

Essential Role of B7-H1 in Double-Stranded RNA-Induced Augmentation of an Asthma Phenotype in Mice

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Clinical and epidemiological studies have shown the contribution of viral infection to the development of allergic asthma. Many RNA viruses, pathogenic for the respiratory tract, generate double-stranded (ds)RNA during their replication. Typical innate immune responses triggered by dsRNA involve the endosomal and cytoplasmic pathways. The former is mediated by Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF), and the latter by IFN- β promoter stimulator 1 (IPS-1). We explored the effect of polyinosinic polycytidilic acid, a synthetic dsRNA, on the development of an asthma phenotype in mice. Administration of dsRNA during ovalbumin sensitization augmented airway eosinophilia and airway hyper-responsiveness after an antigen challenge, which was associated with enhanced induction of IL-13-producing CD8⁺ T cells. The augmentation was induced in IPS-1-deficient mice but not in TRIF-deficient mice. The interactions between dendritic cells (DCs) and T cells are regulated by B7-family costimulatory molecules, including B7-H1 (also known as PD-L1), a putative ligand for programmed death-1 (PD-1). Treatment of bone marrow-derived DCs with dsRNA enhanced B7-H1 expression in a TRIF-dependent manner. Additionally, dsRNA increased B7-H1 expression on DCs in the draining lymph nodes of ovalbumin-sensitized mice. The augmentation of the asthma phenotype was prevented by the treatment of mice with anti-B7-H1 mAb but not with anti-PD-1 mAb. The augmentation was not induced in B7-H1-deficient mice. These results suggest that dsRNA-triggered activation of the innate immune system in sensitization leads to augmentation of the asthma phenotype via IL-13 mainly from CD8⁺ T cells. B7-H1 plays a crucial role in the process without requiring interaction with PD-1.

Keywords: TRIF; IL-13; CD8⁺ T cell; PD-1

Asthma is one of the most common chronic diseases worldwide, imposing a substantial burden on children and adults. Despite prominent advances in the therapeutic modality, clinicians continue to be challenged by the evidence that its prevalence has considerably increased. The pathogenesis of asthma is frequently associated with airway viral infection. The respiratory syncytial

CLINICAL RELEVANCE

This study demonstrates that dsRNA-triggered activation of the innate immune system in the sensitization phase leads to augmentation of the asthma phenotype. B7-H1 plays a crucial role in the augmentation. Further elucidation of the interactions between viral infection and the pathogenesis of asthma may provide a novel strategy for preventing the development of this disease.

virus, the rhinovirus, and the parainfluenza virus are reported to be associated with the development of asthma and its acute exacerbation (1, 2). These viruses have single-stranded RNA as their own genome and then generate double stranded (ds)RNA after infection to their host cells as an intermediate for replication.

Recent progress in molecular immunology has revealed that dsRNA triggers strong innate immune responses via its binding to pattern recognition receptors, such as TLR3 and the family of RNA helicase, namely the retinoic acid-inducible gene I and the melanoma differentiation-associated gene 5 (3–5). TLR3 recognizes dsRNA in the endosome and initiates signaling through the Toll/IL-1R domain-containing adaptor-inducing IFN- β (TRIF) (also known as TICAM-1) (6, 7). On the other hand, retinoic acid-inducible gene I and Mda5 recognize dsRNA in the cytoplasm and initiate signaling through IFN- β promoter stimulator 1 (IPS-1) (also known as MAVS/Cardif/VISA) (8–11). The interaction between dsRNA and pattern recognition receptors initiates cascades to produce type-I IFNs and a variety of proinflammatory cytokines. TRIF and IPS-1 share interferon regulatory factor-3 as their downstream transcriptional factor for the production of type-I IFNs (12). It is conceivable that the innate immune responses modulate the phenotype of adaptive immune responses, including allergy.

The interactions between antigen-presenting cells (APCs) and T cells constitute the first step in the development of allergy. These interactions are characterized by the binding of an array of costimulatory molecules, including B7-family molecules and their putative receptors (13). B7-H1 (PD-L1) shares its receptor, programmed death (PD)-1, with B7-DC (PD-L2) (14–17). PD-1 is inducibly expressed in T cells and B cells during their activation, and its ligation to B7-H1 or B7-DC results in the suppression of the activated status (18). B7-H1 and B7-DC are expressed constitutively or inducibly on APCs (19). In addition, B7-H1 is expressed on the peripheral tissues, including the airways, and the B7-H1 blockade leads to exacerbation of the various disease phenotypes (20–22). These studies have suggested that B7-H1 has an inhibitory role in the pathogenesis of the disease via the

(Received in original form December 14, 2009 and in final form August 12, 2010)

This study was supported in part by a grant-in-aid for scientific research from the Japan Society for the Promotion of Science and by the National Institute of Biomedical Innovation, Japan.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 45, pp 31–39, 2011

Originally Published in Press as DOI: 10.1165/rncmb.2009-0450OC on August 27, 2010

Internet address: www.atsjournals.org

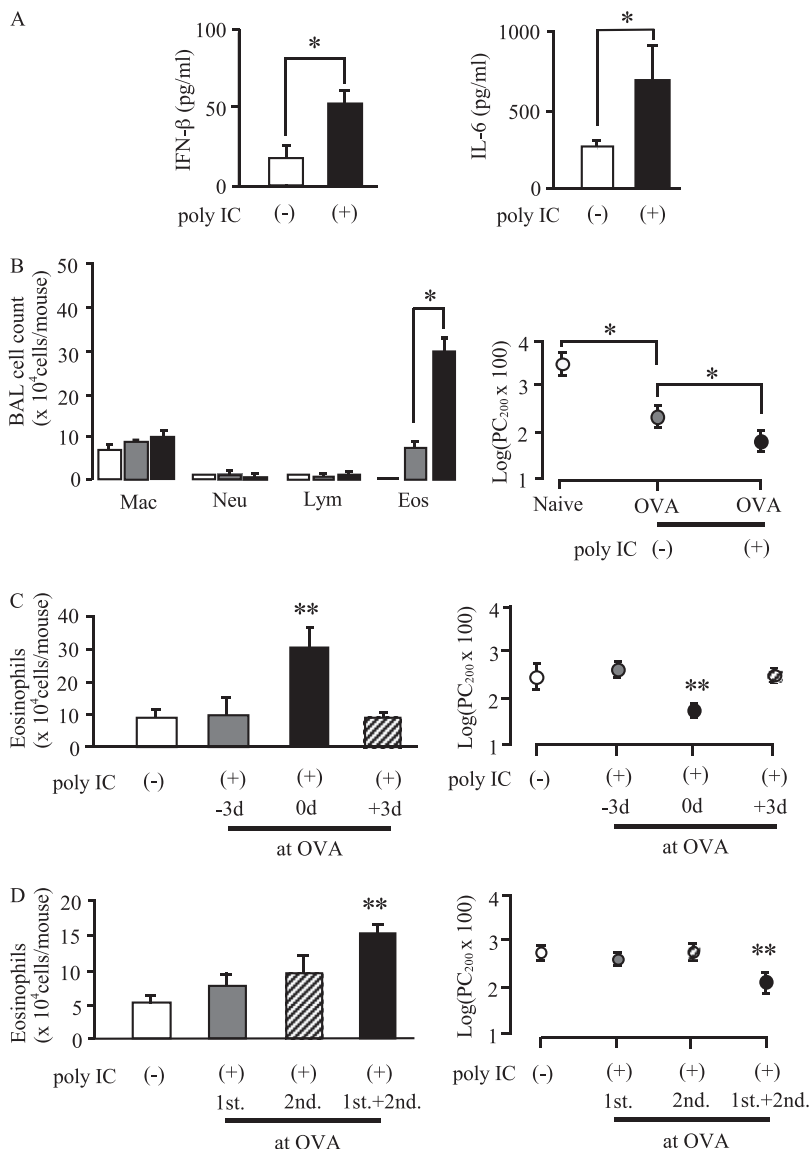


Figure 1. Effect of polyinocinic polycytidilic acid (poly IC) during sensitization on the allergen-induced asthma phenotype. (i) BALB/c mice were administered poly IC intraperitoneally. The sera were collected 5 hours after poly IC, and IFN- β and IL-6 were measured by ELISA. Each group consisted of four mice. (B) Mice were treated with poly IC (black bar) or saline alone (gray bar) 1 hour before each sensitization with ovalbumin (OVA). Nonsensitized/challenged mice served as naive controls (white bar). After the OVA challenge, the eosinophilia in bronchoalveolar lavage fluid (BALF) and the airway hyperresponsiveness (AHR) were measured. Each group consisted of seven to eight mice. Data are representative of three independent experiments. (C) Mice were treated with poly IC on the same day or 3 days before or after each sensitization. After OVA challenge, the eosinophilia in BALF and the AHR were measured. Saline-treated mice with sensitization and challenge served as controls. Each group consisted of five mice. (D) Mice were treated with poly IC at the first or second sensitization or both. After OVA challenge, the eosinophilia in BALF and the AHR were measured. Saline-treated mice with sensitization and challenge served as controls. Each group consisted of five mice. Data are expressed as the means $\pm$ SEM. * P < 0.05 between the indicated two groups. ** P < 0.05 versus saline-treated control mice.

induction and maintenance of peripheral tolerance. On the other hand, it has been reported that the B7-H1 blockade enhances intracellular bacterial infection, suggesting that B7-H1 may provide stimulatory signals for several immune responses (23). The role of B7-H1 in adaptive immune response remains undetermined.

The B7-H1/PD-1 pathway is considered to contribute to the development of immune responses to viral infection (24). We previously showed that treatment of cultured human airway epithelial cells with polyinocinic polycytidilic acid (poly IC), a synthetic dsRNA, enhanced the expression of B7-H1 on the cell surface (25). In the present study, we sought to investigate whether the stimulation with poly IC during allergen sensitization affects the phenotype of asthma after allergen challenge in mice. To determine if that was the case, we investigated whether its process is associated with B7-H1 and PD-1.

MATERIALS AND METHODS

Protocols

Mice were sensitized by intraperitoneal injection of 10 μ g of ovalbumin (OVA) (Sigma-Aldrich, St Louis, MO) and 0.3 mg of Al(OH)₃ (SERVA Electrophoresis, Heidelberg, Germany) on Days 1 and 14 and challenged with 5% OVA in saline mist for 20 minutes on Days 26,

27, and 28. Measurements were conducted on Day 30. Mice received an intraperitoneal injection of poly IC (Sigma-Aldrich) or saline 1 hour before sensitization. Several mice received intraperitoneal injections of 330 μ g of a recombinant mouse IL-13 receptor α 2/Fc chimeric protein (rmIL-13R α 2-Fc) (R&D Systems) or human IgG1 (Sigma-Aldrich) 3 hours before challenge (26–28). Subgroups of mice received intraperitoneal injections (250 μ g per animal) of anti-mouse B7-H1 mAb (MIH6), anti-mouse PD-1 mAb (RMP1–14), or control rat IgG (Sigma-Aldrich) 6 hours before sensitization.

Airway Hyperresponsiveness and Bronchoalveolar Lavage

Anesthetized mice were ventilated to measure airway responsiveness to acetylcholine via tracheostomy (29). The data were expressed as the provocative concentration 200, the concentration at which airway pressure was 200% of its baseline. Lungs were lavaged with 1 ml of saline via a tracheal cannula. Total cell counts were performed.

Lung Histology

Lungs were fixed with 10% formalin, and tissue sections were stained with hematoxylin and eosin (H&E) or with Alcian blue/periodic acid-Schiff.

Determination of Cytokines

IL-4, IL-5, IL-10, IL-13, and IFN- γ in the supernatants of bronchoalveolar lavage fluid (BALF) and the cultured supernatant from lymph

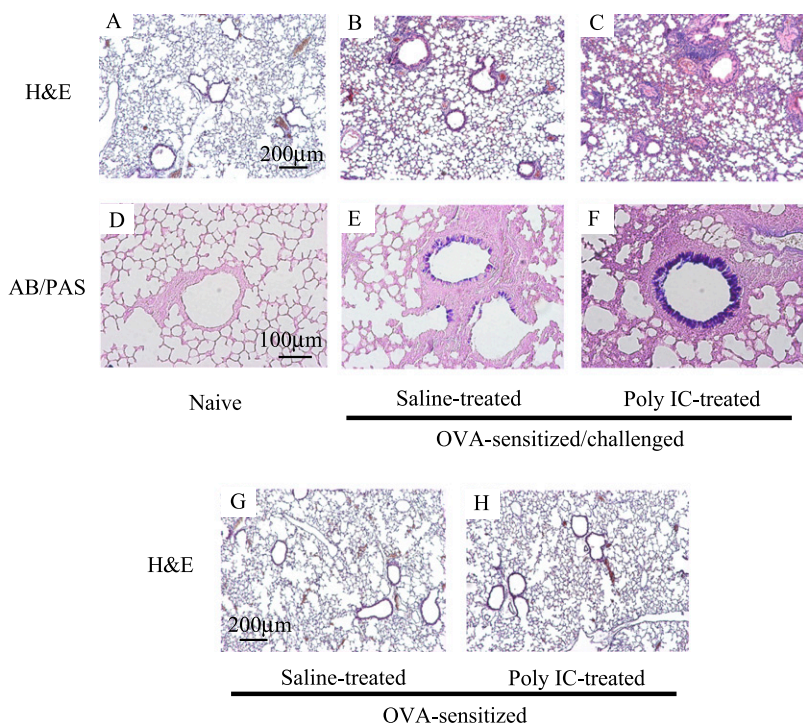


Figure 2. Effects of poly IC on lung inflammation. BALB/c mice were treated with poly IC or saline alone before each OVA sensitization. After OVA challenge, the lungs were collected and processed for H&E staining and Alcian blue/periodic acid-Schiff (AB/PAS) staining. (A and D) Unsensitized/unchallenged mice served as naive controls. (B and E) Saline-treated mice. (C and F) Poly IC-treated mice. (G and H) BALB/c mice were treated with poly IC or saline alone before each OVA sensitization. Just before OVA challenge, the lungs were processed for H&E staining. (G) Saline-treated mice. (H) Poly IC-treated mice. Data are representative of five mice of each group.

node cells and IL-6 and IFN- β in the serum were quantified using ELISA kits (BioSource, Camarillo, CA, or R&D Systems, Minneapolis, MN).

Collection of Lung Cells and Lymph Node Cells

Lungs were minced, and the single-cell suspensions were prepared. Draining lymph node (DLN) cells were collected from thoracic lymph nodes and dissociated into a single-cell suspension. Details are provided in the online supplement.

Determination of IL-13 Production by DLN Cells

DLN cells (4×10^6 cells per well) were cultured with OVA at 0, 10, 100, and 1,000 ng/ml. The supernatants were analyzed for IL-13 after 72 hours of stimulation.

Preparation of Bone Marrow-Derived Dendritic Cells

Bone marrow-derived dendritic cells (BMDCs) were prepared with recombinant mouse GM-CSF (R&D Systems) at 10 ng/ml for 4 to 6 days after the initiation of culture. On Day 7, BMDCs were stimulated with or without poly IC at 30 μ g/ml for 24 hours.

Flow Cytometry

To detect IL-13-producing T cells, lung cells (4×10^6 cells per well) were stimulated with anti-mouse CD3 mAb (145-2C11) at 5 μ g/ml plus anti-mouse CD28 mAb (37.51) at 2 μ g/ml for 48 hours. Surface staining was performed using PerCP-labeled anti-CD4 (RM4-5) or anti-CD8 α (53-6.7) and allophycocyanin-labeled anti-CD3 (145-2C11) mAbs. Intracellular IL-13 staining was performed using a kit (eBioscience, San Diego, CA). To detect B7-H1, BMDCs, lung cells, and DLN cells were stained with FITC-labeled anti-CD11c mAb (HL3), PE-labeled anti-B7-H1 mAb (MIH6), and PerCP-labeled anti-CD8 α (53-6.7). Flow cytometry was performed using a FACSCalibur (BD Biosciences). Details are provided in the online supplement.

Data Analysis

Parametric data were analyzed using the unpaired *t* test or ANOVA with Bonferroni's correction. Nonparametric data were analyzed using the Mann-Whitney *U* test or the Kruskal-Wallis test. *P* values less than 0.05 were considered significant.

RESULTS

Poly IC in Sensitization Augments the Asthma Phenotype after Allergen Challenge

In the preliminary dose-ranging experiments, an intraperitoneal injection of 10 μ g of poly IC to naive BALB/c mice activated the innate immune system, as typically demonstrated by the elevation of IFN- β and IL-6 in the sera collected 5 hours after the poly IC injection (Figure 1A), without a lethal outcome. To simulate a mild impact of viral dsRNA on the host immune system, the above dosage was chosen for the following study. When BALB/c mice were treated with poly IC 1 hour before each sensitization with OVA (Figure 1B), the eosinophilia in BALF and airway hyperresponsiveness (AHR) after OVA inhalation challenge were augmented significantly more than in saline-treated mice. The activation of an innate immune system is known to occur within a few hours and to resolve within a few days (30). To elucidate whether such a temporal kinetic is critical for the augmentation, the effect of poly IC was examined at various time points. The augmentation appeared in the mice that had been treated with poly IC on the same day at each sensitization but not 3 days before or after (Figure 1C), which is consistent with the kinetics of innate immune responses. An adaptive immune system is characterized by the phenomenon in which repeated antigenic stimuli amplify the magnitude of effector-phase responses associated with immune memory (31). If dsRNA affects the process of adaptive immune responses, its effect would be associated with the above phenomenon (32). Indeed, the augmentation of an asthma phenotype was shown in the mice that had been treated with poly IC at both the first and second sensitization but not at one or the other alone (Figure 1D). These results suggest that the dsRNA-triggered activation of an innate immune system may affect the ongoing adaptive immune responses and augment the phenotype of asthma.

The effect of poly IC was further examined by histology. The OVA sensitization and challenge caused the infiltration of inflammatory cells composed mainly of mononuclear cells and

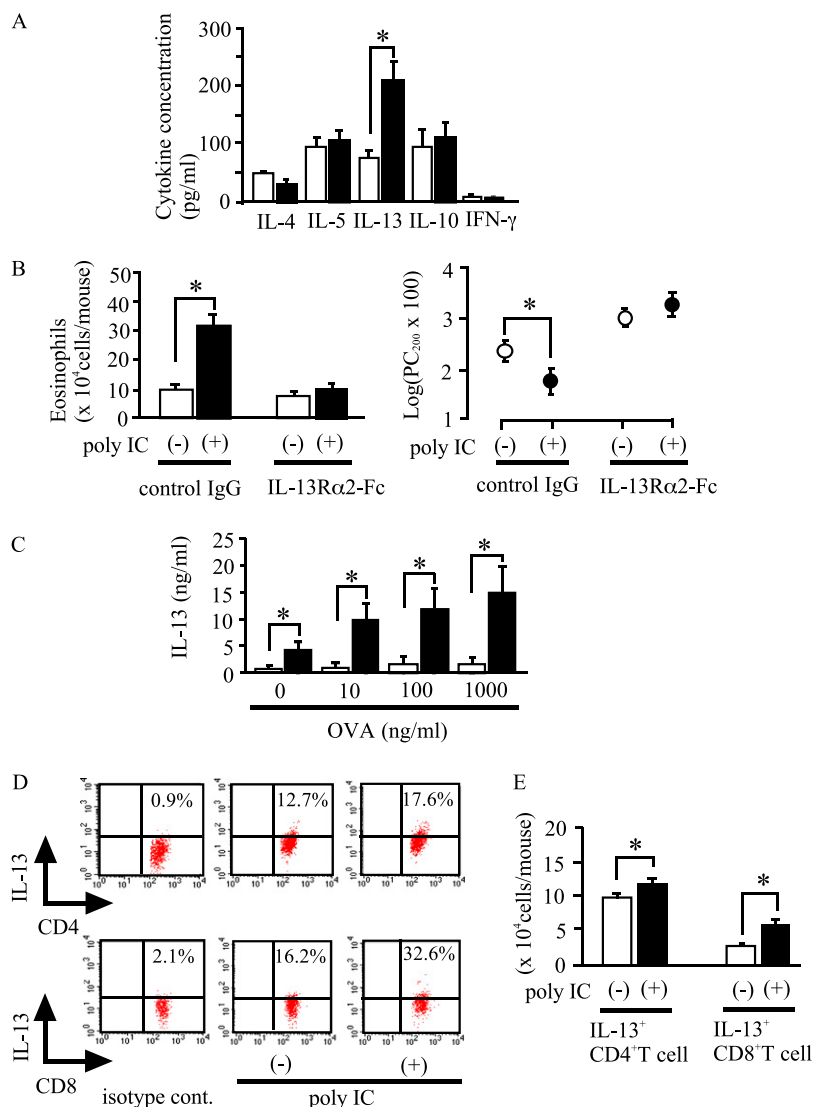


Figure 3. Effect of poly IC on cytokine responses. BALB/c mice were treated with poly IC or saline alone before each OVA sensitization. (A) After OVA challenge, the cytokine contents in BALF were measured by ELISA. Each group consisted of seven to eight mice. Data are representative of two independent experiments. (B) During the OVA challenge, mice were treated with rmlIL-13 α_2 -Fc or control human IgG1, and the eosinophilia in BALF and the AHR were measured. Each group consisted of five to seven mice. (C) After the OVA challenge, draining lymph node cells were collected and stimulated *ex vivo* with OVA. IL-13 in the cultured supernatant was measured by ELISA. White bars indicate saline-treated mice; black bars indicate poly IC-treated mice. Each group consisted of five mice. (D and E) After OVA challenge, IL-13-producing CD4⁺ and CD8⁺ T-cell subsets in the lungs were evaluated by intracellular cytokine staining. The numbers of dot plots indicate the typical percentage of IL-13-positive cells for each T-cell subset. Statistical assessment was performed for enumerated data as absolute numbers per animal. Each group consisted of six mice. Data are expressed as the means $\pm$ SEM. * $P < 0.05$ between the indicated two groups.

eosinophils in the airways (Figures 2A and 2B). The inflammation accompanied with increase in Alcian blue/periodic acid-Schiff-positive cells suggested the presence of goblet cell hyperplasia (Figures 2D and 2E). Treatment with poly IC induced more prominent inflammation and goblet cell hyperplasia (Figures 2C and 2F). Previous studies reported that poly IC administered directly into the airways has direct proinflammatory effects on the airways (33, 34). However, no signature of inflammation was seen in the lung histology of OVA-sensitized mice, regardless of intraperitoneal poly IC treatment, that were killed on Day 25, just before OVA challenge (Figures 2G and 2H). Accordingly, there was no difference in the cellular profiles in the BALF between poly IC-treated and untreated mice (data not shown). These results suggest that the augmentation by poly IC may be not due to its direct effect on the airways.

Poly IC-Induced Augmentation of the Asthma Phenotype Is Mediated by IL-13

The cytokine profiles in BALF after challenge were analyzed (Figure 3A). The concentration of IL-13 in poly IC-treated mice was significantly higher than that in saline-treated mice, whereas the concentrations of IL-4, IL-5, IL-10, and IFN- γ were comparable. IL-13 is known to be a representative cytokine causing the asthma phenotype (26, 27, 29). Its selective overproduction might

be responsible for the augmentation. Thus, we examined the effect of an IL-13 inhibitor, rmlIL-13 α_2 -Fc (Figure 3B). Under rmlIL-13 α_2 -Fc treatment during the OVA challenge, neither the eosinophilia in BALF nor AHR in poly IC-treated mice was different from that in saline-treated mice. Under the control human IgG1 treatment, the asthma phenotype was significantly augmented in poly IC-treated mice. These results indicate that excessive IL-13 is responsible for the augmentation of the asthma phenotype.

When whole cells were collected from the DLN cells of OVA-challenged mice and then stimulated *ex vivo* with OVA, the concentration of IL-13 in the cultured supernatant was significantly higher in the supernatants from poly IC-treated mice than in the supernatants from saline-treated mice (Figure 3C). This finding implies that T cells may be a potent source of IL-13. To determine which T-cell subset is responsible for the overproduction of IL-13 in the lungs, IL-13-producing CD4⁺ and CD8⁺ T-cell subsets were evaluated by intracellular cytokine staining (Figure 3D). In terms of absolute numbers per animal, there were significantly larger numbers of IL-13-producing CD4⁺ and CD8⁺ T cells in poly IC-treated mice than in saline-treated mice (Figure 3E). However, the net effect of poly IC on the number differed between subsets. The number of IL-13-producing CD8⁺ T cells in poly IC-treated mice was 2.2-fold larger than in saline-treated

mice, whereas that of IL-13-producing CD4⁺ T cells in poly IC-treated mice was increased only 1.2-fold than in saline-treated mice. These results suggest that the excessive IL-13 may be produced from CD8⁺ T cells.

TRIF Mediates Poly IC-Induced Augmentation of Airway Eosinophilia

To determine which signaling pathway is involved in the augmentation, the effects of poly IC were compared among TRIF^{-/-} mice, IPS-1^{-/-} mice, and wild-type C57BL/6 mice. First, the concentrations of IFN- β and IL-6 in the sera were assessed 5 hours after a single intraperitoneal injection of poly IC to their naive mice (Figure 4A). The injection significantly increased the concentrations of IFN- β and IL-6 in wild-type mice and IPS-1^{-/-} mice but not in TRIF^{-/-} mice. Next, the effect of poly IC on the asthma phenotype was evaluated. Because it had been confirmed that AHR is unaffected by the treatment with poly IC in the mice on a C57BL/6 background (data not shown), the asthma phenotype was evaluated by eosinophilia in BALF in the subsequent experiments. In wild-type mice and IPS-1^{-/-} mice, the poly IC treatment significantly augmented the eosinophilia in BALF, but it did not in TRIF^{-/-} mice (Figure 4B). Thus, the TRIF-mediated

pathway is essential for the poly IC-induced augmentation of airway eosinophilia.

TRIF Mediates Poly IC-Induced Augmentation of B7-H1 Expression on BMDCs

To explore the direct effect of poly IC on the expression of B7-H1 on APCs, BMDCs were prepared from naive C57BL/6 mice (Figure 5A). There was a minimal expression of B7-H1 on Day 7 after the initiation of culture. When BMDCs were stimulated on Day 7 with poly IC for 24 hours, the expression of B7-H1 was strongly up-regulated, whereas that of unstimulated control mice was not. Similar results were obtained from BMDCs derived from BALB/c mice (data not shown). The stimulation with poly IC significantly up-regulated the expression of B7-H1 on BMDCs derived from IPS-1^{-/-} mice at a comparable level to that from wild-type mice. However, the stimulation with poly IC did not up-regulate the expression of B7-H1 on BMDCs derived from TRIF^{-/-} mice (Figure 5B). These results suggest that the TRIF-mediated pathway is essential for poly IC-induced augmentation of B7-H1 expression on BMDCs.

Poly IC in Sensitization Augments B7-H1 Expression on CD8 α^+ Dendritic Cells in DLN Cells

To examine whether the intraperitoneal administration of poly IC affects the expression status of B7-H1 in the lungs and DLN cells, whole cell samples were collected from the lungs and DLN cells on Day 15, 1 day after the second OVA sensitization with or without poly IC treatment. Flow cytometric analysis revealed that the expression of B7-H1 on CD8 α^+ dendritic cells (DCs) in DLN cells, but not the lungs, was significantly up-regulated by poly IC treatment, whereas B7-H1 expression on CD8 α^- DCs in the lungs and DLN cells was not affected by poly IC (Figures 5C and 5D).

B7-H1 Is Essential for Poly IC-Induced Augmentation of the Asthma Phenotype

To elucidate the *in vivo* roles of B7-H1, the effect of B7-H1 blockade was examined in the asthma model. Treatment of BALB/c mice with anti-B7-H1 mAb during OVA sensitization prevented the poly IC-induced augmentation of airway eosinophilia, AHR, and the concentration of IL-13 in BALF after OVA challenge, whereas treatment with control IgG did not (Figure 6A). Consistent results were obtained in the study using OVA-sensitized/challenged B7-H1^{-/-} mice on a BALB/c background because no augmentation of airway eosinophilia and AHR was induced by poly IC (Figure 6B). B7-H1 shares its receptor, PD-1, with B7-DC. The effect of anti-B7-H1 mAb was examined in OVA-sensitized/challenged B7-DC^{-/-} mice on a BALB/c background (Figure 6C). Poly IC treatment significantly augmented airway eosinophilia and AHR even in B7-DC^{-/-} mice. The augmentation was prevented by the treatment with anti-B7-H1 mAb. These results exclude the involvement of B7-DC in the process of augmentation. Treatment of mice with anti-PD-1 mAb during OVA sensitization did not prevent the poly IC-induced augmentation of airway eosinophilia or AHR (Figure 6D). Thus, the pathogenic role of B7-H1 may be independent of its interaction with PD-1.

DISCUSSION

The present study showed that dsRNA-triggered activation of an innate immune system in a sensitization phase leads to the augmentation of an asthma phenotype. This effect is initiated via the TRIF-mediated pathway and is operated through augmented production of IL-13. Although IL-13 is produced from

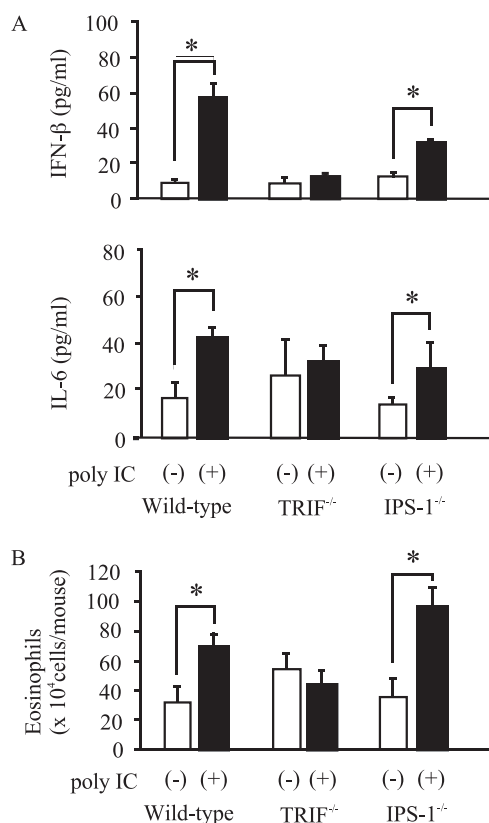


Figure 4. Role of Toll/IL-1R domain-containing adaptor inducing IFN- β (TRIF) and IFN- β promoter stimulator 1 (IPS-1) in poly IC-induced augmentation of airway eosinophilia. (A) Naive TRIF^{-/-} mice, IPS-1^{-/-} mice, and wild-type (WT) C57BL/6 mice were administered poly IC. The sera were collected 5 hours after poly IC, and the IFN- β and IL-6 contents were measured by ELISA. Each group consisted of five mice. (B) TRIF^{-/-} mice, IPS-1^{-/-} mice, and WT mice were treated with poly IC or saline alone before each OVA sensitization. After OVA challenge, the eosinophilia in BALF was measured. Each group consisted of seven to nine mice. Data are expressed as the means $\pm$ SEM. * P < 0.05 between the indicated two groups.

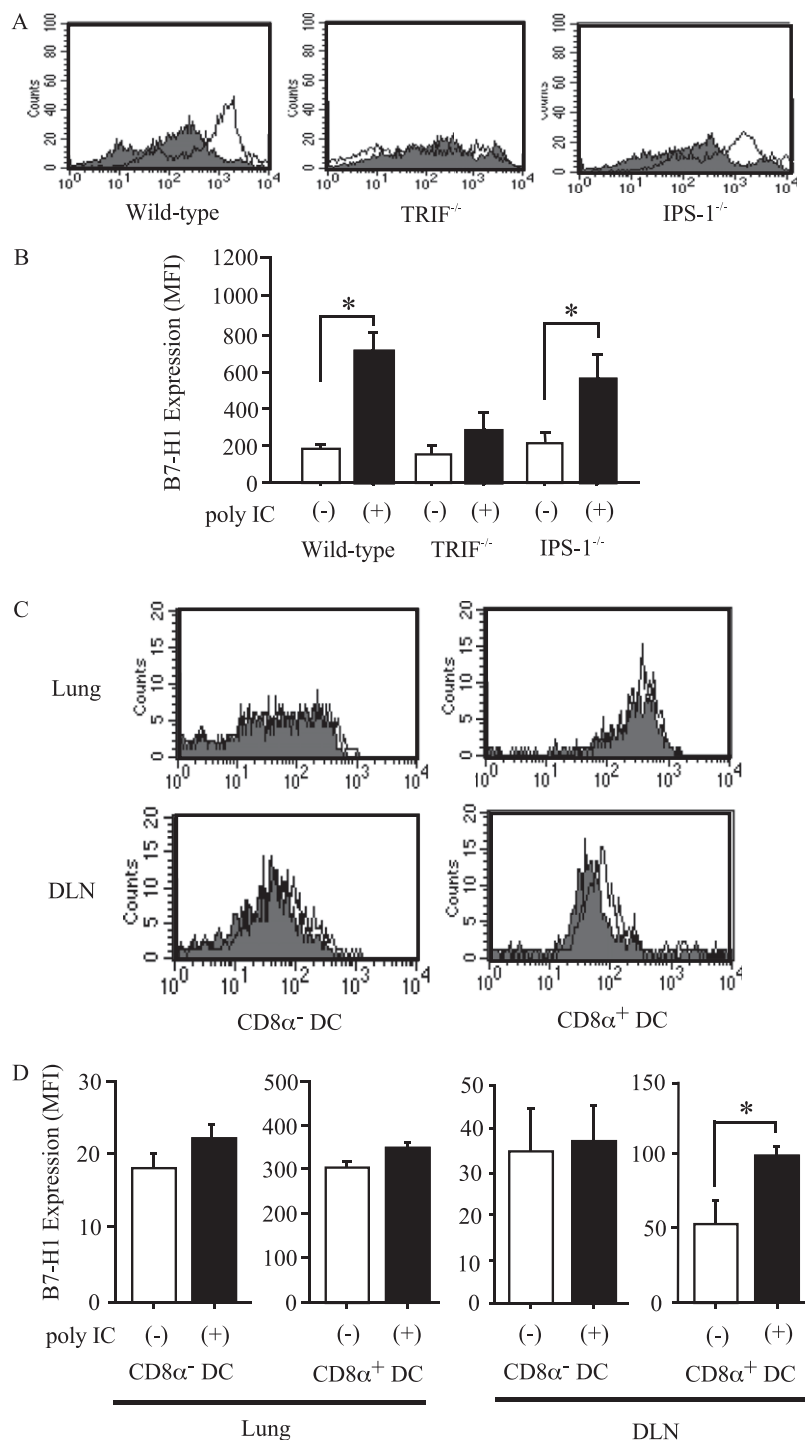


Figure 5. Effect of poly IC on B7-H1 expression on dendritic cells (DCs) *in vitro* and *in vivo*. Bone marrow-derived DCs from WT C57BL/6 mice, TRIF^{-/-} mice, and IPS-1^{-/-} mice were stimulated on Day 7 after the initiation of culture with poly IC (30 μ g/ml) for 24 hours. The expression of B7-H1 was evaluated by flow cytometry. (A) Typical histograms are shown. Shaded histograms indicate unstimulated controls; lined histograms indicate poly IC-stimulated samples. (B) Mean fluorescent intensities (MFIs) of B7-H1. Each group consisted of five to eight mice. BALB/c mice were treated with poly IC or saline alone before each OVA sensitization. One day after the second sensitization, the lungs and draining lymph node (DLN) cells were collected, and the expression of B7-H1 on CD8 α ⁻ and CD8 α ⁺ DC subsets was evaluated. (C) Typical histograms. Shaded histograms indicate saline-treated mice; lined histograms indicate poly IC-treated mice. (D) The MFIs of B7-H1 are shown. Each group consisted of three mice. Data are expressed as the means $\pm$ SEM. * $P < 0.05$ between the indicated two groups.

a variety of cells, including T cells, NK cells, mast cells, and eosinophils, the present study revealed that T cells, especially CD8⁺ T cells, may be among the main producers of excessive IL-13. A similar protocol of OVA sensitization showed that IL-13-producing CD8⁺ T cells are required for AHR and airway inflammation (35). Another study using a cockroach allergen model of asthma demonstrated that IL-4 and IL-13 from CD8⁺ T cells are required for AHR (36). In humans, the pathogenic relevance of IL-13-producing CD8⁺ T cells has been implicated in allergic asthma (37).

Given that the induction of IL-13-producing CD8⁺ T cells is enhanced by dsRNA stimulation, identifying the molecule responsible for the induction is important. The basic character of

antigen-specific T subsets is determined by the initial interactions between APCs and naive T cells. Costimulatory molecules are key molecules for these interactions (13). In the present study, the expression of B7-H1 on BMDCs was markedly up-regulated by dsRNA stimulation. This up-regulation was dependent on the TRIF-mediated pathway, which is consistent with the essential role of TRIF in dsRNA-induced augmentation of airway eosinophilia. Next, the expression of B7-H1 on CD8 α ⁺ DCs in the thoracic DLN cells was up-regulated by intraperitoneal poly IC *in vivo*. In addition, the blockade of B7-H1 during the sensitization phase prevented dsRNA-induced augmentation of airway eosinophilia, AHR, and IL-13 in BALF. These consistent results were obtained in B7-H1-deficient mice. These results provide

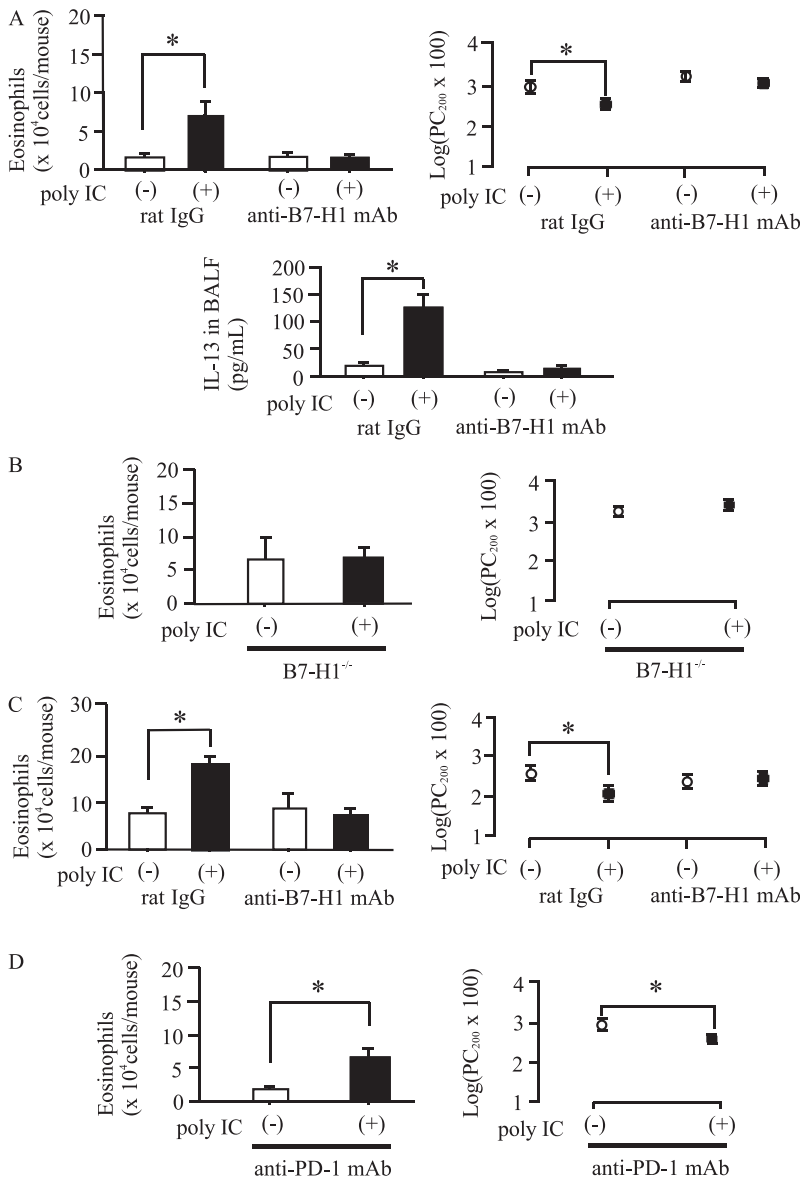


Figure 6. Effect of B7-H1 blockade and PD-1 blockade on the poly IC-induced augmentation of the asthma phenotype. (A) BALB/c mice were treated with anti-B7-H1 mAb during OVA sensitization. After OVA challenge, the eosinophilia in BALF, AHR, and the concentration of IL-13 in BALF were measured. Each group consisted of six mice. (B) B7-H1^{-/-} mice on a BALB/c background were treated with poly IC or saline alone before each OVA sensitization. After OVA challenge, eosinophil counts in BALF and the AHR were performed. Each group consisted of five mice. (C) B7-DC^{-/-} mice on a BALB/c background were treated with anti-B7-H1 mAb during OVA sensitization. After OVA challenge, the eosinophilia in BALF and the AHR were measured. Each group consisted of five to eight mice. (D) BALB/c mice were treated with anti-PD-1 mAb during OVA sensitization. After OVA challenge, the eosinophilia in BALF and the AHR were measured. Each group consisted of eight mice. Data are expressed as the mean $\pm$ SEM. * $P < 0.05$ between the indicated two groups.

strong evidence that B7-H1 may be an important molecule for the dsRNA-induced augmentation of an asthma phenotype. Previous studies suggest that B7-H1 is associated with chronic viral diseases because virus-induced up-regulation of B7-H1 inhibits the activation of virus-specific cytotoxic CD8⁺ T lymphocytes, leading to the persistence of infection (38, 39). In such cases, B7-H1 may work as a coinhibitory molecule rather than as a costimulatory molecule with regard to its mode of action. In contrast, the present study revealed a costimulatory property of B7-H1 that has a pathogenic role in the development of a virus-associated disorder.

The ligation of PD-1 by B7-H1 or B7-DC leads to the inhibition of T-cell activation (15, 17, 18), which fails to explain the stimulatory effect of B7-H1 on the induction of antigen-specific IL-13-producing T cells. Treatment of anti-PD-1 mAb did not prevent dsRNA-induced augmentation of an asthma phenotype. We previously found that the treatment of OVA-sensitized BALB/c mice with anti-B7-DC mAb during the effector phase, but not the sensitization phase, augmented airway eosinophilia and AHR after OVA challenge (40). In that model, the treatment of anti-PD-1 mAb did not affect the outcome of the asthma phenotype. Several investigators have proposed that, in addition

to PD-1, there may be a second receptor for B7-H1/B7-DC that is capable of delivering a stimulatory signal to T cells (14, 41, 42). Our findings are consistent with this proposal. Alternatively, the stimulatory effect of B7-H1 might be due to its unique property to bind B7-1 (CD80) (43). B7-H1 is expressed not only on APCs but also on T cells and nonimmune cells. B7-H1 on subsets of T cells might bind B7-1 on bystander cells during APC/T-cell interaction and then promote the induction of antigen-specific IL-13-producing T cells. This assumption is unlikely because B7-H1/B7-1 interaction delivers an inhibitory signal to activated T cells. Finally, a recent report has shown that B7-H1 on naive T cells is required to condition immature DCs to undergo efficient maturation when they encounter microbial infection (44). In turn, the mature DCs stimulate a robust T-cell response against the infecting pathogen. It remains uncertain whether these mechanisms contributed to the development of dsRNA-induced augmentation of an asthma phenotype in the present study.

Although the present study did not directly clarify how antigen-specific IL-13-producing CD8⁺ T cells can be induced by dsRNA stimulation and B7-H1 expression, previous investigations indicated that the induction largely depends on cross-priming (45). Cross-priming is a process by which CD8⁺ T-cell

responses, typically CD8⁺ T lymphocyte responses, can be generated against soluble antigens that are not expressed directly within APCs. This process involves the degradation of antigens to peptides by the proteasome systems and the processing of antigenic peptides to the endoplasmic reticulum, leading to the production of antigenic-peptide-MHC class I complexes. Cross-priming typically occurs when DCs capture soluble antigens in the presence of danger signals, such as dsRNA (46). Previous studies have shown that murine CD8 α ⁺ DCs exclusively express TLR3 and that the binding of dsRNA to TLR3 markedly promotes cross-priming (47). Type I IFNs are known to enhance cross-priming through the up-regulation of costimulatory molecules and the production of proinflammatory cytokines (48). These studies support our findings that B7-H1 was selectively up-regulated in CD8 α ⁺ DCs in DLN cells by poly IC. Although B7-H1 on CD8 α ⁺ DCs from spleen and DLN cells were up-regulated by intraperitoneal poly IC in tumor immunotherapy (49), depletion or blockade of B7-H1 increased the magnitude of effector CD8⁺ T-cell expansion, which is in contrast to our results. The role of B7-H1 in allergy may be different from that in antitumor immunity. Accumulating evidence is consistent with the essential role of the TRIF-mediated pathway in dsRNA-induced augmentation of an asthma phenotype.

In summary, dsRNA-triggered activation of the innate immune system in the sensitization phase leads to IL-13-mediated augmentation of the asthma phenotype. B7-H1 plays a crucial role in the augmentation without requiring direct interaction with PD-1. Further elucidation of the process will help us to understand the interactions between viral infection and the pathogenesis of asthma, which may provide a novel strategy for preventing the development of this disease.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank Lieping Chen (The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD) and Tahir Shin (San Antonio Cancer Institute, University of Texas Health Science Center at San Antonio, TX) for providing B7-H1^{-/-} mice and B7-DC^{-/-} mice, respectively, and Yuki Yoshiura, Tomoko Yoshimura, and Ayako Hashizume for technical assistance.

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