

T_H2 and T_H1 lung inflammation induced by airway allergen sensitization with low and high doses of double-stranded RNA

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Background: Although respiratory viral infections in early childhood can enhance the development of airway allergen sensitization, the exact mechanisms of the effects of viral infections on the adaptive immune response to inhaled allergens are controversial.

Objective: We sought to evaluate the effects of double-stranded RNA (dsRNA) on airway sensitization to inhaled allergens.

Methods: Novel mouse models were created through simultaneous airway sensitization to an allergen and low or high doses of dsRNA. The mouse models were applied to Toll-like receptor 3–, IL-13–, IL-4–, signal transducer and activator of transcription (STAT) 6–, IFN- γ –, and T-box expressed in T cells (T-bet)–deficient mice to evaluate underlying pathophysiologic mechanisms in the development of allergic lung inflammation.

Results: We found that airway allergen sensitization with dsRNA induced lung inflammation that was not present in Toll-like receptor 3–deficient mice. Moreover, lung inflammation enhanced by low-dose dsRNA was impaired in IL-13–deficient mice, whereas lung inflammation by high-dose dsRNA was impaired in IFN- γ –deficient mice. The models also demonstrated that low-dose dsRNA enhanced IL-4 expression during allergen sensitization and that inflammation enhanced by low-dose dsRNA was not present in IL-4– or STAT6–deficient mice. In contrast, the present study showed that high-dose

dsRNA enhanced IFN- γ expression during allergen sensitization and that the development of lung inflammation enhanced by high-dose dsRNA was impaired in T-bet–deficient mice.

Conclusion: These findings suggest that airway allergen exposure during respiratory viral infections might induce asthma induced by both T_H1 and T_H2 immune responses to inhaled allergens.

Clinical implications: Targeting both T_H1 and T_H2 lung inflammation might be important in the treatment of virus-associated asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Double-stranded RNA, virus, allergic inflammation, T_H1, T_H2

The development of asthma is linked not only to respiratory allergen sensitization but also to viral respiratory tract infections in early childhood.^{1,2} Viral infection during the first 3 years of life greatly enhances the risk of asthma in children,³ and experimental data in animal experiments support this idea.⁴ These studies suggest that specific interactions between respiratory allergies and viral infections in respiratory tracts exist in which respiratory viral infections in early childhood might enhance the development of airway allergen sensitization.

Recent longitudinal studies have shed light on the expression patterns of childhood asthma that persist into adult life, and distinct asthma phenotypes, such as atopy-associated (or atopic) and virus-associated (or nonatopic) asthma, have been identified.⁵ In terms of the lung inflammation in asthmatic patients, children with virus-associated asthma have significantly lower levels of eosinophils than children with atopic asthma,^{6,7} suggesting that different underlying pathophysiologic mechanisms exist in these 2 asthmatic groups. The respiratory tract is commonly infected by a range of viruses, and double-stranded RNA (dsRNA) is produced by many viruses during their replicative cycles.^{8,9} Antiviral immune responses have innate and adaptive components, and dsRNA stimulates innate immune responses, through Toll-like receptor 3 (TLR3), retinoic acid-inducible protein 1 (RIG-I), and melanoma differentiation-associated gene 5 (MDA5), which results in the production of proinflammatory and immune-modulating mediators, such

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Abbreviations used

AHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
dsRNA:	Double-stranded RNA
IL-4R:	IL-4 receptor
IP-10:	IFN- γ -inducible protein 10
MDA5:	Melanoma differentiation-associated gene 5
OVA:	LPS-depleted ovalbumin
PKR:	Double-stranded RNA-dependent protein kinase
Poly(I:C):	Polyinosine-polycytidylic acid
RIG-I:	Retinoic acid-inducible protein I
STAT:	Signal transducer and activator of transcription
T-bet:	T-box expressed in T cells
TLR3:	Toll-like receptor 3
WT:	Wild-type

as type I IFNs (eg, IFN- α and IFN- β), IFN- γ , and IL-12.¹⁰⁻¹² However, the exact mechanisms of different doses of dsRNA on the adaptive immune response to inhaled allergens is wholly unknown. Here we hypothesized that airway allergen sensitization with different doses of dsRNA would induce distinct adaptive immune responses to inhaled allergens.

METHODS

Animals

TLR3-deficient (B6129S1) and wild-type (WT) control (B6129SF1/J); IL-4-deficient, signal transducer and activator of transcription (STAT) 6-deficient, IFN- γ -deficient, and WT control (C57BL/6); and T-box expressed in T cells (T-bet)-deficient and WT control (BALB/c) mice were purchased from Jackson Laboratories (Bar Harbor, Me). IL-13-deficient mice (C57BL/6) were kindly donated by Dr J. A. Elias (Yale University School of Medicine, New Haven, Conn). The breeding of these null mutant and WT mice has been previously described.^{13,14} Animal study protocols were approved by the Institutional Animal Care and Use Committee of Seoul National University, Korea.

A novel mouse model of dsRNA-enhanced asthma

For more data on a novel mouse model of dsRNA-enhanced asthma, see Fig 1, A. LPS-depleted ovalbumin (OVA) was prepared, as previously described.¹⁵ Six-week-old WT mice were sensitized with 4 intranasal doses of 75 μ g of OVA on days 0, 1, 2, and 7 to generate a mouse asthma model. OVA sensitization was performed in either the absence or presence of 0.1 μ g (low dose) or 10 μ g (high dose) of polyinosine-polycytidylic acid (poly[I:C]), a dsRNA (Sigma-Aldrich, St Louis, Mo). Mice were then challenged 4 times intranasally with 50 μ g of OVA on days 14, 15, 21, and 22. Evaluation of methacholine airway hyperresponsiveness (AHR) and lung inflammation was performed on days 23 and 24, respectively.

Evaluations of methacholine AHR, lung inflammation, and mucus production

Pulmonary function testing was assessed in conscious, unrestrained mice by using noninvasive whole-body plethysmography (Allmedicus, Seoul, Korea), as previously described.¹⁶ Lung and bronchoalveolar lavage (BAL) samples were obtained, as previously described.¹⁶ The total numbers of inflammatory cells were counted

after dilution of cell pellets with 50 μ L of PBS. After Diff-quick staining (Dade Behring, Duding, Switzerland) of BAL pellets in a cyto-spin preparation, the types of inflammatory cells were determined by counting 300 cells, which were classified as macrophages, lymphocytes, neutrophils, or eosinophils. The lung was perfused with cold PBS through the right ventricle until the pulmonary vasculature was clean. The whole lung was inflated with fixatives for histology. Hematoxylin and eosin and diastase-resistant periodic acid-Schiff stains were used on lung sections after pressure fixation with Streck solution (Streck Laboratories, La Vista, Neb). The same microscopic magnification was used on all sample slides for comparison.

Evaluation of immunologic parameters

BAL proteins and serum OVA-specific IgG1, IgG2a, and IgE levels were determined as previously described.¹⁶ For cell-surface marker analysis and intracellular staining, single-cell suspensions from the lung were prepared as described previously.¹⁷ Briefly, cells isolated from lung tissue were distributed into tubes (1×10^6 cells per tube) for staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD11b, anti-NK1.1, anti-CD14, anti-CD3, and anti-CD8 (BD Pharmingen, San Diego, Calif). Isolated cells were stained with anti-IL-4 and anti-IFN- γ antibody (BD Pharmingen) for 30 minutes to determine intracellular IL-4 and IFN- γ levels. The cells were analyzed with a FACSCalibur (BD Biosciences, Franklin Lakes, NJ). Results were processed with CellQuest software (BD Biosciences).

Statistics

Significant differences among treatments were assessed by using the Student *t* test, ANOVA, or Wilcoxon rank sum test. For multiple comparisons, ANOVA was initially used, and if significant differences were found, individual *t* tests or Wilcoxon rank sum tests between pairs of groups were performed.

RESULTS

Airway allergen sensitization with dsRNA, irrespective of doses, induces noneosinophilic lung inflammation after an allergen challenge

To evaluate the effects of different levels of dsRNA on the adaptive immune response to allergens, mice were sensitized with OVA plus low (0.1 μ g) or high (10 μ g) doses of poly(I:C) and analyzed after intranasal OVA challenge, as shown in Fig 1, A. The present study showed that total inflammatory cell counts in BAL fluids were higher in mice from the OVA plus poly(I:C) groups, irrespective of poly(I:C) doses, than in mice sensitized with PBS, OVA, or low or high doses of poly(I:C) alone. In particular, macrophage and neutrophil counts were higher in the OVA plus poly(I:C) groups than in the other groups (Fig 1, B). Lung histologic findings also showed that peribronchiolar and perivascular infiltrations by mononuclear inflammatory cells were also markedly increased in the OVA plus poly(I:C) groups compared with the other groups (Fig 1, C). However, the present study showed that mucus production was not found in mice sensitized with OVA plus low or high doses of poly(I:C) (data not shown).

TLR3 signaling plays an essential role in the development of methacholine AHR and lung inflammation enhanced by dsRNA exposure

Based on the knowledge that TLR3 is one of the key pattern-recognition receptors for dsRNA in innate

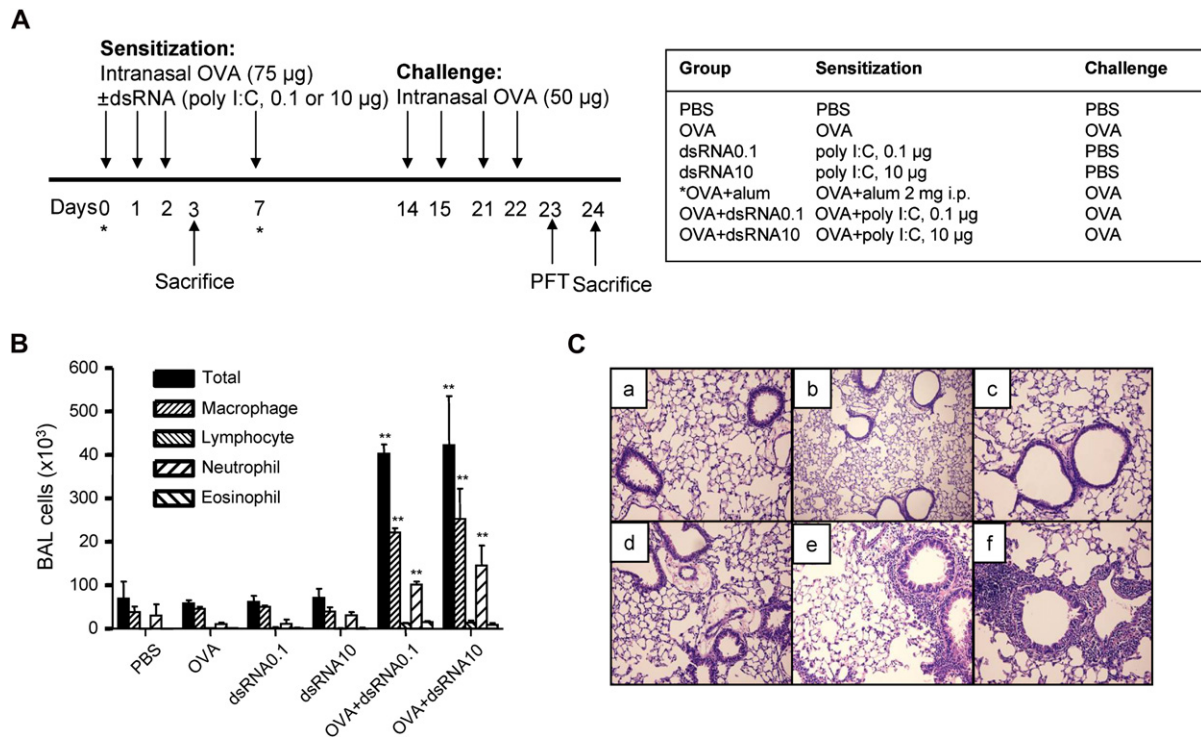


FIG 1. Lung inflammation and immunologic parameters in mice sensitized with allergens plus dsRNA. For all figures, an *asterisk* indicates a *P* value of less than .05 compared with the PBS and OVA groups, and a *double asterisk* indicates a *P* value of less than .05 versus the other groups. In (B), 1 representative experiment of 5 replicates is shown. **A**, Protocol for our mouse models of asthma. **B**, BAL cellularity after allergen challenge. **C**, Lung histology after allergen challenge (a, PBS; b, OVA; c, dsRNA0.1; d, dsRNA10; e, OVA plus dsRNA0.1; f, OVA plus dsRNA10; hematoxylin and eosin stain, original magnification $\times 200$).

immunity,¹⁸ we applied the aforementioned mouse models to TLR3-deficient and same-aged WT control mice. We found that methacholine AHR was enhanced in mice intraperitoneally sensitized with OVA plus alum and intranasally sensitized with OVA plus low or high doses of poly(I:C) more than in mice intranasally sensitized with OVA alone (Fig 2, A). However, AHR enhanced by low or high doses of poly(I:C) did not develop in TLR3-deficient mice, although AHR enhanced by intraperitoneal OVA plus alum sensitization remained unaffected in TLR3-deficient mice (Fig 2, A). In addition, BAL cellularity enhanced by low or high doses of poly(I:C) did not develop in TLR3-deficient mice, whereas BAL cellularity enhanced by intraperitoneal OVA plus alum sensitization was similar between TLR3-deficient and WT control mice (Fig 2, B). In terms of OVA-specific antibody production, we found that OVA-specific IgE production enhanced by low-dose poly(I:C) did not develop in TLR3-deficient mice, although OVA-specific IgE production enhanced by intraperitoneal OVA plus alum sensitization did not decrease (Fig 2, C). In addition, serum OVA-specific IgG2a levels enhanced by high-dose poly(I:C) was also not observed in TLR3-deficient mice (Fig 2, C).

IL-13 is a key cytokine in the development of lung inflammation in the low-dose dsRNA model

To evaluate the role of IL-13 in the development of lung inflammation enhanced by dsRNA, we applied the above-described mouse models to IL-13-deficient and same-aged WT control mice. In this experiment BAL cellularity was significantly lower in IL-13-deficient mice sensitized with OVA plus low-dose poly(I:C) but not lower in these mice when sensitized with OVA plus high-dose poly(I:C) compared with WT mice sensitized in the same manner (Fig 3, A). Furthermore, the production of TGF- β 1 (a key downstream cytokine of the T_H2 cytokines) in the low-dose dsRNA model was also lower in IL-13-deficient mice relative to the WT mice (Fig 3, B). In terms of the role of IL-13 on allergen-specific antibody production, our data show that OVA-specific IgE production enhanced by low-dose poly(I:C) in WT mice decreased in IL-13-deficient mice (Fig 3, C). In contrast, OVA-specific IgG2a levels were higher in IL-13-deficient mice sensitized with OVA plus low-dose poly(I:C) mice than in WT mice sensitized in the same manner, although the levels were similar between IL-13-deficient and WT mice sensitized with OVA plus high-dose poly(I:C) (Fig 3, C).

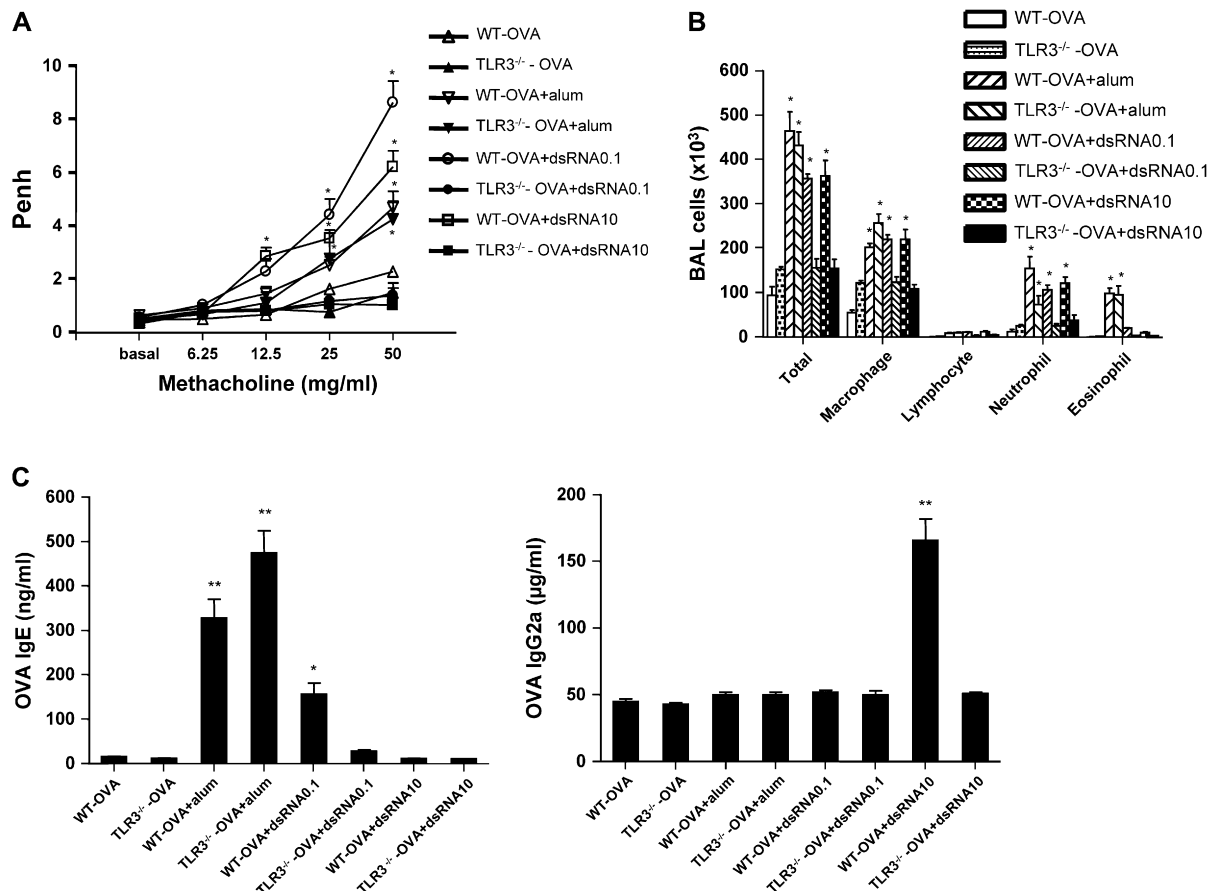


FIG 2. The role of TLR3 on the development of AHR and lung inflammation enhanced by dsRNA. For all figures, 1 representative experiment of 5 replicates is shown. An *asterisk* indicates a *P* value of less than .05 compared with the OVA groups, and a *double asterisk* indicates a *P* value of less than .05 versus the other groups. Methacholine AHR (A) and BAL cellularity (B) after OVA challenge are shown. Serum OVA-specific IgE (C) and IgG2a (D) levels after OVA challenge are shown. *Penh*, Enhanced pause.

IL-4 and STAT6 signaling is a key pathway in the development of lung inflammation in the low-dose dsRNA model

During the sensitization period, we found that lung infiltration of NK1.1⁺ cells was enhanced in mice sensitized with OVA plus low-dose poly(I:C). In contrast, CD11b⁺NK1.1⁻ cells increased in the lungs of mice sensitized with OVA plus high-dose poly(I:C) (data not shown). In terms of T_H2-polarizing cytokines, NK1.1⁺ IL-4⁺ cells were more infiltrated in mice sensitized with OVA plus low-dose poly(I:C) relative to the other groups (Fig 4, A). Based on these findings, we hypothesized that IL-4 and STAT6 signaling is a key pathway in the development of lung inflammation in the low-dose dsRNA model. To test this, we applied the above-described mouse models to IL-4- and STAT6-deficient and same-aged WT control mice and then evaluated lung inflammation after allergen challenge. In this experiment BAL cellularity enhanced by low-dose poly(I:C) was significantly lower in IL-4- or STAT6-deficient mice sensitized with OVA plus low-dose poly(I:C) but was similar in these mice when sensitized with OVA plus high-dose poly(I:C)

compared with that seen in WT mice sensitized in the same manner (Fig 4, B). Furthermore, BAL TGF-β1 levels enhanced by low-dose poly(I:C) did not increase in IL-4- or STAT6-deficient mice relative to WT mice (Fig 4, C). However, the upregulation of IFN-γ-inducible protein 10 (IP-10) by high-dose poly(I:C) was not enhanced in IL-4- or STAT6-deficient mice (Fig 4, C). As for allergen-specific antibody production, our data show that OVA-specific IgE production enhanced by low-dose poly(I:C) in WT mice was not observed in IL-4- or STAT6-deficient mice (Fig 4, D). In contrast, OVA-specific IgG2a levels were enhanced in both IL-4-deficient and STAT6-deficient mice compared with those seen in WT mice in the low-dose dsRNA model, although the levels were similar among these mice in the high-dose dsRNA model (data not shown).

IFN-γ is a key cytokine in the development of AHR and noneosinophilic lung inflammation in the high-dose dsRNA model

In terms of T_H1-polarizing cytokines, we found that lung infiltration of CD14⁺IFN-γ⁺ cells was enhanced in

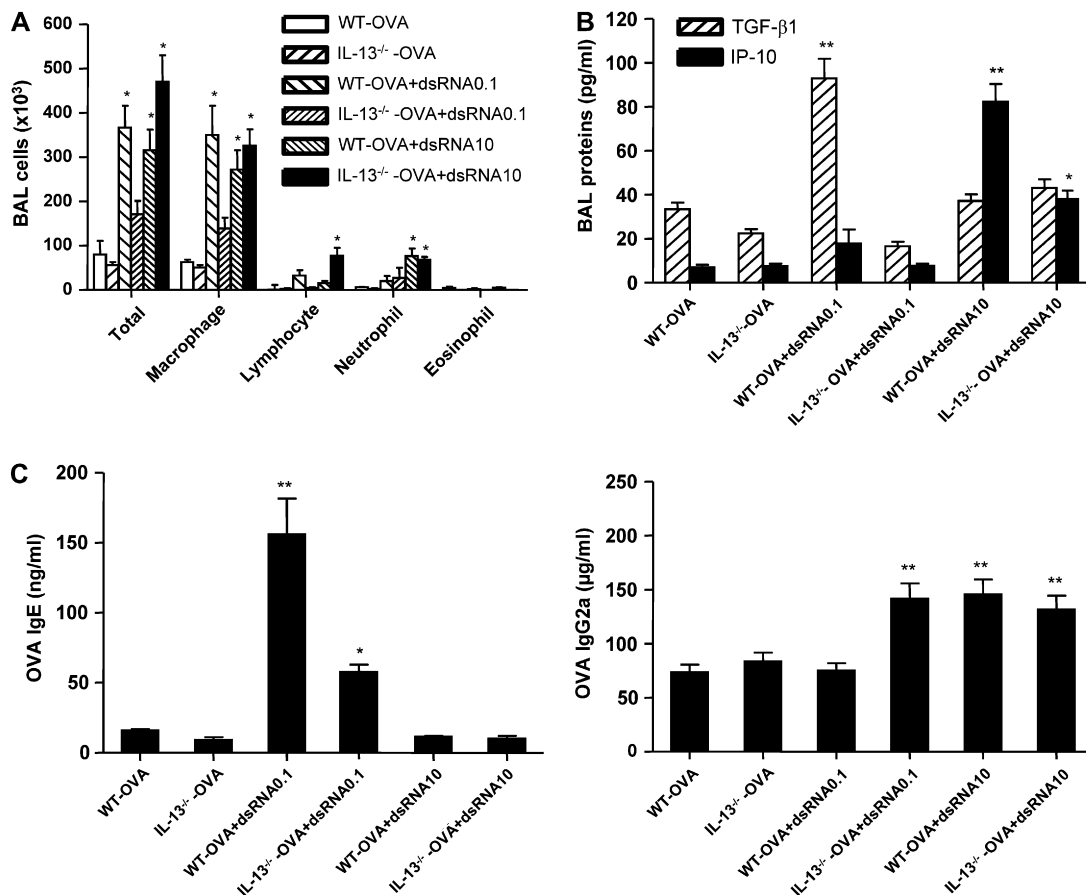


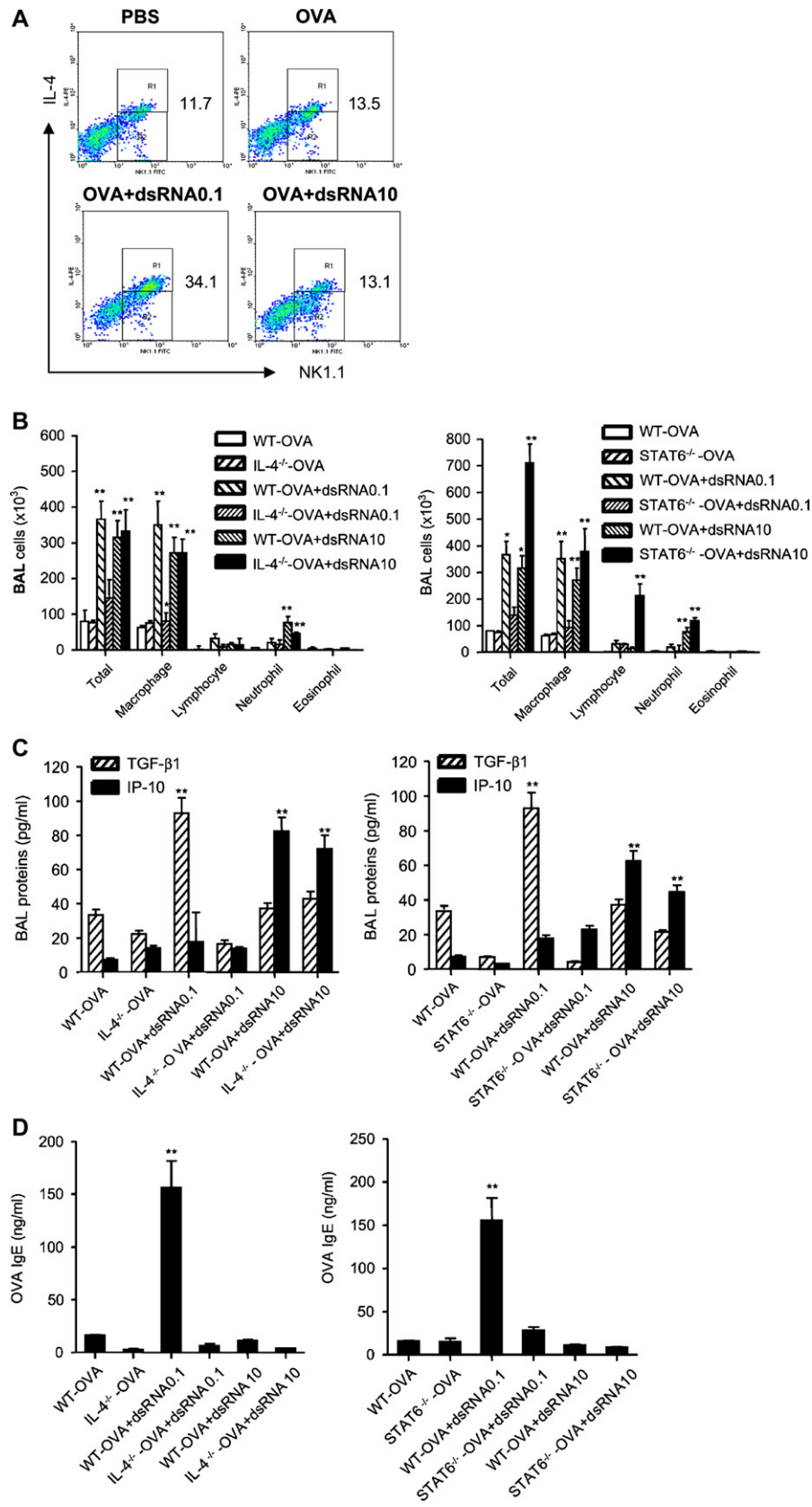
FIG 3. The role of IL-13 on the development of lung inflammation enhanced by dsRNA. For all figures, 1 representative experiment of 5 replicates is shown. An *asterisk* indicates a *P* value of less than .05 compared with the OVA groups, and a *double asterisk* indicates a *P* value of less than .05 versus the other groups. Cellularity (**A**) and protein levels (**B**) in BAL fluids after OVA challenge are shown. Serum OVA-specific IgE (**C**) and IgG2a (**D**) levels after OVA challenge are shown.

mice sensitized with OVA plus high-dose poly(I:C) (Fig 5, A). In addition, BAL fluid IL-12p40 levels were also significantly higher in mice sensitized with OVA plus high-dose poly(I:C) than in mice sensitized with PBS or OVA alone or OVA plus low-dose poly(I:C) (Fig 5, B). Based on these findings, we hypothesized that T_H1 immune response is a key pathophysiology in the development of asthma phenotypes enhanced by high-dose dsRNA. To test this postulate, we administered the high-dose dsRNA model to IFN- γ -deficient and same-aged WT control mice and then evaluated AHR and lung inflammation after allergen challenge. The present study showed that methacholine AHR was not developed in IFN- γ -deficient mice sensitized with OVA plus high-dose poly(I:C), although it developed in WT control animals sensitized in the same manner (Fig 5, C). BAL macrophage and lymphocyte counts were significantly lower in IFN- γ -deficient mice sensitized with OVA plus high-dose dsRNA compared with WT mice sensitized in the same manner (Fig 5, D), although eosinophil counts were enhanced in IFN- γ -deficient mice. Lung histologic findings also showed that peribronchiolar and perivascular macrophage and

lymphocyte infiltrations were induced, although to a lesser degree, in OVA plus high-dose dsRNA-sensitized IFN- γ -deficient mice versus WT mice sensitized in the same manner as shown in Fig 5, D. These phenotype changes in IFN- γ -deficient mice were accompanied by downregulation of IP-10 and upregulation of TGF- β 1 production (Fig 5, E), but the production of T_H2 cytokines, such as IL-4 and IL-13, was not enhanced in IFN- γ -deficient mice (data not shown). Furthermore, serum OVA-specific IgG2a levels did not increase in OVA plus high-dose dsRNA-sensitized IFN- γ -deficient mice but did increase in WT control animals, despite an increase in OVA-specific IgG1 levels in OVA plus high-dose dsRNA-sensitized IFN- γ -deficient mouse serum (Fig 5, F).

Lung inflammation enhanced by high-dose dsRNA is mainly dependent on the T-bet signaling pathway

Finally, we investigated the role of T-bet in the development of lung inflammation in the high-dose dsRNA model because T-bet is well-known to be a transcription factor that controls IFN- γ and IL-12R β 2 expression in



CD4⁺ T cells.¹⁹ Our data show that the enhanced infiltration of macrophages, lymphocytes, and neutrophils by high-dose dsRNA exposure was not observed in T-bet-deficient mice (Fig 6, A). These phenotypes were accompanied by downregulation of IP-10 production, whereas TGF- β 1 production was enhanced in T-bet-deficient mice, irrespective of the addition of dsRNA (Fig 6, B). Moreover, the enhanced OVA-specific IgG2a production by high-dose dsRNA was impaired in T-bet-deficient mice, although IgG1 production was not altered in these mice (Fig 6, C).

DISCUSSION

Considering the functional differentiation of helper T-cell subsets, a reduction in infectious diseases during early childhood has been emphasized as a risk factor for the increasing prevalence of asthma.²⁰ However, the fact that asthma in infants and young children, especially the first attack, often develops with or just after viral respiratory tract infections suggests that viral respiratory tract infection is a risk factor for the development of asthma.^{2,3} The default immune response to inert inhaled proteins is the development of immune tolerance.^{21,22} Dahl et al⁴ demonstrated through the use of animal experiments that airway allergen sensitization after influenza A infection induced both T_H1 and T_H2 responses to allergens. Antiviral immune responses have innate and adaptive components.²³ dsRNA is produced by many viruses during their replicative cycles and is a key pathogen-associated molecular pattern of single-stranded RNA viruses, including respiratory viruses like rhinovirus, respiratory syncytial virus, and influenza A and B.^{8,9} To evaluate the effects of respiratory viral infections on allergen sensitization and the subsequent development of allergic inflammation, we developed novel mouse models that use dsRNA as an adjuvant. We found that airway allergen sensitization with dsRNA induced noneosinophilic lung inflammation with the upregulation of allergen-specific IgE or IgG2a production. These findings suggest that airway allergen exposure during respiratory viral infections induces T-cell priming more than tolerance.

The innate immune system senses viral infection by recognizing a variety of viral components, including dsRNA.^{12,24} Some synthetic dsRNAs, such as poly(I:C), have similar activities to that of dsRNA. Some of the immunostimulatory activity of dsRNA is believed to be elicited by the activation of dsRNA-dependent protein kinase (PKR).⁸ However, it was shown that the responses to viral infections were apparently normal in PKR-deficient

mice, indicating that additional molecules other than PKR might be responsible for the recognition of dsRNA.²⁵ TLR3, located in endosome, and the cytoplasmic helicase proteins RIG-I and MDA5 have been implicated in the recognition of poly(I:C) and the subsequent induction of antiviral responses.^{18,26,27} A recent *in vivo* study using mice lacking RIG-I, MDA5, or TRIF (Toll/IL-1 receptor-domain-containing adaptor, a key signaling molecule of TLR3) suggest that MDA5 is essential for poly(I:C)-induced IFN- α production and TLR3 signaling is critical for IL-12 production in response to poly(I:C).¹² Our present study showed that lung inflammation and AHR enhanced by low or high doses of dsRNA were impaired in TLR3-deficient mice. Taken together, the effects of dsRNA on the adaptive immune response to inhaled allergens might be linked to a TLR3-mediated pathway, and immune-modulating mediators other than type I IFN are critical in the development of adaptive immune response enhanced by low or high doses of dsRNA.

The present study showed that airway allergen sensitization with high-dose dsRNA induced T_H1 lung inflammation with enhanced AHR, which was abolished in the absence of IFN- γ . This finding indicates that T_H1 immune response to inhaled allergens is a key pathophysiologic mechanism on the development of asthma phenotypes enhanced by high-dose dsRNA. A substantial advance in elucidating T_H1 lineage commitment and IFN- γ expression has been provided with the isolation of a novel protein, T-bet.²⁸ T-bet is a member of the T-box family of transcription factors that regulate several developmental processes. T-bet expression is strongly correlated with IFN- γ expression; ectopic expression of T-bet *in vitro* leads to strong transactivation of the IFN- γ gene reporter construct.²⁸ Therefore T-bet appears to function by initiating a T_H1 differentiation program. Thus we evaluated the role of T-bet in the development of lung inflammation in the high-dose dsRNA model based on the data that high-dose dsRNA induces enhanced expression of IFN- γ and IL-12 during the allergen sensitization period. Indeed, our findings indicated that the development of lung inflammation and allergen-specific IgG2a production were impaired in T-bet-deficient mice. Taken together, high levels of dsRNA appear to promote T_H1 polarization through the IFN- γ /T-bet/IL-12 pathway.

Meanwhile, the present study indicated that airway allergen sensitization with low-dose dsRNA induced lung inflammation, which was impaired in IL-13-deficient mice. This finding indicates that T_H2 cytokines, especially IL-13, are key mediators in the development of lung inflammation enhanced by low-dose dsRNA. Moreover, the present study showed that IL-4 expression was

FIG 4. The role of the IL-4 and STAT6 signaling pathway on the development of lung inflammation enhanced by dsRNA. For all figures, 1 representative experiment of 5 replicates is shown. An *asterisk* indicates a *P* value of less than .05 compared with the OVA groups, and a *double asterisk* indicates a *P* value of less than .05 versus the other groups. **A**, Intracellular staining of IL-4 in NK1.1⁺ lung cells during allergen sensitization. Data are expressed as the percentage of IL-4⁺ cells among the total cells. Cellularity (**B**) and protein levels (**C**) in BAL fluids and serum OVA-specific IgE levels (**D**) after OVA challenge in IL-4- or STAT6-deficient mice are shown.

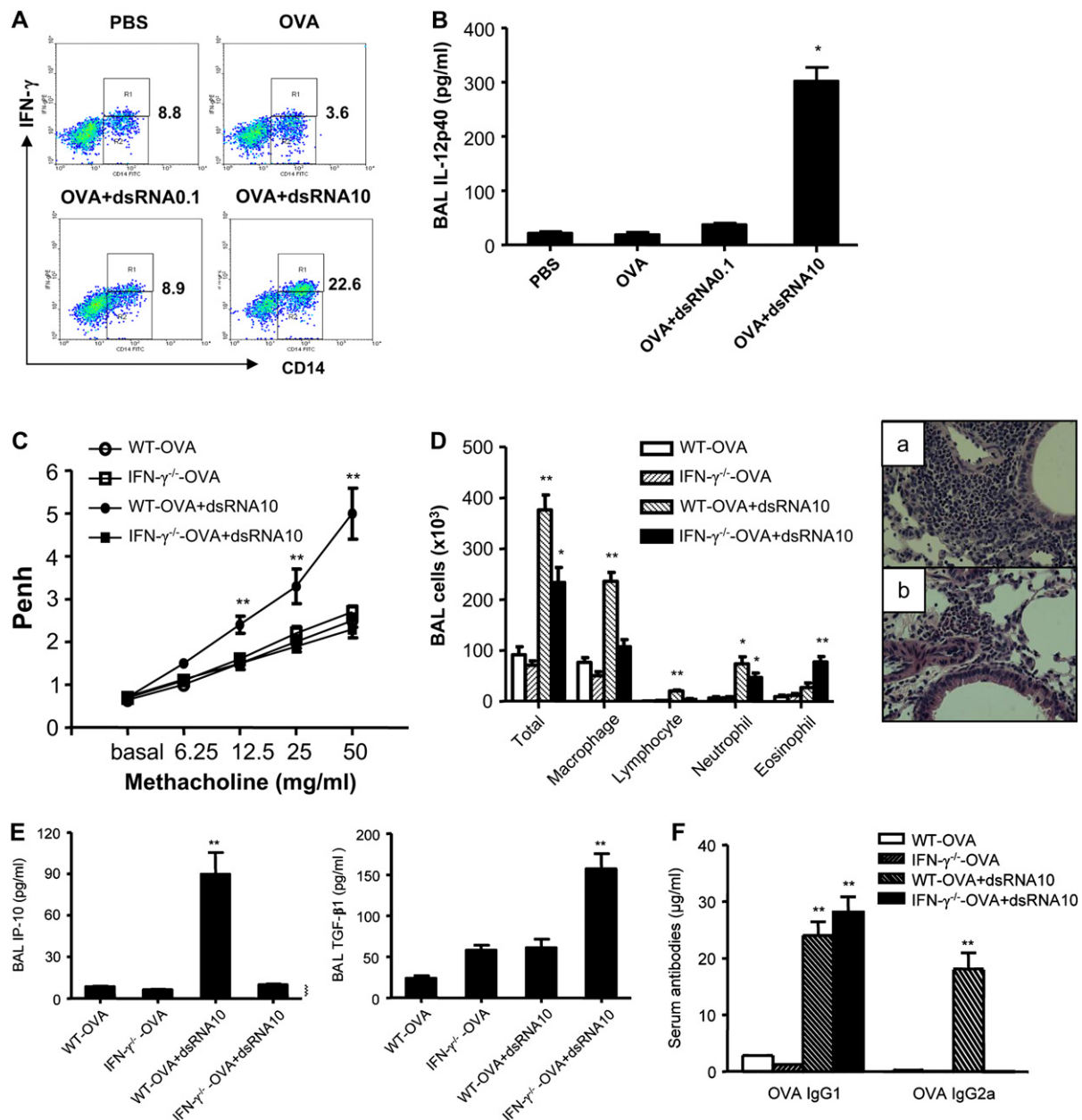


FIG 5. The role of IFN- γ on the development of lung inflammation enhanced by high-dose dsRNA. For all figures, 1 representative experiment of 5 replicates is shown. An *asterisk* indicates a P value of less than .05 compared with the OVA groups, and a *double asterisk* indicates a P value of less than .05 versus the other groups. **A**, Intracellular staining of IFN- γ in CD14 $^{+}$ lung cells during allergen sensitization. Data are expressed as the percentage of IFN- γ^{+} cells among the total cells. **B**, BAL IL-12p40 levels during the sensitization period. Methacholine AHR (**C**) and BAL fluid cellularity and lung histology (**D**) after OVA challenge in IFN- γ -deficient mice is shown (*a*, WT-OVA plus dsRNA10; *b*, IFN- $\gamma^{-/-}$ -OVA plus dsRNA10; hematoxylin and eosin stain, original magnification $\times 400$). **E**, BAL fluid IP-10 and TGF- β 1 levels after OVA challenge in IFN- γ -deficient mice are shown. **F**, Serum OVA-specific IgG1 and IgG2a levels after OVA challenge in IFN- γ -deficient mice are shown. *Penh*, Enhanced pause.

upregulated during the allergen sensitization period. *In vitro* studies of helper T cell differentiation have found that T_H2 cells are typically generated by activating naive T cells through T-cell antigen receptor cross-linking in the presence of exogenous IL-4. The IL-4 receptor (IL-4R), which consists of the common cytokine-receptor γ subunit

and the IL-4-binding IL-4R α chain, is expressed on naive T cells. IL-4R signals are transduced by the transcription factor STAT6, which together with nuclear factor of activated T cells, activating protein-1, nuclear factor κ B, and other T-cell antigen receptor-induced signals, activates the transcription of IL-4 and IL-13.²⁹ The resulting autocrine

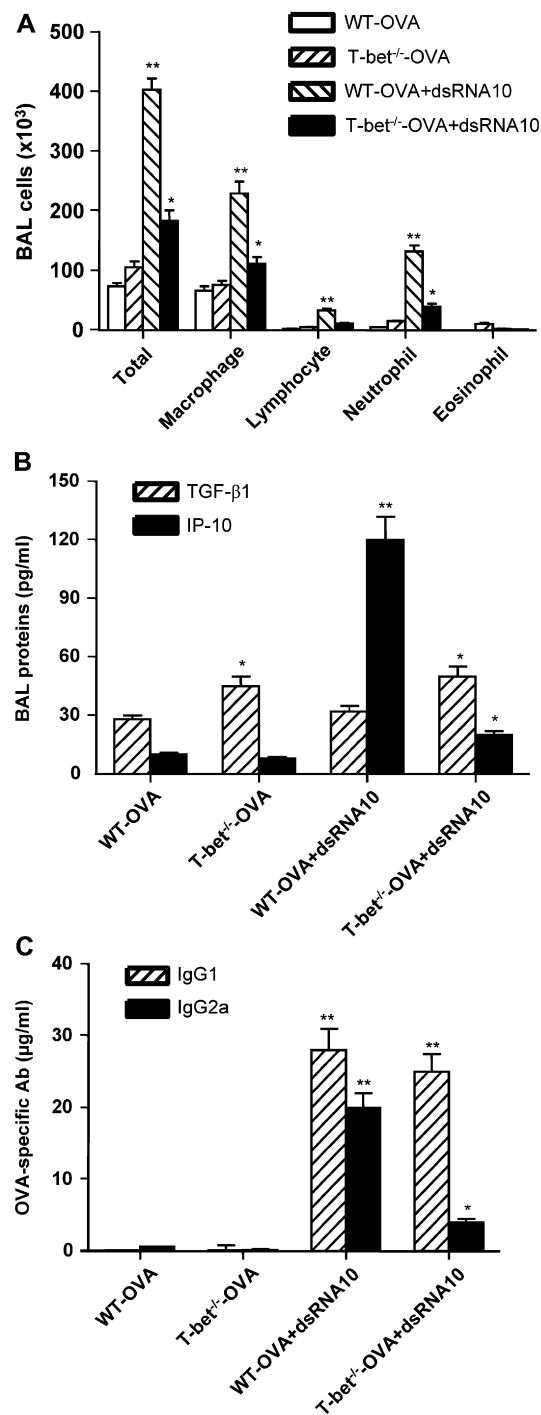


FIG 6. The role of T-bet on the development of lung inflammation enhanced by high-dose dsRNA. For all figures, 1 representative experiment of 5 replicates is shown after OVA challenge. An asterisk indicates a *P* value of less than .05 compared with the OVA groups, and a double asterisk indicates a *P* value of less than .05 versus the other groups. Cellularity (A) and proteins (B) in BAL fluids after allergen challenge in T-bet-deficient mice are shown. C, Serum OVA-specific IgG1 and IgG2a levels after allergen challenge in T-bet-deficient mice.

feedback loop acts positively to favor T_H2 differentiation.^{19,29} Indeed, the present study also showed that lung inflammation and allergen-specific IgE production enhanced by low-dose dsRNA were found to be impaired in STAT6-deficient mice. These findings suggest that allergic lung inflammation enhanced by low-dose dsRNA is mainly dependent on the IL-4 and STAT6 pathway.

In summary, we demonstrated that airway allergen sensitization with dsRNA-induced allergic lung inflammation occurred in concert with the upregulation of allergen-specific IgE or IgG2a production and that low and high doses of dsRNA enhanced T_H2 and T_H1 inflammation, respectively, which were mediated by the IL-4 and STAT6 pathway and the IFN-γ and T-bet pathway, respectively.

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