helms, M. N., X. J. Chen, S. Ramosevac, D. C. Eaton, and L. Jain. 2006. Dopamine regulation of amiloride-sensitive sodium channels in lung cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 290: L710–L722.
49. Helms, M. N., J. Self, H. F. Bao, L. C. Job, L. Jain, and D. C. Eaton. 2006. Dopamine activates amiloride-sensitive sodium channels in alveolar type I cells in lung slice preparations. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 291: L610–L618.
50. Antonini, A., and P. Barone. 2008. Dopamine agonist-based strategies in the treatment of Parkinson's disease. *Neurol. Sci.* 29(Suppl 5): S371–S374.
51. Ilani, T., D. Ben-Shachar, R. D. Strous, M. Mazor, A. Sheinkman, M. Kotler, and S. Fuchs. 2001. A peripheral marker for schizophrenia: increased levels of D3 dopamine receptor mRNA in blood lymphocytes. *Proc. Natl. Acad. Sci. USA* 98: 625–628.
52. Kwak, Y. T., M. S. Koo, C. H. Choi, and I. Sunwoo. 2001. Change of dopamine receptor mRNA expression in lymphocyte of schizophrenic patients. *BMC Med. Genet.* 2: 3.
53. Barbanti, P., G. Fabbrini, A. Ricci, G. Bruno, R. Cerbo, E. Bronzetti, F. Amenta, and G. Luigi Lenzi. 2000. Reduced density of dopamine D2-like receptors on peripheral blood lymphocytes in Alzheimer's disease. *Mech. Ageing Dev.* 120: 65–75.
54. Barbanti, P., G. Fabbrini, A. Ricci, R. Cerbo, E. Bronzetti, B. Caronti C. Calderaro, L. Felici, F. Stocchi, G. Meco, et al. 1999. Increased expression of dopamine receptors on lymphocytes in Parkinson's disease. *Mov. Disord.* 14: 764–771.
55. Barbanti, P., G. Fabbrini, A. Ricci, M. P. Pascali, E. Bronzetti, F. Amenta, G. L. Lenzi, and R. Cerbo. 2000. Migraine patients show an increased density of dopamine D3 and D4 receptors on lymphocytes. *Cephalgia* 20: 15–19.
56. Hietala, J., T. Seppälä, J. Lappalainen, and E. Syvälahti. 1992. Quantification of SCH 39166, a novel selective D1 dopamine receptor antagonist, in rat brain and blood. *Psychopharmacology (Berl.)* 106: 455–458.
57. Monsma, F. J., Jr., L. C. Mahan, L. D. McVittie, C. R. Gerfen, and D. R. Sibley. 1990. Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. *Proc. Natl. Acad. Sci. USA* 87: 6723–6727.