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A viral PAMP double-stranded RNA induces allergen-specific Th17 cell response in the airways which is dependent on VEGF and IL-6

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Abstract

Background: Innate immune response by a viral pathogen-associated molecular pattern dsRNA modulates the subsequent development of adaptive immune responses. Although virus-associated asthma is characterized by noneosinophilic inflammation, the role of Th17 cell response in the development of virus-associated asthma is still unknown.

Objective: To evaluate the role of the Th17 cell response and its underlying polarizing mechanisms in the development of an experimental virus-associated asthma.

Methods: An experimental virus-associated asthma was created via airway sensitization with ovalbumin (OVA, 75 μg) and a low (0.1 μg) or a high (10 μg) doses of synthetic dsRNA [polyinosine–polycytidylic acid; poly(I:C)]. Transgenic (IL-17-, IL-6-deficient mice) and pharmacologic [a vascular endothelial growth factor receptor (VEGFR) inhibitor] approaches were used to evaluate the roles of Th17 cell responses.

Results: After cosensitization with OVA and low-dose poly(I:C), but not with high-dose poly(I:C), inflammation scores after allergen challenge were lower in IL-17-deficient mice than in wild-type (WT) mice. Moreover, inflammation enhanced by low-dose poly(I:C), but not by high-dose poly(I:C), was impaired in IL-6-deficient mice; this phenotype was accompanied by the down-regulation of IL-17 production from T cells from both lymph nodes and lung tissues. Airway exposure of low-dose poly(I:C) enhanced the production of VEGF and IL-6, and the production of IL-6 was blocked by treatment with a VEGFR inhibitor (SU5416). Moreover, the allergen-specific Th17 cell response and subsequent inflammation in the low-dose poly(I:C) model were impaired by the VEGFR inhibitor treatment during sensitization.

Conclusions: Airway exposure of low-level dsRNA induces an allergen-specific Th17 cell response, which is mainly dependent on VEGF and IL-6.

Asthma is a very common chronic respiratory disease in which the airways occasionally constrict and become inflamed, often in response to inhaled allergens (1). Recent longitudinal studies have shed light on the expression patterns of childhood asthma that persists into adult life, and distinct asthma phenotypes such as atopy-associated (atopic) and virus-associated (nonatopic) asthma have been identified (2). Respiratory infection by any of several common respiratory viruses during the first 3 years of life greatly enhances the risk that a child will develop asthma (3, 4). After viral infections, pattern-recognition receptors (PRRs), such as Toll-like receptor 3 (TLR3) and retinoic-acid-inducible protein I (RIG-I), recognize pathogen-associated molecular patterns (PAMPs), including double-stranded (ds) RNA (5–7). Innate immune response-mediated PRRs after recognition of viral PAMPs cause inflammation and the production of pro-inflammatory and immune-modulating mediators (8–10). In recent animal experiments, we demonstrated that airway cosensitization with allergens and a viral PAMP dsRNA induce asthma phenotypes that may mimic virus-associated asthma (11).

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The Th2 hypothesis of asthma pathogenesis posits that an increase in the type-2 T-helper (Th2) cell response results in eosinophilic inflammation in patients with asthma (12). However, eosinophil levels in the airways are significantly lower in children with virus-associated asthma than in children with atopic asthma (13, 14), suggesting that immunological mechanisms other than the Th2 cell response contribute to the pathogenesis of virus-associated asthma. Th17 cells, which produce interleukin (IL)-17, were recently identified (15–17). IL-17 has pleiotropic activities (18), is involved in the proliferation, maturation, and chemotaxis of neutrophils (19, 20), and has been detected in target tissues of patients with asthma (21, 22). Although ample evidence suggests that Th17 cells are potent inducers of neutrophilic inflammation (15, 20, 23, 24), the role of IL-17 in the pathogenesis of virus-associated asthma is wholly unknown.

In this study, we examined the hypothesis that Th17 cell response is important in the development of noneosinophilic or neutrophilic inflammation characterized by virus-associated asthma. To test this, the role of Th17 cell response in the development of noneosinophilic inflammation was first evaluated in a virus-associated asthma model using IL-17-deficient mice. Next, to investigate the underlying immunological mechanisms of Th17 cell polarization, transgenic and pharmacologic approaches were performed to examine the roles of vascular endothelial growth factor (VEGF) and IL-6 in a virus-associated asthma model.

Methods

Mice and reagents

IL-17-deficient mice (BALB/c background) were gifts from Y.C. Sung at Pohang University of Science and Technology (POSTECH; Pohang, Republic of Korea). IL-6-deficient mice (C57BL/6 background) and wild-type (WT) mice (BALB/c and C57BL/6 background) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were bred in a pathogen-free facility at POSTECH, and all live animal experiments were approved by the POSTECH Ethics Committee.

Synthetic dsRNA polyinosine–polycytidylic acid [poly(I:C)] was purchased from Calbiochem (La Jolla, CA, USA). Ovalbumin (OVA) and SU5416 (a VEGF receptor inhibitor) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Protocols for a virus-associated asthma mouse model and pharmacologic intervention

A virus-associated asthma mouse model (Fig. 1) was generated by cosensitization of mice with poly(I:C) and allergens, as described previously (11). Briefly, 6-week-old mice were treated intranasally with 75 μg OVA plus a low (0.1 μg) or a high (10 μg) poly(I:C) on days 0, 1, 2, and 7 and then challenged with 50 μg OVA on days 14, 15, 21, and 22. Innate immune responses to poly(I:C) were evaluated after sensitization on day 0, and allergen-specific adaptive immune responses were examined 6 h after allergen challenge on day 21. Asthma phenotypes, such as lung inflammation, were examined 24 h after the final allergen challenge on day 22.

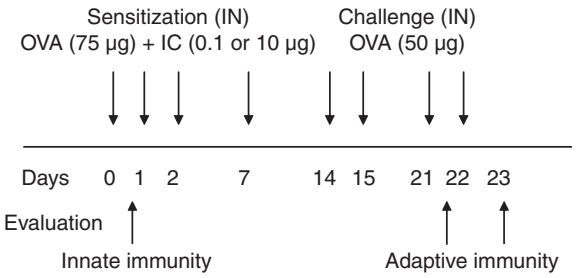

Figure 1 A study protocol for the generation of an experimental virus-associated asthma. A virus-associated asthma mouse model was generated by cosensitization of mice with ovalbumin (OVA) and poly(I:C) and then challenged with OVA alone. Innate immune response was evaluated after sensitization on day 0, and T-cell response and inflammation evaluated after allergen challenge on day 21 and day 22, respectively.

To block VEGFR-mediated signaling, mice were treated with the VEGFR inhibitor SU5416 or with a sham control (dimethyl sulfoxide [DMSO] vehicle) during sensitization (on days 0, 1, 2, and 7).

Cellularity in bronchoalveolar lavage (BAL) fluid

Bronchoalveolar lavage fluid cellularity was analyzed as described previously (11). Briefly, cell pellets were diluted in 50 μl phosphate-buffered saline (PBS), and 300 inflammatory cells were counted and classified as macrophages, lymphocytes, neutrophils, or eosinophils.

Lung tissue histology

Lung sections were stained with hematoxylin and eosin (H&E) after pressure fixation with Streck solution (Streck Laboratories, La Vista, NE, USA). All sample slides were compared at the same magnification. Lung inflammation was measured by assessing the degree of peribronchiolar and perivascular inflammation as described previously (11).

Immune response in lung-draining lymph nodes (LNs)

After harvest, lung-draining LNs were cultured (4×10^6/ml) in 24-well plates at 37°C in Dulbecco's modified Eagle medium in the presence of OVA (100 μg/ml) or anti-CD3 and anti-CD28 antibodies (1 μg/ml each). Levels of cytokines produced by the restimulated T cells were determined using culture supernatant fractions collected 72 h after OVA stimulation or 12 h after anti-CD3/CD28 antibody stimulation.

Cytokine measurements

Levels of cytokines in BAL fluid and supernatant fractions of cultured cells were measured using enzyme-linked

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immunosorbent assays (ELISA) in accordance with the manufacturer's instructions (R&D Systems, Mineapolis, MN, USA).

Statistical analysis

Analysis of variance (ANOVA) was used to determine the statistical significance of differences among all groups. Significant differences among treatments were assessed using Student's *t*-test, ANOVA, or Wilcoxon's rank sum test. For multiple comparisons, ANOVA was used first, and if significant differences were found, individual *t*-tests or Wilcoxon's rank sum tests were performed between pairs of groups. Differences were considered statistically significant at $P < 0.05$.

Results

Role of IL-17 in lung inflammation induced by airway cosensitization with allergens and low- or high-dose poly(I:C)

In our previous study, we found that airway cosensitization with allergens and low- or high-dose poly(I:C) (0.1 or 10 μg, respectively) induced noneosinophilic inflammation in the airways (11). Furthermore, the inflammation associated with low- or high-dose poly(I:C) was causally related to the Th2 and Th1 cell responses, respectively (11). In this study, we evaluated the role of the Th17 cell response in the development of lung inflammation in our virus-associated asthma mouse model (Fig. 1). When we evaluated inflammation in IL-17-deficient and WT mice 24 h after the final allergen challenge, BAL cellularity analysis showed less macrophage and neutrophil infiltration in IL-17-deficient mice sensitized with OVA plus low-dose poly(I:C) than in WT mice sensitized in the same manner; in contrast, IL-17-deficient and WT control mice exhibited similar levels of inflammatory cell infiltration when they were sensitized with OVA plus high-dose poly(I:C) (Fig. 2A). Inflammation scores based on lung histology yielded similar results; peribronchiolar and perivascular infiltration by inflammatory cells was less extensive in IL-17-deficient mice sensitized with OVA plus low-dose poly(I:C) than in WT mice sensitized in the same manner, but when OVA was used with high-dose poly(I:C), rather than with low-dose poly(I:C), the IL-17-deficient and WT mice exhibited similar phenotypes (Fig. 2B).

In analyzing the production of Th17 cell downstream cytokines, we found that sensitization with OVA plus low-dose poly(I:C) enhanced BAL fluid levels of TNF-α and IL-6 in WT mice, but not in IL-17-deficient mice; however, sensitization with OVA plus high-dose poly(I:C) had no effect on levels of these cytokines in either group (Fig. 2C). In contrast, the levels of IP-10 (a IFN-γ downstream chemokine) in BAL fluids were higher in IL-17-deficient mice sensitized with OVA plus low- or high-dose poly(I:C) than in

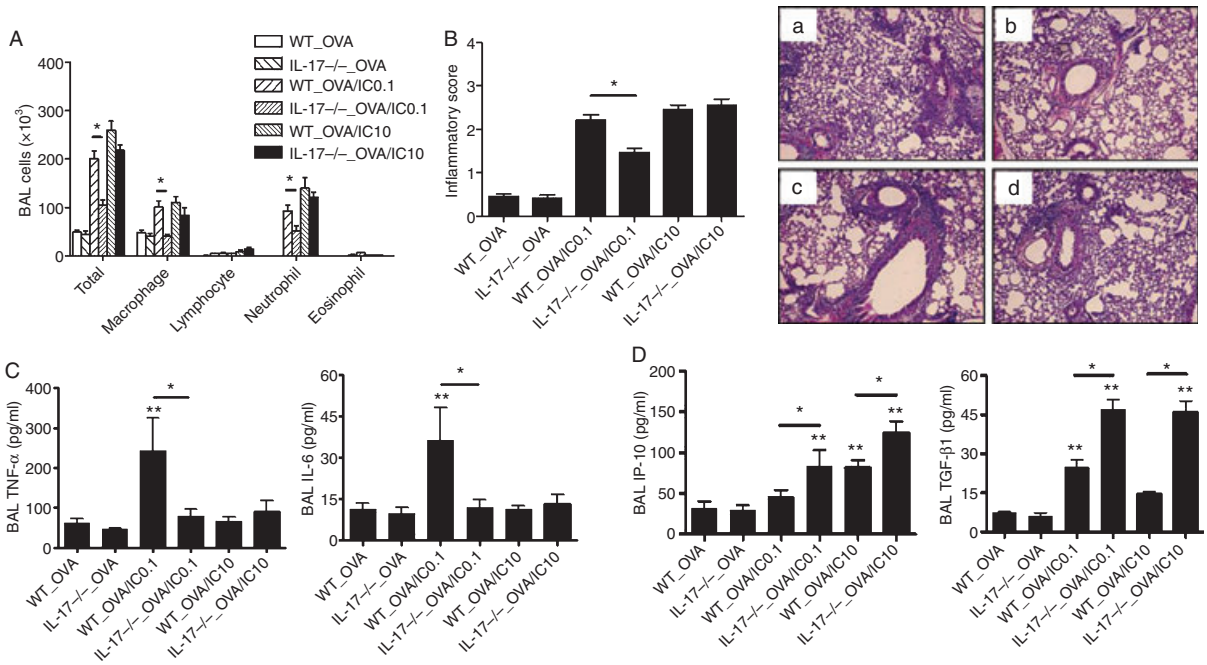

Figure 2 IL-17 plays a key role in the development of noneosinophilic lung inflammation induced by airway cosensitization with allergen and low-dose (but not high-dose) poly(I:C). Each group of mice ($n = 5$ each) was evaluated 24 h after the final allergen challenge on day 22. For all figures: OVA, mice sensitized with ovalbumin (OVA) alone; OVA/IC0.1, mice sensitized with OVA plus low-dose (0.1 μg) poly(I:C); and OVA/IC10, mice sensitized with OVA plus high-dose (10 μg) poly(I:C). (A) Bronchoalveolar lavage (BAL) cellularity. $*P < 0.05$. (B) Inflammatory score (left panel) and representative lung histologic findings (right panel: a, WT_OVA/IC0.1; b, IL-17$^{-/-}$_OVA/IC0.1; c, WT_OVA/IC10; d, IL-17$^{-/-}$_OVA/IC10; H&E staining, ×200). $*P < 0.05$. (C) TNF-α and IL-6 levels in BAL fluids. $*P < 0.05$; $**P < 0.05$ relative to OVA group. (D) IP-10 and TGF-β_1 levels in BAL fluids. $*P < 0.05$; $**P < 0.05$ relative to OVA group.

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WT mice sensitized in the same manner; similarly, the level of transforming growth factor (TGF)-β_1 (a downstream mediator of Th2 cytokines) in BAL fluids was enhanced in the IL-17-deficient mice, irrespective of the poly(I:C) dose (Fig. 2D).

In summary, these data suggest that the airway exposure to allergens and low-dose dsRNA induced Th17 cell response and subsequent noneosinophilic inflammation.

Role of IL-6 in the development of lung inflammation induced by airway sensitization with allergens and low- or high-dose poly(I:C)

Studies on the mechanisms by which Th17 cells develop from naïve T cells have shown that IL-6 acts synergistically with TGF-β to induce IL-17 expression in T cells *via* the STAT3-RORγt pathway (25). In this *in vitro* studies, we found that mouse macrophages (RAW264.7 cells) produced increased levels of both TGF-β_1 and IL-6 after stimulation with 10 μg/ml poly(I:C) (data not shown). This finding led to evaluate the role of IL-6 in the development of Th17 cell response and inflammation in the virus-associated asthma model. When we evaluated lung inflammation in IL-6-deficient or WT mice 24 h after the final allergen challenge, BAL cellularity analysis showed significantly lower levels of macrophage and neutrophil infiltration induced by airway cosensitization with OVA plus low-dose poly(I:C) in the IL-6-deficient mice, whereas inflammatory cell infiltration levels were similar in IL-6-deficient and WT mice sensitized with OVA plus high-dose poly(I:C) (Fig. 3A). Perivascular and peribronchiolar infiltration by inflammatory cells in allergen/poly(I:C)-cosensitized mice, as determined by histologic analysis, was less extensive in IL-6-deficient mice than in WT mice when the low dose of poly(I:C) was used, but not when the high dose was used (Fig. 3B). Bronchoalveolar lavage fluid levels of the Th17-related cytokines IL-17 and TNF-α were higher in WT mice sensitized with OVA + low-dose poly(I:C) than in WT mice sensitized with OVA alone or OVA + high-dose poly(I:C), but the enhanced production of IL-17 and TNF-α by low-dose poly(I:C) was not detected in the IL-6-deficient mice (Fig. 3C). In contrast, levels of the Th1- and Th2-related cytokines IP-10 and TGF-β_1 were higher in IL-6-deficient mice sensitized with OVA plus low- or high-dose poly(I:C) than in WT mice sensitized in the same manner (Fig. 2D). Taken together, these findings suggest that IL-6 is an important mediator in the development of Th17-type inflammation induced by airway exposure to allergens and low-level dsRNA.

Role of IL-6 in development of Th17 and Th2 cell responses in the low-dose poly(I:C) model

Our previous and current findings suggest that inflammation induced by allergens and low-level dsRNA is dependent on both Th17 and Th2 cell responses (11). This led to evaluate the role of IL-6 in the development of Th17 and Th2 cell responses

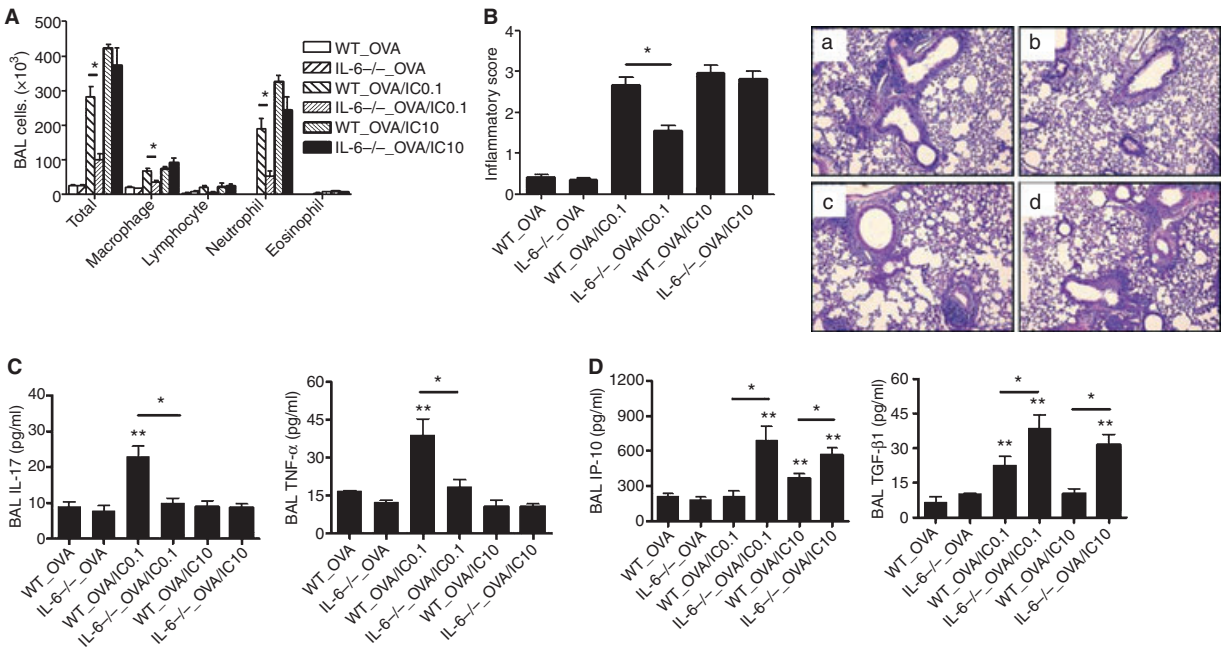

Figure 3 IL-6 plays a key role in the development of lung inflammation enhanced by low-dose (but not high-dose) poly(I:C). For all figures, each group of mice (n = 5 each) was evaluated 24 h after the final allergen challenge on day 22. *P < 0.05; **P < 0.05 relative to ovalbumin (OVA) group. (A) Bronchoalveolar lavage (BAL) cellularity. (B) Inflammatory score (upper panel) and representative lung histologic findings (lower panel: a, WT_OVA/IC0.1; b, IL-6_OVA/IC0.1; c, WT_OVA/IC10; d, IL-6_OVA/IC10; H&E stain, ×200). (C) Levels of the Th17-related cytokines IL-17 and TNF-α in BAL fluid. (D) Levels of the Th1- and Th2-related cytokines IP-10 and TGF-β_1 in BAL fluid.

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in the low-dose poly(I:C) model. To test this notion, we measured the expression of Th17 and Th2 cytokines in IL-6-deficient and WT mice 6 h after allergen challenge on day 21 in the low-dose poly(I:C) model. We found that IL-17 production by T cells from lung-draining LNs after stimulation with anti-CD3 and anti-CD28 antibodies was higher for WT mice cosensitized with OVA + low-dose poly(I:C) than for WT mice sensitized with OVA alone; again, the enhanced IL-17 production was almost abolished in the absence of IL-6 (Fig. 4 A). Similarly, in WT mice but not in IL-6-deficient mice, IL-17 production by lung T cells after stimulation with anti-CD3/CD28 antibodies was higher after cosensitization with OVA plus low-dose poly(I:C) than after sensitization with OVA alone (Fig. 4A). Production of the Th2 cytokine IL-4 by LN T cells after anti-CD3/CD28 antibody stimulation was unaffected by the absence of IL-6 but was higher after cosensitization with OVA plus low-dose poly(I:C) than after sensitization with OVA alone; however, the production of IL-4 from lung T cells in the low-dose poly(I:C) model was enhanced in the absence of IL-6 (Fig. 4B). The production of IL-10 (a key cytokine of Treg cells) by both LN and lung T cells was enhanced in the absence of IL-6, when exposed to OVA + low-dose poly(I:C) (Fig. 4C). These data together indicate that IL-6 is a key cytokine in the development of the Th17 cell response in the lung and Th2 and Treg cell responses were enhanced in absence of IL-6.

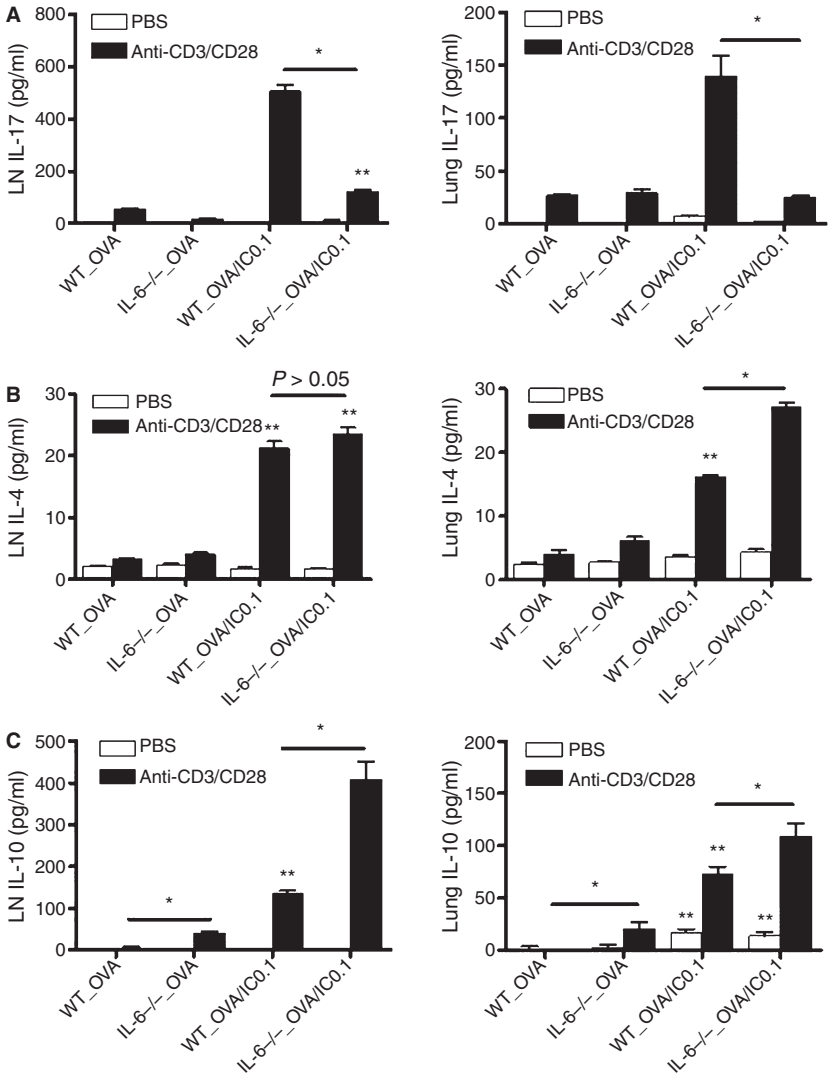

Figure 4 IL-6 plays a key role in the development of the Th17 cell response induced by airway sensitization with allergens and low-dose poly(I:C). Each group of mice ($n = 4$ each) was evaluated 6 h after allergen challenge on day 21. Cells were isolated from lung-draining lymph nodes (left panels) and lung tissues (right panels) and incubated with phosphate-buffered saline or anti-CD3 and anti-CD8 antibodies for 12 h, and the levels of IL-17 (A), IL-4 (B), and IL-10 (C) in the supernatant fractions were determined. $^{*}P < 0.05$; $^{**}P < 0.05$ relative to OVA group.

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Role of VEGF in the induction of IL-6 production by airway exposure to low-dose poly(I:C)

In the present *in vitro* experiments, VEGF production by mouse macrophages (RAW264.7) was enhanced from 2 h through 24 h after stimulation with 10 μg/ml poly(I:C) relative to stimulation with PBS alone (Fig. 5A). In addition, in *in vivo* experiments, BAL fluid levels of VEGF were increased significantly over basal levels from 2 h through 8 h after airway application of a single 0.1 μg dose of poly(I:C); in addition, BAL fluid levels of IL-6 also increased significantly, but the increase in IL-6 was delayed by 8 h relative to that of VEGF (Fig. 5B). Moreover, the *in vitro* application of recombinant VEGF to mouse macrophages increased IL-6 production in a dose-dependent manner (Fig. 5C). Recently, we found that lipopolysaccharide (LPS)-induced VEGF in the airways is a key upstream mediator on the production of IL-6 and subsequent development of Th17 cell response in context of inhalation of LPS-containing allergens (26). This led to the notion that VEGF is a essential upstream mediator on the production of IL-6 during innate immune response in the experimental virus-associated asthma. To test this notion, IL-6 production was evaluated 12 h after airway application of a single 0.1 μg dose of poly(I:C) in the presence of the VEGFR inhibitor (SU5416) or a sham control (DMSO). This study showed that intervention with SU5416 (10 μg) completely abolished the enhancement of IL-6 production by low-dose poly(I:C) exposure (Fig. 5D), suggesting that VEGFR-mediated signaling is crucial on the production of IL-6 in the experimental virus-associated asthma.

Role of poly(I:C)-induced VEGF in the development of Th17 cell response and inflammation in the low-dose poly(I:C) model

Based on the result of VEGFR dependency of IL-6 production, we evaluated the role of low-dose poly(I:C)-induced VEGF in the development of Th17 cell response and subsequent inflammation. To test this, the VEGFR inhibitor (SU5416, 10 μg) was treated during sensitization in the low-dose poly(I:C) model; lung inflammation was evaluated 24 h after the final allergen challenge on day 22; T cell responses were evaluated 6 h after allergen challenge on day 21, as shown in Fig. 1. As for the role of VEGF on the development of Th17-type inflammation, analysis of BAL cellularity showed significantly less extensive macrophage, lymphocyte, and neutrophil infiltration in the SU5416-treated mice cosensitized with OVA plus low-dose poly(I:C) than in sham-treated mice cosensitized in the same manner (Fig. 6A). Histologic inflammatory scores also indicated decreased peribronchiolar and perivascular infiltration by inflammatory cells in the SU5416-treated mice (Fig. 6B). Moreover, SU5416 treatment completely blocked the induction of IL-17 in mice cosensitized with OVA plus low-dose poly(I:C) (Fig. 6C); in addition, it also abolished the induction of increased BAL fluid levels of TNF-α and IL-6 (Fig. 6D). In contrast, the level of TGF-β_1 (a key downstream mediator of Th2 cytokines) was higher in SU5416-treated mice than in sham-treated mice (Fig. 6D). These data indicate that VEGF induced by low-dose poly(I:C) during sensitization is a key mediator in the development of Th17-type inflammation after allergen challenge.

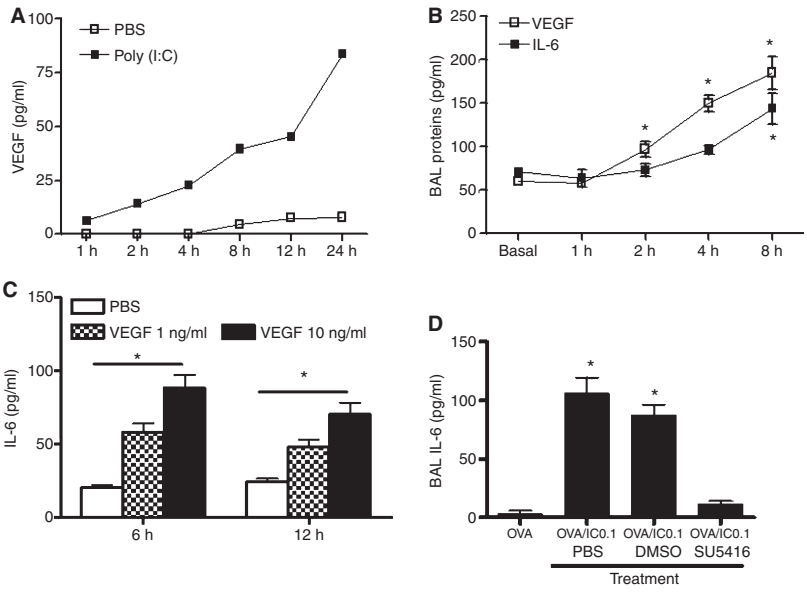

Figure 5 Poly(I:C)-induced production of IL-6 depends primarily on vascular endothelial growth factor (VEGF). (A) *In vitro* time-dependent production of VEGF after stimulation with 10 μg/ml poly(I:C) or phosphate-buffered saline. (B) *In vivo* time-dependent production of VEGF and IL-6 after airway application of 0.1 μg/ml poly(I:C). $*P < 0.05$ compared with basal level. (C) *In vitro* production of IL-6 after stimulation with various doses of recombinant VEGF. $*P < 0.05$. (D) Levels of IL-6 in bronchoalveolar lavage fluid collected 12 h after airway application of 0.1 μg poly(I:C) to mice treated with a VEGFR inhibitor (SU5416, 10 μg) or sham (dimethyl sulfoxide) during sensitization. $*P < 0.05$ compared with other groups.

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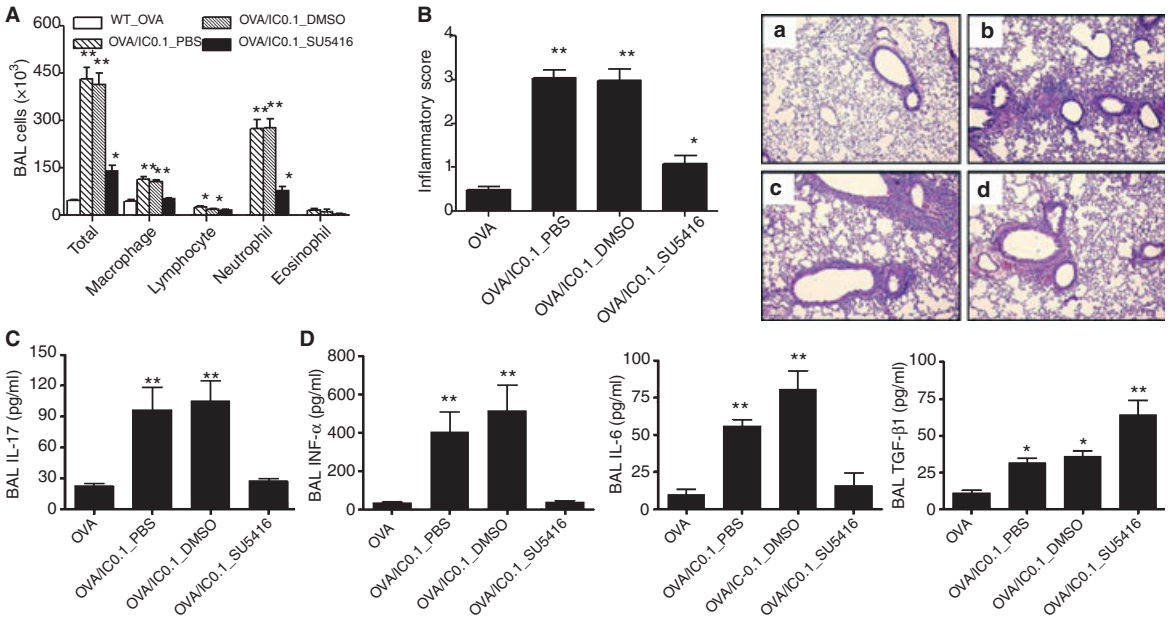

Figure 6 Vascular endothelial growth factor induced by poly(I:C) during sensitization plays a key mediating role in the development of Th17-type inflammation. Evaluation in groups of SU5416- or dimethyl sulfoxide (DMSO)-treated mice ($n = 5$ each) was performed 24 h after the final allergen challenge on day 22. (A) Bronchoalveolar lavage (BAL) cellularity. (B) Inflammatory score based on lung histology (left panel) and representative lung histologic findings (right panel: a, ovalbumin (OVA); b, OVA/IC0.1_PBS; c, OVA/IC0.1_DMSO; d, OVA/IC0.1_SU5416; H&E staining, × 200). (C) IL-17 levels in BAL fluid. (D) Levels of the Th17-related cytokines TNF-α and IL-6 and the Th2-related cytokine TGF-β_1. $^{*}P < 0.05$ compared with OVA group; $^{**}P < 0.05$ compared with other groups.

With regard to the role of VEGF on the development of Th17 cells, we found that IL-17 production by LN T cells after nonspecific stimulation with anti-CD3/CD28 antibodies [which was enhanced by cosensitization with OVA plus low-dose poly(I:C)] was significantly reduced by SU5416 treatment; in addition, IL-17 production by LN T cells after incubation with an allergen (OVA)-specific stimulus was completely abolished by SU5416 treatment (Fig. 7A). Moreover, SU5416 treatment

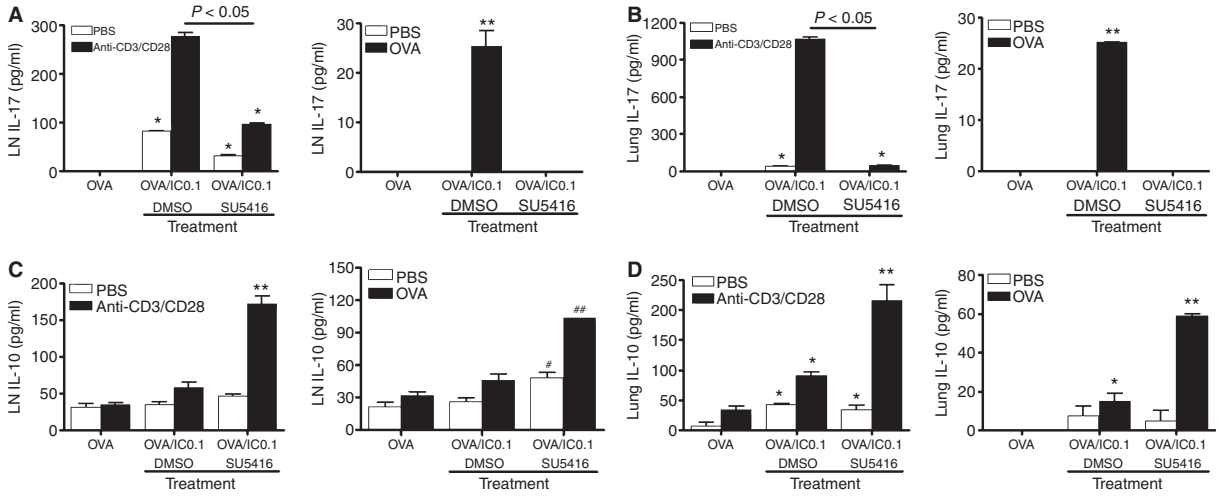

Figure 7 Vascular endothelial growth factor plays a key mediating role in the development of allergen-specific Th17 cell response in the low-dose poly(I:C) model. Evaluation in groups of SU5416- or dimethyl sulfoxide-treated mice ($n = 4$ each) was performed 6 h after allergen challenge on day 21. Levels of IL-17 (A, B) and IL-10 (C, D) in supernatant fractions of T cells isolated from lung-draining lymph nodes (A, C) and from lung tissues (B, D) were determined after the cells were stimulated with anti-CD3 and anti-CD28 antibodies (left panels) or with an allergen [ovalbumin (OVA), right panels]. $^{*}P < 0.05$; $^{\#}P < 0.05$ compared with OVA group; $^{\#\#}P < 0.05$ compared with other groups.

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during sensitization abolished the production of IL-17 by lung T cells after incubation with a nonspecific (anti-CD3/CD28 antibodies) or allergen-specific (OVA) stimulus (Fig. 7B). In contrast, the production of IL-10 by LN T cells after incubation with a nonspecific or allergen-specific stimulus was enhanced by SU5416 treatment during sensitization (Fig. 7C), as was the production of IL-10 by lung T cells (Fig. 7D). Together, these data strongly suggest that VEGF induced by low-dose poly(I:C) during sensitization is crucial in the development of allergen-specific Th17 cell response.

Discussion

Evidence that inflammatory mechanisms other than eosinophilic inflammation are involved in the pathogenesis of asthma is increasing (11, 27). Only 50% of asthma cases, at most, appear to be attributable to eosinophilic inflammation (27). A major proportion of noneosinophilic asthma cases may develop subsequent to viral respiratory infection. Our previous animal studies demonstrated that noneosinophilic asthma induced by airway exposure to allergens and low-level dsRNA is dependent on the Th2 response. Given that Th2 cytokines induce eosinophilic inflammation, immune mechanisms other than the Th2 cell response may also be involved in the development of noneosinophilic inflammation in this asthma animal model. Indeed, in this study, we found that airway exposure of low-level dsRNA with allergens induced Th17 cell response and noneosinophilic inflammation, suggesting that the allergen-specific Th17 cell response is also important in the development of asthma subsequent to viral respiratory infection.

Although immunologists have divided helper T cells into two functional subsets, Th1 and Th2 (28), for the last 20-plus years, T cells producing IL-17 (Th17 cells) were recently identified (15). Explorations of the mechanisms by which these Th17 cells differentiate from naïve T cells have shown that IL-6, in synergy with TGF-β, induces Th17 polarization via the STAT3-RORγt pathway (25). Recently, we also demonstrated that LPS-induced IL-6 in the airways was crucial in the development of allergen-specific Th17 cells in context of inhalation of LPS-containing allergens (26). The results of this study show that the *in vivo* and *in vitro* production of IL-6 is enhanced by airway exposure to low-level dsRNA. Moreover, we found that the dsRNA-induced Th17 cell response was completely abolished in IL-6-deficient mice. These findings together suggest that the viral PAMP dsRNA-induced IL-6 is essential for development of Th17 cells in the airways after viral respiratory infection.

Vascular endothelial growth factor, a potent mediator of vascular remodeling and angiogenesis in the lung (29, 30), is produced during the innate immune response to viral respiratory infection (30, 31). In addition, over-expression of VEGF in the airways augments T-cell priming to inhaled allergens and enhances the expression of costimulatory molecules by lung dendritic cells (29). Our previous study showed that VEGF is a key mediator in the development of Th17 cell response in context of LPS-containing allergen inhalation (26). In this study, *in vivo* levels of both VEGF and the Th17-polarizing cytokine IL-6 were increased by airway application of low-dose poly(I:C), but the increase in IL-6 production was delayed 6 h relative to that of VEGF; moreover, the *in vivo* and *in vitro* production of IL-6 was completely blocked by treatment with the VEGFR inhibitor. Moreover, we also observed that blocking VEGFR-mediated signaling during sensitization abolished the development of dsRNA-induced Th17 cell response. Taken together, these findings suggest that the VEGF–IL-6 axis induced by dsRNA- or LPS-induced innate immune response is crucial in the development of allergen-specific Th17 cell response.

What are the roles of T-cell-derived cytokines in the development of noneosinophilic lung inflammation? Much evidence has demonstrated that neutrophilic inflammation is related to the Th1 cell response rather than to the Th2 cell response, particularly in severe asthma or chronic obstructive pulmonary disease (11, 32). Indeed, our previous study showed that allergen-induced neutrophilic inflammation in the high-dose poly(I:C) model is dependent on IFN-γ. In addition, IL-17 acts *in vitro* and *in vivo* as a potent mediator of the development of neutrophilic inflammation (19). Unfortunately, the precise role of Th17 cells in the development of allergen-induced neutrophilic inflammation after viral respiratory infection is still poorly understood. In our low-dose poly(I:C) model, allergen-induced neutrophilic inflammation was partially dependent on the Th17 cell response. Thus, both allergen-specific Th1 and Th17 cell responses appear to be responsible for the development of noneosinophilic inflammation in virus-associated asthma pathophysiology.

In summary, we have demonstrated that the viral PAMP dsRNA induced allergen-specific Th17 cell response in the airways and VEGF and IL-6 induced by low-level dsRNA during sensitization are responsible for the differentiation of naïve T cells into Th17 cells.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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