
Amelioration of experimental allergic rhinitis with suppression of topical immune responses by lack of IL-27/WSX-1 signaling

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Background: Allergic rhinitis is 1 of the most common atopic diseases with strong similarity to asthma. Interleukin (IL) 27 is an immunosuppressive cytokine, and lack of the IL-27 receptor (WSX-1) resulted in exacerbation of allergic airway hyperresponsiveness.

Objective: To address the role of IL-27/WSX-1 in the rhinitis model compared with the asthma model.

Methods: Wild-type or *WSX-1*^{-/-} female mice were immunized intraperitoneally 4 times with ovalbumin adsorbed to aluminum potassium sulfate at a 1-week interval. The sensitized mice were then challenged for 14 days with ovalbumin intranasally from days 22 to 35. Clinical scores, serum antigen specific IgE levels, and cytokine production in the nasal lavage fluid were examined. Cytokine and chemokine expression in the cervical lymph nodes, nasopharynx-associated lymphoid tissues, and nasal mucosa was also examined.

Results: *WSX-1*^{-/-} mice developed augmented immune responses in the serum (IgE production), cervical lymph nodes (cytokine and chemokine expression), and nasopharynx-associated lymphoid tissues (cytokine and chemokine expression), whereas local responses, such as clinical scores and nasal lavage fluid cytokine production, were reduced in *WSX-1*^{-/-} mice. Expression of some chemokines was also reduced in the nasal mucosal tissues of *WSX-1*^{-/-} mice.

Conclusion: In contrast to the immunosuppressive role observed in the asthma model, IL-27/WSX-1 topically plays an exacerbating role in the pathogenesis of allergic rhinitis, presumably through differential expression of chemokines.

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INTRODUCTION

Allergic rhinitis is 1 of the most common atopic diseases with similarity to asthma. Incidence of allergic rhinitis has been markedly increasing in industrial countries, causing an important medical and social problem.¹ Allergic rhinitis is regarded as a T_H2-mediated disorder characterized by antigen-specific IgE production, infiltration of inflammatory cells into the nasal mucosa, and several nasal symptoms, such as sneezing, itching, and rhinorrhea.^{1,2} In allergic states, the allergen triggers a T_H2-predominant immune response, leading to production of antigen-specific IgE. Subsequent exposure to the allergen provokes IgE-mediated stimulation of

mast cells to release chemical mediators.³ Among T_H2 cytokines, interleukin (IL) 4 participates in T_H2 cell differentiation,⁴ IgE isotype class switching,⁵ and airway remodeling, including enhanced collagen synthesis by fibroblasts.⁶ IL-5 promotes differentiation and migration of eosinophils.^{7–9} When released by mast cells and other cells, chemokines such as CCL5 (RANTES), CCL11 (eotaxin 1), and CCL17 (TARC) trigger recruitment of inflammatory cells such as eosinophils and T_H2 cells, thereby contributing to the induction of allergic responses.^{1,10}

WSX-1 is a class 1 cytokine receptor with homology to glycoprotein 130 of the IL-6 receptor.¹¹ WSX-1 is highly expressed in the spleen, thymus, and lymph nodes and particularly in CD4⁺ T cells, as well as in CD8⁺ T cells and natural killer cells.^{11–13} In addition, WSX-1 is reportedly expressed in various types of cells, including endothelial cells and epithelial cells,¹⁴ implicating WSX-1-mediated regulation of cell activity in various tissues and cells. IL-27, a heterodimeric cytokine consisting of p28 and Epstein-Barr virus-induced gene 3 (*EBI3*), has been identified as a ligand for WSX-1.¹⁵ IL-27 stimulation of naive CD4⁺ T cells induces proliferation and interferon γ (IFN- γ) production, demonstrating its critical role in T_H1 differentiation.¹³ However, recent lines of evidence also revealed a new role for IL-27 as a suppressor of inflammatory responses.¹⁶ In experimental allergic asthma model, WSX-1-deficiency resulted in exac-

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erbation of the disease with augmented production of T_H2 -type cytokines, such as IL-4 and IL-5.¹⁷

In the current study, we examined the role of IL-27/WSX-1 in the regulation of allergic rhinitis. We found that in the allergic rhinitis model, local symptoms such as clinical scores and cytokine production, including IL-4 and IL-5 in nasal lavage fluid (NLF), were lower in WSX-1-deficient mice than in wild-type (WT) mice, although systemic features, including serum IgE levels and cytokine production by cervical lymph node cells, were higher in WSX-1-deficient mice. Expression of some chemokines was lower in the nasal mucosa tissues of WSX-1-deficient mice than in WT mice. These results demonstrated that IL-27/WSX-1 topically plays an exacerbating role in the pathogenesis of allergic rhinitis, presumably through differential expression of chemokines.

MATERIALS AND METHODS

Animals

WSX-1^{-/-} mice were generated as described previously¹³ and were backcrossed more than 9 times to C57BL/6 mice. Mice were housed in microisolator cages and were used between 8 and 14 weeks of age. Age- and sex-matched WT C57BL/6 mice (Seac Yoshitomi, Fukuoka, Japan) were used as controls. All experiments were approved by the institutional animal research committee of Saga University and conformed to the animal care guidelines of the American Physiologic Society.

Sensitization and Intranasal Challenge With Ovalbumin

WT or WSX-1^{-/-} female mice were immunized intraperitoneally 4 times with 20 μ g of ovalbumin (0.2 mL of 100 μ g/mL in saline) adsorbed to aluminum potassium sulfate (Imject Alum; Pierce, Rockford, Illinois) at a 1-week interval. The sensitized mice were then challenged for 14 days with ovalbumin (10 μ L of 25 mg/mL of ovalbumin per mouse) intranasally from days 22 to 35. Twenty-four hours after the last challenge, measurements of airway responsiveness and other parameters were performed as follows. The protocol for the induction of rhinitis is shown in Figure 1. The protocol for the sensitization and aerosol challenge for asthma induction was described previously.¹⁷

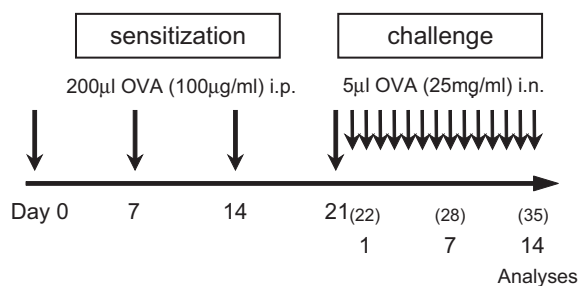


Figure 1. Protocols for sensitization and challenge. Wild-type (WT) and WSX-1^{-/-} mice were sensitized with intraperitoneal injection of ovalbumin (OVA) in alum on days 0, 7, 14, and 21 and then challenged on days 22 to 35 with intranasal administration of OVA.

Clinical Symptoms

On day 22 (after 4 rounds of intraperitoneal sensitization) and on days 28 and 35 (on days 7 and 14 after nasal challenges, respectively), nasal symptoms were evaluated for each mouse by counting the number of sneezes and nasal itching motions (nasal rubbing) for 10 minutes after ovalbumin intranasal challenge.¹⁸

Determination of Antigen-Specific Antibodies in Serum

Serum samples were collected within 24 hours after the last nasal challenge. Amounts of total or antigen-specific IgE in serum samples were measured with a mouse ovalbumin IgE enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Shibukawa, Japan).

NLF Analyses and Histologic Evaluation

For NLF analyses, the nasal cavities were gently lavaged with 150 μ L of saline 3 times 24 hours after the last nasal challenge. The fluid was collected for determination of cytokines with ELISA kits (R&D Systems, Minneapolis, Minnesota). To avoid tissue damage by lavage, different sets of mice were prepared similarly and killed, and their heads removed, fixed with 4% paraformaldehyde phosphate-buffered saline, decalcified, and embedded in paraffin.

NALT Cell and Nasal Mucosal Tissues Preparation

Nasopharynx-associated lymphoid tissue (NALT) removal was performed as described elsewhere.^{3,10,18} Briefly, after exsanguinations of the mice, the lower jaw and cheek muscles were removed, and NALT was exposed by carefully peeling away the palate. NALT localized on the posterior part of the palate was teased out with syringe needles in ice-cold RPMI 1640 culture medium supplemented with 10% fetal bovine serum, and then the cells were passed through a cell strainer. Nasal mucosal tissues were then removed and specimens cut into 3- to 4-mm³ pieces and passed through a cell strainer.

Real-Time Reverse-Transcription Polymerase Chain Reaction Analysis

Twenty-four hours after the last ovalbumin challenge, total RNA was isolated from nasal mucosa tissue, NALT, and draining lymph node (cervical lymph node) lymphocytes using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, California) and reverse transcribed to complementary DNA with random primers. Reverse-transcription polymerase chain reaction was performed using the following appropriate pairs of primers (sense and antisense): IL-5, 5'-CGC TCA CCG AGC TCT GTT G-3' and 5'-CCA ATG CAT AGC TGG TGA TTT TT-3'; IFN- γ , 5'-TCA AGT GGC ATA GAT GTG GAA GAA-3' and 5'-TGG CTC TGC AGG ATT TTC ATG-3'; IL-4, 5'-ACA GGA GAA GGG ACG CCAT-3' and 5'-GAA GCC CTA CAG ACG AGC TCA-3'; WSX-1, 5'-GGA CCA GGA AAC CAT TGG AGT-3' and 5'-GTT GAG CTT GTC CAG GCT GTC-3'; p28, 5'-GGC CAT GAG GCT GGA TCTC-3' and 5'-AAC ATT TGA ATC CTG CAG CCA -3'; and EBI3, 5'-ACC CAT TGA

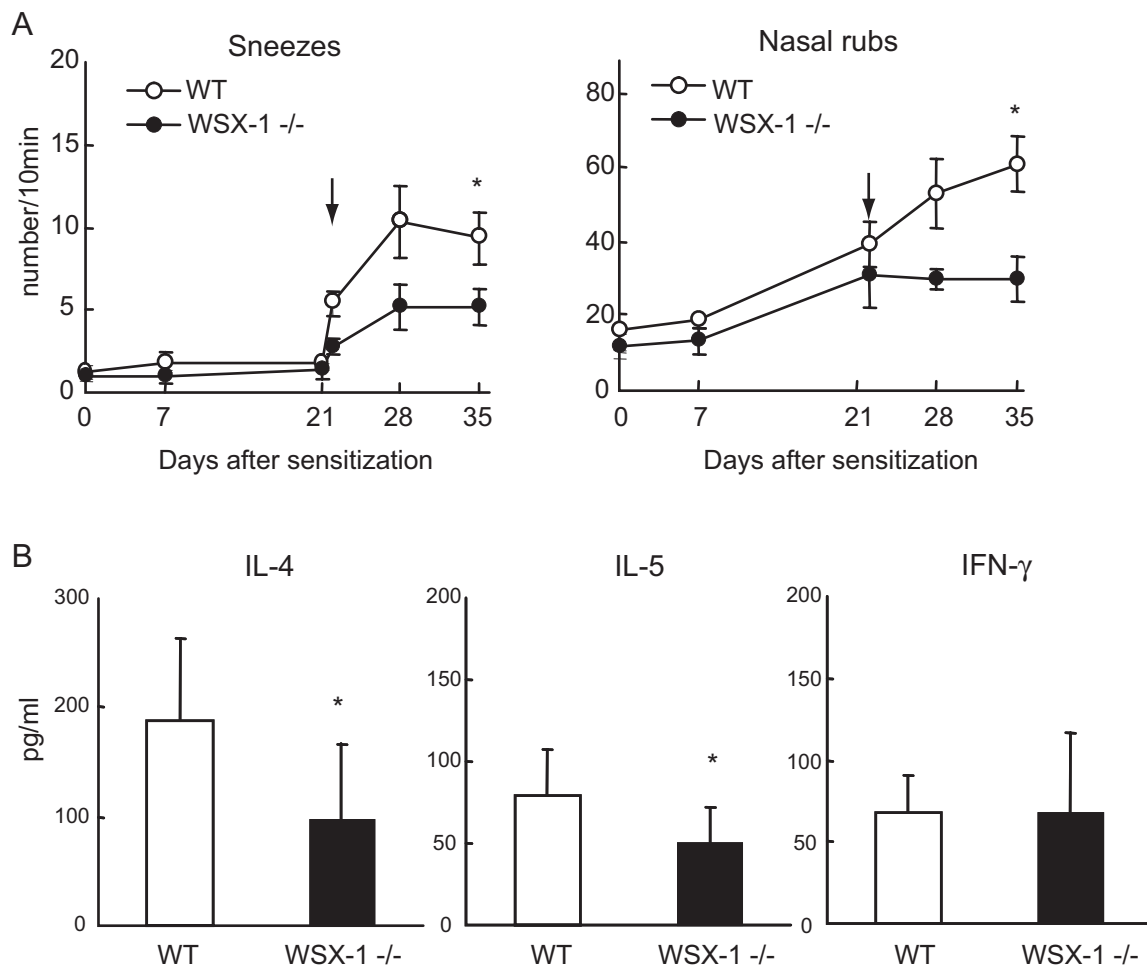


Figure 2. Reduced clinical symptoms of rhinitis and T_H2 cytokine production in $WSX-1^{-/-}$ mice after ovalbumin challenge. A, Mice were immunized and challenged with ovalbumin as described in the "Materials and Methods" section. Clinical scores such as sneezes and nasal rubs were measured for 10 minutes after the last intranasal challenge (on day 35) in wild-type (WT) and $WSX-1^{-/-}$ mice. Experiments were repeated 5 times with similar results. B, Twenty-four hours after the last ovalbumin challenge, nasal lavage fluid was collected from ovalbumin-challenged WT and ovalbumin-challenged $WSX-1^{-/-}$ mice. The levels of interleukin (IL) 4, IL-5, and interferon γ (IFN- γ) were determined by enzyme-linked immunosorbent assay. * $P < .05$. Experiments were repeated 3 times with similar results.

AGC CAC GAC TT-3' and 5'-AGC TGA CAC CTG GAT GCA ATA CT-3'; CCL5, 5'-CTC CCT GCT TTG CCT AC-3' and 5'-ACA CAC TTG GCG GTT CCT TC-3'; CCL11, 5'-GGC TCC ATC CCA ACT TCC TG-3' and 5'-ATC TCT TTG CCC AAC CTG GTC-3'; CCL17, 5'-CAG GGA TGC CAT CGT GTT TCT-3' and 5'-GGT CAC AGG CCG CTT TAT GTT-3'. As an internal control for RNA input and reverse transcripts, we measured the level of β -actin messenger RNA expression with real-time polymerase chain reaction (ABI PRISM 7000; Applied Biosystems, Foster City, California).

Antigen-Induced Cytokine Production by Cervical Lymph Node $CD4^+$ T Lymphocytes

Cervical lymph nodes were removed from ovalbumin-sensitized mice 24 hours after the last ovalbumin challenge. Pu-

rified $CD4^+$ T lymphocytes (1×10^5 per well) were cultured in the presence of irradiated (30 Gy) syngeneic spleen cells (1×10^6 per well) and ovalbumin (0, 50, and 500 $\mu\text{g/mL}$). The supernatants were collected and analyzed for the production of IFN- γ , IL-4, and IL-5 using ELISA development kits according to the manufacturer's direction (R&D Systems). After the 96-hour culture, cells were incubated for 6 hours with [^3H]-thymidine (MP Biomedicals, Solon, Ohio) and added at a final concentration of 1 μCi per well, and then incorporated radioactivity was measured with a 1205 Beta-plate (Wallac/PerkinElmer, Waltham, Massachusetts).

Data Analysis

Differences among groups were analyzed using unpaired t tests or an analysis of variance together with a Student-Newman-Keuls analysis. Nonparametric data were analyzed

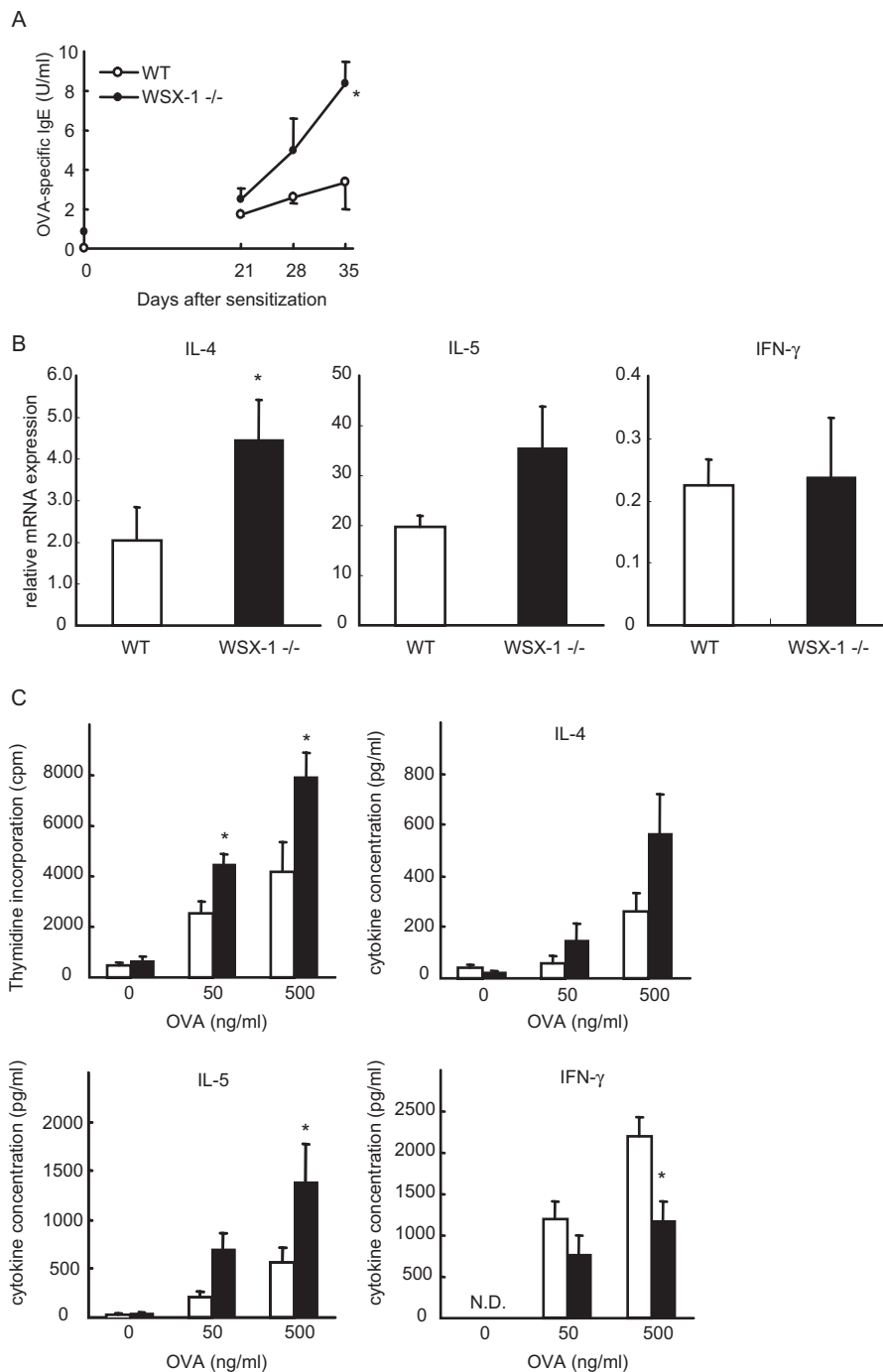


Figure 3. Augmented systemic immune responses in *WSX-1^{-/-}* mice. A, Antigen-specific IgE production in ovalbumin (OVA)-challenged mice were determined. Serum samples were collected from OVA-challenged wild-type (WT) (open circles) and *WSX-1^{-/-}* (closed circles) mice 24 hours after the last OVA challenge or on day 0 before immunization. OVA-specific IgE amounts were determined by enzyme-linked immunosorbent assay. B, Expression of cytokine messenger RNA (mRNA) in cervical lymph node CD4⁺ T cells were evaluated by real-time reverse-transcription polymerase chain reaction 24 hours after the last OVA challenge on day 35. Open columns, WT mice; closed columns, *WSX-1^{-/-}* mice. C, Antigen-specific proliferation and cytokine production of cervical lymph node CD4⁺ T cells were measured. CD4⁺ T cells from OVA-challenged WT (open columns) and OVA-challenged *WSX-1^{-/-}* (closed columns) mice were cultured for 4 days with indicated concentration of OVA. Error bars indicate SEM (n = 4 to 5 mice per group). **P* < .05 over WT sample. Experiments were repeated 4 times with similar results. IFN γ indicates interferon γ ; IL, interleukin.

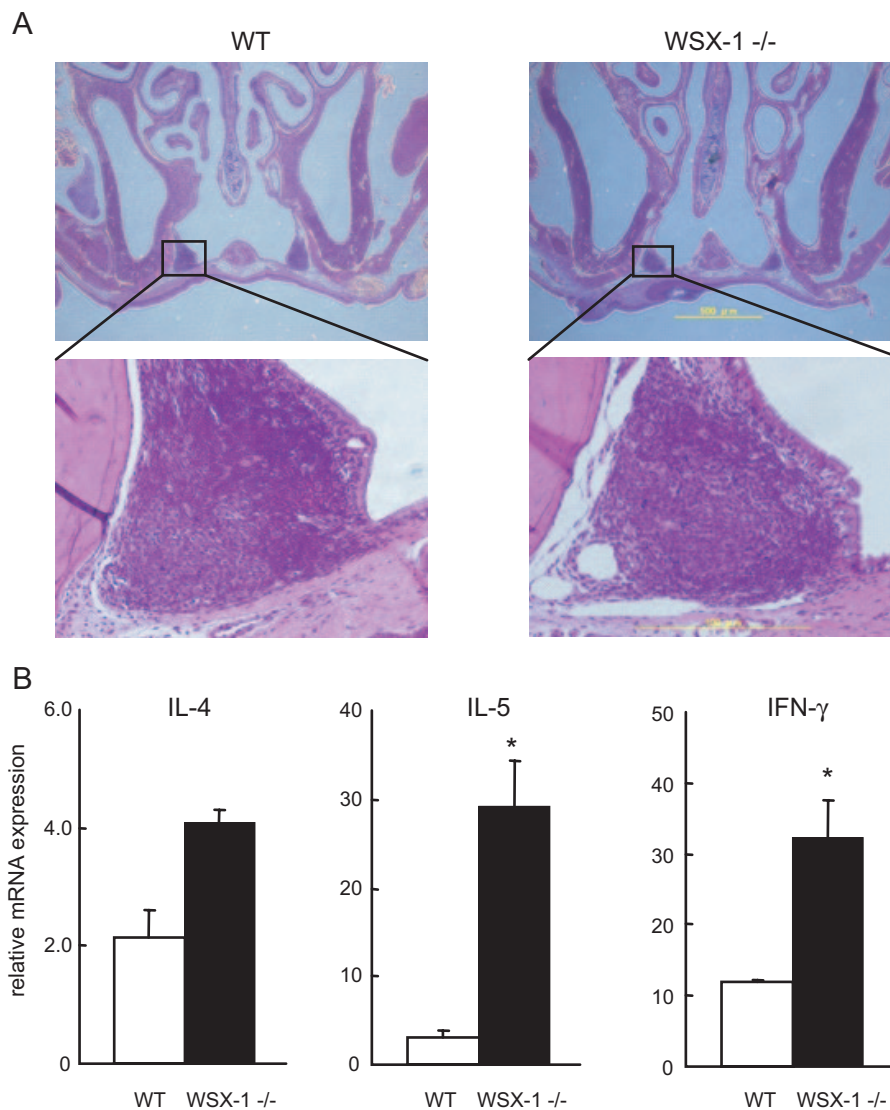


Figure 4. Augmented cytokines expression by nasopharynx-associated lymphoid tissue lymphocytes in *WSX-1^{-/-}* mice. A, Histologic analysis of nasal mucosal tissues and nasopharynx-associated lymphoid tissues. Mice were immunized and challenged with ovalbumin (OVA) as described in the “Materials and Methods” section. Tissue specimen from wild-type (WT) and *WSX-1^{-/-}* mice were removed on day 35, fixed, and stained with hematoxylin-eosin. Original magnification was $\times 40$ and $\times 100$ for lower and higher magnifications, respectively. B, Expression of cytokine messenger RNA (mRNA) was evaluated as in the legend to Figure 3B. Error bars indicate SEM ($n = 4-5$ mice per group). * $P < .05$ compared with the WT sample. Experiments were repeated 3 times with similar results. IFN γ indicates interferon γ ; IL, interleukin.

using the Kruskal-Wallis test, followed by the Mann-Whitney test. $P < .05$ was considered statistically significant.

RESULTS

Reduced Clinical Symptoms of Allergic Rhinitis in WSX-1-Deficient Mice

To clarify the role of IL-27/WSX-1 in the control of allergic diseases in the upper respiratory tract, the allergic rhinitis mouse model was used.¹⁸ Sneezes and nasal rubs were observed after the last intranasal challenge. As shown in Figure

2A, up to the seventh nasal challenge (on day 28), WT mice and *WSX-1^{-/-}* mice showed no significant symptomatic difference after intranasal challenge. However, after 14 days of continuous exposure on day 35, the frequency of sneezes and nasal rubs was significantly lower in *WSX-1^{-/-}* mice than in WT mice.

Next, we determined whether clinical score and nasal inflammation in *WSX-1^{-/-}* mice reflected altered cytokine profiles in the nasal cavity. After ovalbumin challenge, significantly higher amounts of IL-4 and IL-5 were produced in

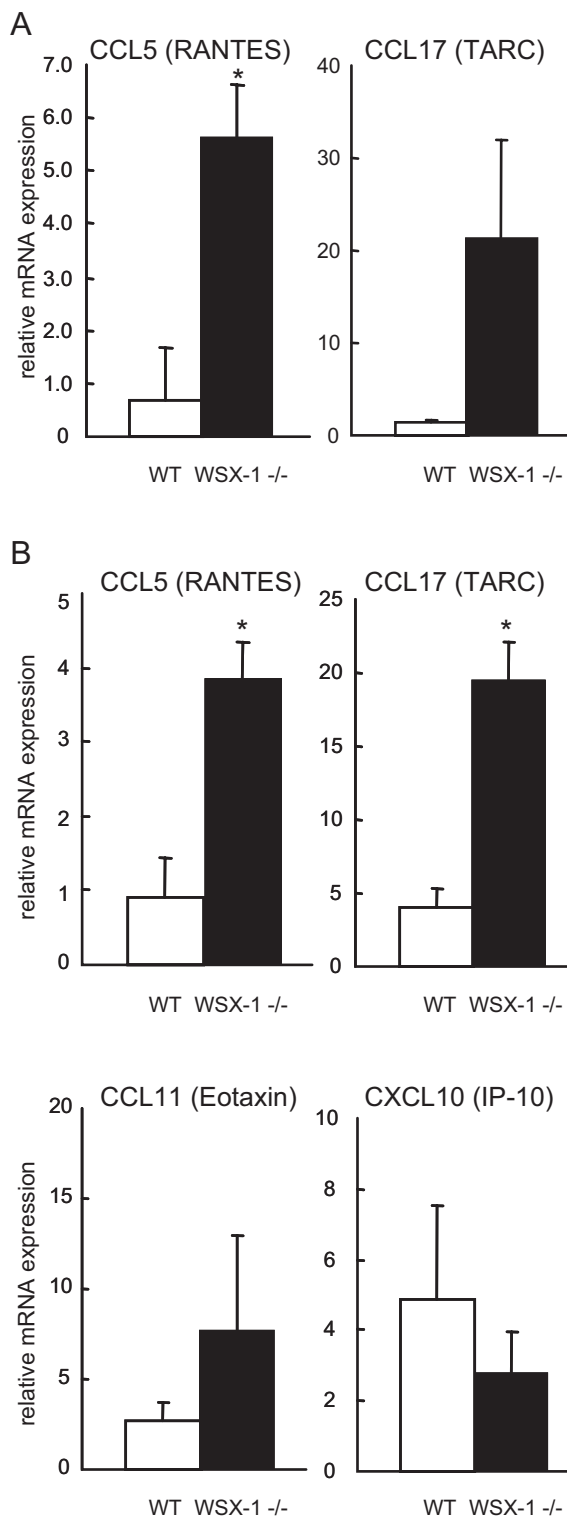


Figure 5. Augmented chemokine expression by nasopharynx-associated lymphoid tissue and cervical lymph node T cells in *WSX-1*^{-/-} mice. Expression of indicated chemokines was evaluated by real-time reverse-transcription polymerase chain reaction in nasopharynx-associated lymphoid tissue

both groups of mice. On day 35, the levels of IL-4 and IL-5 in NLF samples from *WSX-1*^{-/-} mice were significantly lower than those in samples from WT mice (Fig 2B). IFN- γ production was not different between *WSX-1*^{-/-} and WT mice. These data demonstrated that clinical symptoms such as clinical scores and cytokine production of IL-4 and IL-5 in the nasal cavity were reduced in the absence of IL-27/WSX-1 signaling.

Augmented Systemic Immune Responses in *WSX-1*^{-/-} Mice

The reduced rhinitis symptoms and cytokine production in NLF in *WSX-1*^{-/-} mice was rather unexpected, because *WSX-1*^{-/-} mice similarly immunized and challenged with ovalbumin in aerosol for airway hyperresponsiveness induction exhibited higher systemic and local immune responses.¹⁷ We thus examined systemic immune responses in the immunized and challenged mice. Development of serum antigen-specific IgE levels was determined because it is important in both allergic asthma and allergic rhinitis pathogenesis, especially in children.^{19–21} In response to ovalbumin challenge, ovalbumin specific IgE in serum samples increased in both the WT and *WSX-1*^{-/-} mice. The level, however, was higher in *WSX-1*^{-/-} mice than in WT mice (Fig 3A).

Similarly, expressions of IL-4 messenger RNA in CD4⁺ T cells from cervical lymph nodes were higher in *WSX-1*^{-/-} mice than in WT mice (Fig 3B). IFN- γ expression was comparable between WT and *WSX-1*^{-/-} mice. However, induction of IFN- γ was low in *WSX-1*^{-/-} and WT mice. For additional confirmation of cytokine overproduction by *WSX-1*-deficient CD4⁺ T cell, we evaluated the antigen-induced proliferation and cytokine production of CD4⁺ T cells purified from cervical lymph nodes of ovalbumin-challenged mice. As shown in Figure 3C, proliferation of CD4⁺ T cells in response to antigen stimulation was significantly higher in *WSX-1*^{-/-} T cells than in WT T cells. Likewise, *WSX-1*^{-/-} T cells from ovalbumin-challenged mice produced more IL-5 than the WT T cells in response to ovalbumin in vitro. Although not significant, the production of IL-4 also tended to be higher in *WSX-1*^{-/-} T cells compared with WT T cells. IFN- γ production was significantly lower in *WSX-1*^{-/-} T cells than in WT T cells, presumably because of the reciprocal suppression of T_H1 responses by augmented T_H2 responses. Augmentation of mainly T_H2 responses in *WSX-1*^{-/-} mice was consistent with the data observed in the previous study for asthma induction.¹⁷

(A) and cervical lymph node CD4⁺ T cells (B) from wild-type (WT) (open columns) and *WSX-1*^{-/-} (closed columns) mice. Twenty-four hours after the last ovalbumin challenge on day 35, total RNAs were isolated from nasopharynx-associated lymphoid tissue or cervical lymph node CD4⁺ T cells and examined for chemokine expression using specific pairs of primers. Error bars indicate SEM (n = 4–5 mice per group). **P* < .05 over WT samples. Experiments were repeated 3 times with similar results. mRNA indicates messenger RNA.

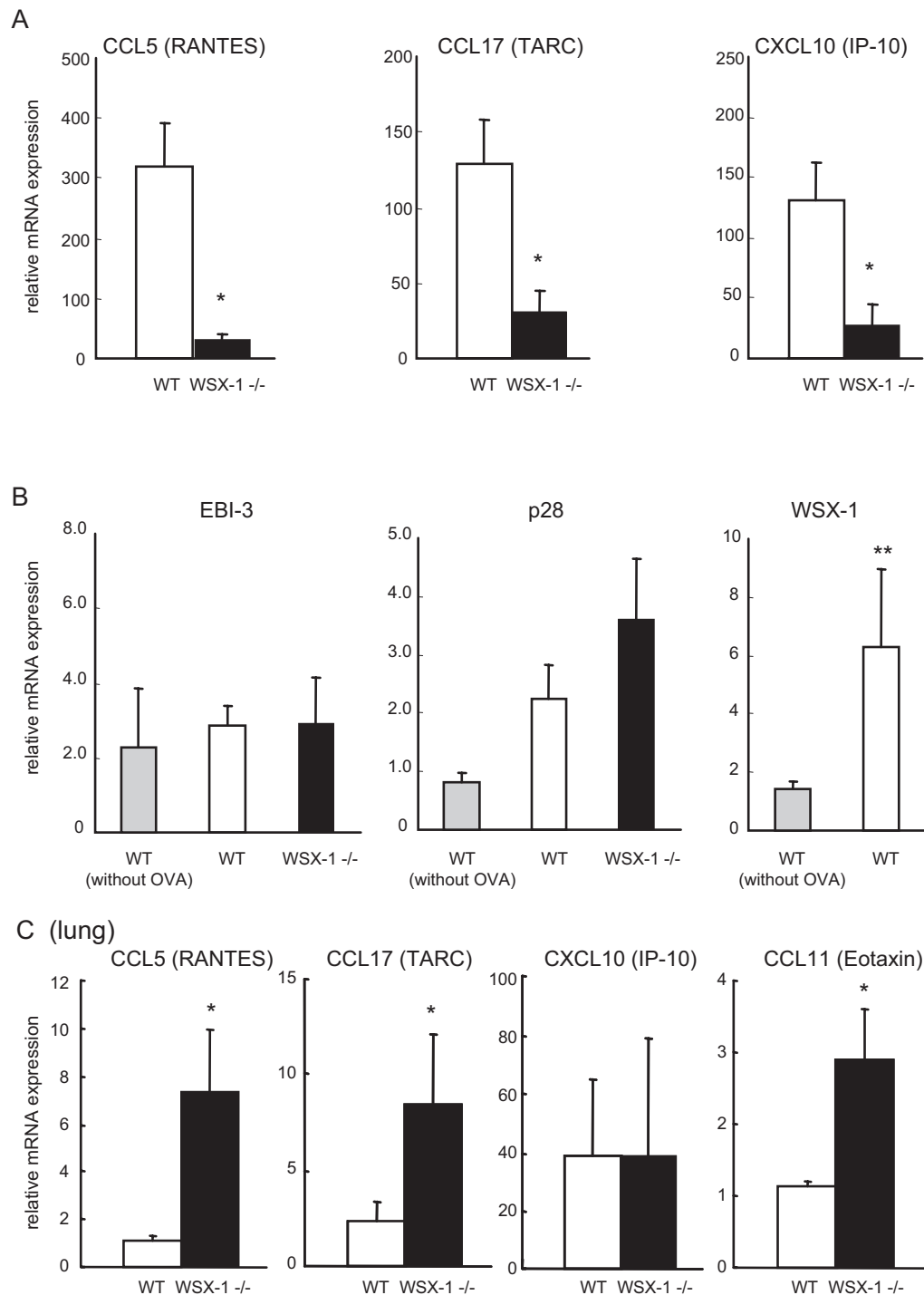


Figure 6. Reduced chemokine expression in ovalbumin (OVA)-challenged *WSX-1^{-/-}* mice. Expression of chemokines (A) and IL-27 subunit, EBI-3 and p28, and *WSX-1* (B) in nasal mucosal tissues was evaluated by real-time reverse-transcription polymerase chain reaction. A, Twenty-four hours after the last OVA challenge on day 35, total RNAs were isolated from nasal mucosal tissues of OVA-challenged wild-type (WT) (open columns) or *WSX-1^{-/-}* (closed columns) mice and examined for expression of indicated chemokines. B, Total RNAs were isolated from nasal mucosal tissues of untreated WT (gray columns), OVA-challenged WT (open columns), and challenged *WSX-1^{-/-}* (closed columns) mice and examined for expression of EBI-3, p28, and *WSX-1*. C, Expression of chemokines in lungs of WT (open columns) or *WSX-1^{-/-}* (closed columns) mice sensitized and aerosol challenged for asthma induction was similarly. Error bars indicate SEM (n = 4–5 mice per group). **P* < .05 compared with WT sample. ***P* < .05 compared with untreated WT sample. Experiments were repeated 3 times with similar tendency. mRNA indicates messenger RNA.

Augmented Cytokine and Chemokine Expression by NALT Cells From WSX-1^{-/-} Mice

NALT acts as an important inductive site for the generation of antigen specific IgA-committed B cells. In addition, although NALT possesses a predominance of naive CD4⁺ cells, the antigen-specific T_H1 and/or T_H2 immune responses can be induced in NALT through intranasal administration of antigens and mucosal adjuvant. Thus, NALT is thought to be a key secondary lymphoid structure for the upper respiratory tract.^{22,23} Given the discrepancy of the local immune responses observed in the current rhinitis model and previous asthma model, we next asked if NALT was responsible for the altered local immune responses observed in the rhinitis model. Development and histologic structure of the nasal mucosa tissues and NALT in *WSX-1^{-/-}* mice seemed normal before challenge (data not shown), and there was no apparent histologic differences in mucosa and NALT organization even after ovalbumin challenge (Fig 4A). Expressions of IL-5 and IFN- γ messenger RNA in the NALT of challenged *WSX-1^{-/-}* mice were higher than in those of WT mice (Fig 4B). IL-4 expression also tended to be higher in the NALT of *WSX-1^{-/-}* mice. These results were similar to those obtained from cervical lymph nodes T cells (Fig 3B), confirming that *WSX-1* deficiency resulted in augmented immune responses even in the NALT.

Chemokines are a group of structurally related chemotactic cytokines that signal through 7-transmembrane G protein-coupled receptors expressed by leukocytes. Some chemokines are involved in the chemotaxis of specific cell populations through their receptor binding. For instance, chemokine receptor (CCR) 3 is expressed in eosinophils, for which CCL11 works as a potent chemoattractant. CCR3 is also expressed by T_H2 cells, mast cells, and basophils. CCL11 and CCL17 induce selective migration of T_H2 cells. CCL5 also binds to CCR3, whereas CCL17 interacts with CCR4. CCR4 is expressed by T_H2 cells.^{24–26} We thus investigated chemokine expression, including CCL5, CCL11, CCL17, and CXCL10, a T_H1-related chemokine, in NALT lymphocytes and cervical lymph node CD4⁺ T cells (Fig 5). Expression of CCL5 in the NALT was augmented in *WSX-1^{-/-}* mice over WT mice (Fig 5A). CCL17 also showed a tendency to be overexpressed in *WSX-1^{-/-}* mice. In cervical lymph node T cells also, expression of CCL5 and CCL17 was higher in *WSX-1^{-/-}* mice and CCL17 tended to be higher in *WSX-1^{-/-}* mice (Fig 5B). Expression of CCL11 and CXCL10 showed no difference between the 2 groups. Thus, *WSX-1*-deficiency also resulted in augmented expression of some chemokines, especially T_H2-related ones.

Reduced Chemokine Expression in Nasal Mucosal Tissues of WSX-1^{-/-} Mice

The results obtained so far suggest that IL-27/*WSX-1* plays exacerbating roles at the local site, such as nasal mucosal tissues. We next investigated chemokine expression in nasal mucosa tissues removed from ovalbumin-challenged mice. Expression of CCL5, CCL17, and CXCL10 was significantly

reduced in the nasal mucosa tissues of challenged *WSX-1^{-/-}* mice compared with those of challenged WT mice (Fig 6A), which was consistent with the local symptoms but was in contrast to systemic symptoms. Then we asked if IL-27/*WSX-1* was expressed and may play some roles in the mucosal tissues. As shown in Figure 6B, EBI-3 (a subunit for IL-27) was expressed even before immunization and expression levels were not altered by immunization or challenge. EBI-3 was expressed in the mucosa of challenged *WSX-1^{-/-}* mice as well. Expression of p28 (the other component of IL-27) was augmented by immunization or challenge in WT mice. Expression of p28 in challenged *WSX-1^{-/-}* mice was increased but not statistically significantly higher than in challenged WT mice. Expression of *WSX-1* in the mucosa of WT mice was augmented by immunization or challenge. Contrary to the results in mucosal tissues of mice challenged for rhinitis induction, expression of chemokines, including CCL5, CCL17, and CCL11, was augmented in *WSX-1^{-/-}* mice over WT mice when mice were sensitized and challenged for asthma induction (Fig 6C). These findings suggested that IL-27/*WSX-1* accumulates in nasal mucosal tissues of ovalbumin-challenged WT mice and plays a role in exacerbating the local symptoms, presumably through differential induction of certain chemokines.

DISCUSSION

T_H2 cytokines, such as IL-4 and IL-5, play crucial roles with strong links to asthma in allergic rhinitis.^{27,28} We previously reported that in an experimental allergic asthma model, *WSX-1* deficiency resulted in exacerbation of the disease with augmented production of T_H2 cytokines.¹⁷ In the current study, we investigate the role of IL-27/*WSX-1* in experimental allergic rhinitis. With the similar pathogenesis of the 2 diseases, we initially predicted that *WSX-1*-deficiency would give rise to augmentation of nasal symptoms in the allergic rhinitis model as well. Contrary to our expectation, clinical symptoms were lower in *WSX-1^{-/-}* mice compared with WT mice in allergic rhinitis (Fig 2). Cytokine production by cervical lymph node cells, however, was higher in *WSX-1^{-/-}* mice. Furthermore, an increased level of serum antigen specific IgE was also observed in *WSX-1^{-/-}* mice (Fig 3). These results suggested that *WSX-1* plays an exacerbating role in systemic development of allergic responses in both rhinitis and asthma models but that IL-27/*WSX-1* signaling plays distinct roles in the rhinitis and asthma models at the effector phases.

To address the *prima facie* discrepancy (ie, distinct effect of IL-27/*WSX-1* at the effector phases of the 2 diseases), pathologic changes and cytokine expression in NALT were examined. It was shown that NALT is a key secondary lymphoid structure for the upper respiratory tract²² and is a functionally distinct lymphoid aggregate when compared with other mucosa-related lymphoid tissues, such as bronchus-associated lymphoid tissue and Peyer patches. NALT contains mainly T_H0 cells with a potential to differentiate into both T_H1 and T_H2 populations.²⁹ NALT is also a key organ for

mucosal tolerance. We thus initially hypothesized that immune responses in NALT of *WSX-1*^{-/-} mice may differ from those in NALT of WT mice. However, nasal mucosal tissues and NALT development in ovalbumin-challenged WT and *WSX-1*^{-/-} mice were histologically comparable. In addition, expression of T_H2 cytokines, such as IL-4 and IL-5, in NALT was higher in *WSX-1*^{-/-} mice than in WT mice, similar to observation in the cervical lymph nodes (Fig 4). In this context, IL-27/WSX-1 exerted similar effects on the NALT and cervical lymph node for induction of allergic immune responses.

Chemokines are a group of proteins that guide migration of cells. Since some chemokine/chemokine receptor signaling induces migration of specific cells, such as T_H1/2 cells, mast cells, and eosinophils, that are involved in the pathogenesis of allergic rhinitis, we next examined that expression of chemokines. The expressions of CCL5, CCL11, and CCL17 by both NALT and cervical CD4⁺ T cell in challenged *WSX-1*^{-/-} mice were higher compared with those in challenged WT mice (Fig 5). These results were again similar to the observation that immune responses in NALT and cervical lymph nodes were augmented by WSX-1-deficiency. Contrary, the chemokine expression (CCL5, CCL17, and CXCL10) in nasal mucosal tissues in challenged *WSX-1*^{-/-} mice was lower compared with that in challenged WT mice. The reduced expression of these chemokines may be responsible for the ameliorated allergic symptoms and lower production of IL-4 and IL-5 in the NLF of *WSX-1*^{-/-} mice (Fig 2B). Interestingly, expression of these chemokines was augmented in the lung of *WSX-1*^{-/-} mice over WT mice sensitized and aerosol challenged for asthma induction (Fig 6C). The augmented expression of these chemokines was compatible with the augmented asthma phenotypes of *WSX-1*^{-/-} mice.¹⁷ Therefore, although similar protocols are used for induction of asthma and rhinitis, the effects of IL-27/WSX-1 for regulation of immune responses are different in the nasal mucosa and in the lung. Although apparent cell migration and accumulation in the mucosa was not detectable in either WT or *WSX-1*^{-/-} mice (Fig 4A) in the current experimental condition, it is reasonable to assume that the reduced chemokine expression in the mucosa of *WSX-1*^{-/-} mice resulted in reduction of migration of rhinitis-associated cells into the site of allergic reaction. WSX-1 is expressed mainly by immune cells, including T cells, natural killer cells, and macrophages.^{12,13} Reportedly, WSX-1 is also expressed in the lung and kidney.¹² Expression of WSX-1 and p28 of IL-27 was enhanced by immunization or challenge in WT mice, whereas substantial expression of EBI-3 was not affected by immunization or challenge. Currently, it is technically impossible to distinguish expression of these genes in mucosal cells from that in immune cells migrated into the mucosa. It is likely that mucosal cells expressed some of *IL-27/WSX-1* gene to be activated for expression of chemokine genes in a way distinct from a situation where IL-27/WSX-1 suppresses activation of immune cells. It may be, of course, possible that IL-27/WSX-1 exerts immunopromoting effects on migrating im-

mune cells in inflamed mucosal environments. In any events, these findings suggest that IL-27/WSX-1 accumulates in nasal mucosal tissues of ovalbumin-challenged WT mice and plays an exacerbating role, presumably through expression of chemokines. The opposing effects of IL-27/WSX-1 in a rhinitis model and an asthma model should be taken into serious account when IL-27/WSX-1 signaling is considered as a potential target for therapy of allergic diseases.

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