JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Allergy and Immunology

Research Article

Requirement for JNK1 in OVA-induced Airway Hyperresponsiveness in vivo

Junya Onuki[1], Eiko Takada[1], Masae Furuhata[1], Katsuko Sudo[2], Kuniaki Shimo[1] and Junichiro Mizuguchi[*1]

[1]*Department of Immunology and Intractable Immunology Research Center, TokyoMedical University, 1-1-6 Shinjuku, Shinjuku-ku, Tokyo, 8402-160, Japan*

[2]*Animal Research Center, Tokyo Medical University, 1-1-6 Shinjuku, Shinjuku-ku, Tokyo, 8402-160, Japan*

**Corresponding author: Dr. Junichiro Mizuguchi, Department of Immunology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402 Japan, Tel: +81-3-090-2319-7415; Fax: +81-3-3341-2941; E-mail: mizujm@icloud.com*

Received: 10-28-2015

Accepted: 11-26-2015

Published: 12-22-2015

Abstract

Background

Bronchial asthma is a chronic inflammatory disease characterized by airway hyperreactivity (AHR) and inflammatory cell infiltration of the airway. c-Jun N-terminal kinase 1 (JNK1), a member of the mitogen-activated protein kinases, is activated by a variety of stimuli including environmental stress and cytokine(s) and plays a crucial role in the induction of inflammation. To assess the role of JNK1 in the induction of bronchial asthma, we examined the production of AHR, inflammatory reaction, and cytokine production using an ovalbumin (OVA)-induced airway inflammation model.

Methods

JNK1-deficient ($JNK1^{-/-}$) and control wild-type (WT) mice were primed with intraperitoneal injection of OVA without alum, followed by intranasal administration of OVA. AHR, inflammatory cell accumulation in the airways, and cytokine production were assessed. AHR was also assessed using the irradiated $JNK1^{-/-}$ and WT mice receiving unprimed WT T plus B cells or cytokine IL-13. Finally, IL-4Rα expression on smooth muscle was determined.

Results

$JNK1^{-/-}$ mice demonstrated impaired AHR relative to WT mice, while comparable levels of accumulation of inflammatory cells in the airways were seen in $JNK1^{-/-}$ and WT mice. Although the levels of Th2 cytokine including IL-4 and IL-5 in the OVA-stimulated $JNK1^{-/-}$ mice were increased relative to the control, $JNK1^{-/-}$ mice demonstrated a reduced sensitivity to induction of AHR by IL-13, probably due to an impaired increase in IL-4Rα expression on smooth muscle following OVA stimulation.

Conclusions

JNK1 regulates an OVA-immunized asthmatic phenotype, especially through upregulation of IL-4Rα on smooth muscle by IL-13 in vivo. Thus, JNK1 would be a promising target for therapeutic intervention in some forms of asthma.

Keywords: Asthma; airway hyperreactivity; c-Jun N-terminal kinase 1; Th2 responses; smooth muscle

Abbreviations

AHR: Airway Hyperresponsiveness;

JNK1: c-Jun N-terminal kinase;

OVA: Ovalbumin;

WT: Wild-type;

Th2: T-helper 2;

IL-4Rs: IL-4 Receptors;

$JNK1^{-/-}$: JNK1-deficient;

mAb: Monoclonal Antibody;

PBS: Phosphate-Buffered Saline;

BALF: Bronchoalveolar Lacage Fluid,

SM: Smooth Muscle

Introduction

One of the basic features of bronchial asthma is chronic airway hyperreactivity (AHR) [1,2], which is initiated by exposure to environmental allergens. The increase in AHR is thought to result from airway inflammation involving several cell types, including T-helper 2 (Th2) cells, eosinophils, and neutrophils [3,4]. Upon activation, T cells exert their effects by producing a variety of cytokines. Th2 cells produce IL-4, IL-5, and IL-13. IL-13 is required for the effector phase of Th2 responses, including eosinophils and AHR [5], whereas IL-4 is involved in the differentiation and maintenance of Th2 cells [6,7]. Both IL-13 and IL-4 signal via IL-4 receptors (IL-4Rs), which consist of types I-R (IL-4Rα and γ_c) and II-R (IL-4Rα and IL-13Rα1) [8]. IL-13 signals via only type II IL-4R, while IL-4 signals via both IL-4Rs. IL-13 may mediate allergen-induced AHR through effects on airway smooth muscle [8-10], which predominantly express type II IL-4R. Th2 responses are regulated by multiple components including transcription factor GATA3 and mitogen-activated protein kinases (MAPKs) [11-13].

There are several classes of MAPKs, including ERK1/ERK2 and JNK1/JNK2/JNK3. JNK1 and JNK2 are expressed in immune cells [14]. MAPKs are activated through a cascade of upstream components in an orderly manner: MAP3K, MAP2K, and MAPK [15]. $CD4^+$ T cells from JNK1-deficient ($JNK1^{-/-}$) mice preferentially differentiate into the Th2 phenotype in response to an anti-CD3 monoclonal antibody (mAb) [16]. However, we recently demonstrated that bronchial asthma induced on exposure of ovalbumin (OVA) without alum was prevented by targeted disruption of the ASK1 gene, a component of MAP3K [17]. Consistent with these findings, treatment with a general inhibitor of JNK SP600125 prevents airway inflammation and AHR on allergen exposure [18].

To explore the role of JNK1 in the induction of bronchial asthma in vivo, we examined whether bronchial asthma is modulated in $JNK1^{-/-}$ mice in response to OVA stimulation. JNK1 is necessary for induction of OVA-induced AHR, at least through upregulation of IL-4Rα expression on bronchial smooth muscle, whereas it is dispensable for allergen-induced airway inflammation. The possible role of JNK1 in the regulation of bronchial asthma will be discussed.

Materials and Methods

Mice

Female $JNK1^{-/-}$ (C57BL/6 background), $Rag2^{-/-}$, and control wild-type (WT) mice at the age of 8–12 weeks were bred and maintained at the animal facility of Tokyo Medical University. Experiments were approved by the Ethical Committee of Animal Experiments of Tokyo Medical University.

OVA-induced airway inflammation

$JNK1^{-/-}$ and WT mice were sensitized by intraperitoneal administration of 10 μg of OVA (grade V; Sigma-Aldrich, St. Louis, MO, USA) suspended in 0.2 mL of phosphate-buffered saline (PBS) on days 0, 3, 5, 7, 9, 11, and 13. Mice were then subjected to an intranasal challenge of 200 μg of OVA in PBS or PBS alone on days 31, 34 and 37. At 24 h after the last OVA administration, AHR, histological sections, cytokine production, and IL-4Rα expression on smooth muscle were examined.

Measurement of AHR

AHR was assessed using an invasive approach as previously described [17]. Briefly, the mean response to each dose of methacholine was determined, and lung resistance (R_L) was calculated by the percent change from the baseline after PBS aerosol treatment.

Histological analysis of lung sections

Histological analysis of lung sections were carried out as previously described [17].

Collection and differentials of bronchoalveolar lavage fluid-derived cells

The airways of the mice were lavaged three times with 1 mL of 2% fetal bovine serum in PBS via a tracheal cannula. Bronchoalveolar lavaged fluid (BALF) was obtained, and differential

cell counts were done using a Sysmex XT-2000iv automated cell counter (Sysmex Corporation, Kobe, Japan), as previously described [17].

Measurement of cytokine levels

The levels of IL-4, IL-5, IL-8, IL-10, IL-12, and IFN-γ in BALF were determined using the Meso Scale Discovery Platform (Gaithersburg, MD, USA), as previously described [17]. IL-13 levels were determined by using an ELISA Ready-SET-Go Kit (eBioscience, San Diego, CA, USA).

Adoptive transfer of T plus B cells into Rag2$^{-/-}$or irradiated JNK1$^{-/-}$ mice

Spleen T and B cells were isolated on an autoMACS ProSeparator (Miltenyibiotech, Gladbach, Germany) together with T cell and B cell isolation kits (Miltenyibiotech), respectively, as previously described [17]. JNK1$^{-/-}$ or WT T cells (1 X 10^7/mouse), together with JNK1$^{-/-}$ or WT B cells (1 X 10^7/mouse), were transferred into Rag2$^{-/-}$ mice. In some experiments, recipient JNK1$^{-/-}$ and WT mice were sublethally irradiated with 800 Rad (Gammacell 40 Exactor, Nordin International Inc., Kanata, Canada), and then intravenously injected with WT T cells (1 X 10^7/mouse) together with B cells (1 X 10^7/mouse). Thirty-six hours after cell transfer, mice were immunized with OVA, followed by assays for AHR and accumulation of inflammatory cells in the airways.

Intranasal administration of IL-13 or IL-4

Mice were anesthetized with 2% isoflurane and then challenged by intranasal administration of IL-13 (5 μg/20 μl PBS with 0.1% BSA/mouse) or IL-4 (Cell Guidance Systems, Cambridge, UK) (5 μg/20 μl in PBS) daily for three consecutive days. BALF and AHR were examined 24 h after administration of IL-13 and IL-4.

Isolation of smooth muscle cells and flow cytometric analysis of IL-4Rα expression

Tracheal smooth muscle cells were isolated by a slight modification of a previously described procedure [19]. Briefly, JNK1$^{-/-}$ and WT mice were euthanized by exposure to gradually increasing concentrations of carbon dioxide. The trachea was removed, minced with scissors, and resuspended in prechilled dissociation solution (135 mM NaCl, 6 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 10 mM HEPES, 10 mM glucose; pH 7.3). The tissues were incubated in dissociation medium containing 10 unit/mL papain (Sigma, St. Louis, MO, USA), 0.5 mM DTT, and 0.15 mg/mL BSA, at 35° C for 3 min, and then transferred to a dissociation medium containing 1 unit/mL collagenase F (Sigma) and 0.15 mg/ml BSA, followed by incubation at 35° C for 5 min. The suspensions were filtered through nylon mesh, followed by centrifugation. Cell yield was determined prior to analysis of flow cytometry.

Cells were stained with anti-IL-4Rα-PE (BioLegend, Pacific Heights, San Diego, CA, USA) and then fixed and permeabilized using a BD Cytofix/Cytoperm Fixation/Permeabilization Solution Kit (BD Bioscience, San Diego, CA, USA), according to the manufacturer's instruction. The cells were then incubated with anti-smooth muscle (SM)-α-actin mAb (Sigma), followed by addition of anti-mouse IgG-FITC (Sigma). Isotype-matched controls were used to define nonspecific background labeling. Forward angle light scatter (FALS) vs. 90° C side scatter (SS) histograms were used to identify intact cells. The cells were analyzed on an Attune Acoustic Focusing Cytometer (Applied Biosystems, Foster City, CA, USA).

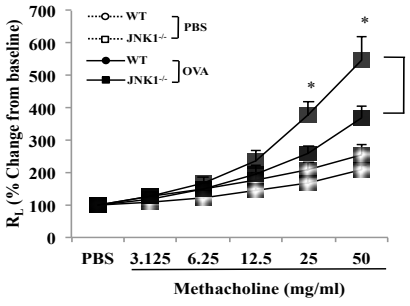
Statistical analysis

Data are expressed as means ± SE for each group. Statistical significance was determined by Student's *t* test.

Results

OVA-induced AHR is diminished in JNK1$^{-/-}$ mice, while inflammatory lesions in airways were comparable to those of control WT mice.

JNK1$^{-/-}$ and control WT mice were intraperitoneally sensitized with OVA in PBS or control vehicle alone and then challenged with OVA or control PBS alone. One day after the final challenged, the mice were assessed for AHR and airway inflammation. A dose-dependent increase in R_L in WT mice was observed in response to the methacholine challenge relative to control PBS alone (Figure 1A). However, the increase in R_L in response to methacholine was diminished in JNK1$^{-/-}$ mice relative to controls.



A

Figure 1. OVA-induced AHR is compromised in JNK1$^{-/-}$ mice, whereas accumulation of airway inflammatory cells and pathological findings are comparable to those of control WT mice. Mice were sensitized by intraperitoneal injection of 10 μg of OVA in PBS, followed by intranasal administration of OVA or control PBS. The day after the final OVA instillation, the mice were assayed for AHR in response to various concentrations of methacholine (A), inflammatory cell accumulation in the airways (B), and pathology in lung sections stained with HE

and PAS (C). (B) Total cells and eosinophils in BALF from JNK1$^{-/-}$ and control WT mice are shown. Data are shown as means ± SE (n=7-9 for OVA, n=4-5 for PBS). Experiments were done twice, with essentially similar results.

Because antigen-induced AHR appears to be closely associated with inflammatory responses [3,6], mice were assayed for BALF cellularity and lung pathology. Surprisingly, the numbers of total cells and eosinophils accumulated in the airways of JNK1$^{-/-}$ mice were comparable to those of WT control mice (Figure 1B). Pathological findings demonstrated that inflammatory lesions in the vascular and perivascular regions in JNK1$^{-/-}$ were almost equal to those of controls following stimulation with OVA (Figure 1C). Comparable levels of goblet cell hyperplasia, as assessed by PAS staining, were also detected in JNK1$^{-/-}$ and control WT mice. These results indicate that the extent of OVA-induced inflammatory responses in JNK1$^{-/-}$ mice was almost equal to those of WT mice, with attenuated levels of AHR.

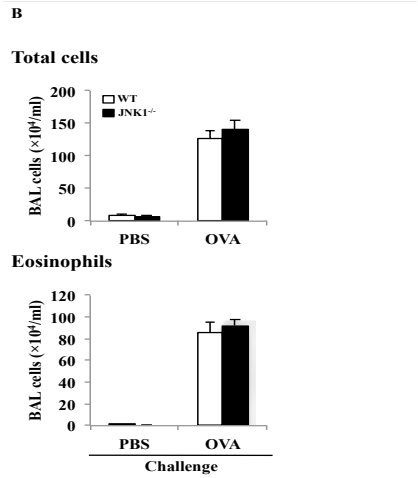


Figure 1B

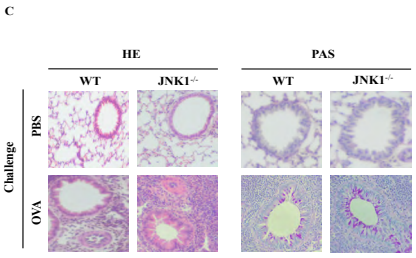


Figure 1C

OVA-Induced Th2 cytokine production enhanced in BALF from JNK1$^{-/-}$ mice

Because airway inflammatory responses are regulated by Th2 cytokines [6], cytokine levels in BALF from OVA-stimulated mice were determined. JNK1$^{-/-}$ mice generated enhanced levels of IL-4 and IL-5, with comparable levels of IL-13 relative to control (Figure 2). Levels of other cytokines including IFN-γ, IL-8, IL-10, IL-12 (Figure 2), IL-2, TGF-β, TNF-α, and IL-1β (Onuki et al. unpublished observation) were almost equal in JNK1$^{-/-}$ and control mice. These results indicate that JNK1 negatively regulates Th2 cytokine production, as previously described [16].

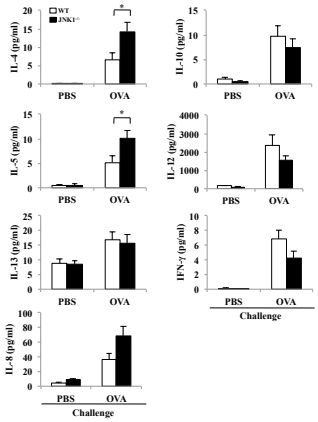


Figure 2. Cytokine profiles of BALF from JNK1$^{-/-}$ and control mice on OVA exposure. BALFs from OVA-stimulated JNK1$^{-/-}$ and control WT mice were assayed for cytokines by ELISA. Data are shown as means ± SE. Experiments were done twice, with essentially similar results.

AHR in Rag2$^{-/-}$ mice receiving JNK1$^{-/-}$ T plus B cells, WT T plus B cells, and their combinations

To determine whether diminished AHR in JNK1$^{-/-}$ mice is due to impaired function of JNK1$^{-/-}$ T and B cells, we examined AHR in Rag2$^{-/-}$ mice injected with T and B cells from either JNK1$^{-/-}$ or WT mice and their combinations. The Rag2$^{-/-}$ mice receiving JNK1$^{-/-}$ T and B cells demonstrated equal levels of AHR relative to control WT T and B cells and their combinations (Figure 3). Airway inflammatory responses were also comparable in JNK1$^{-/-}$ and control mice (S1), suggesting that JNK1$^{-/-}$ immune cells have the capacity to induce AHR upon OVA exposure relative to that of control cells.

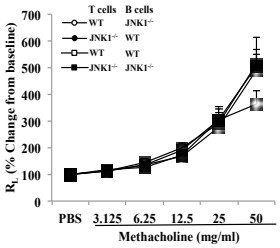
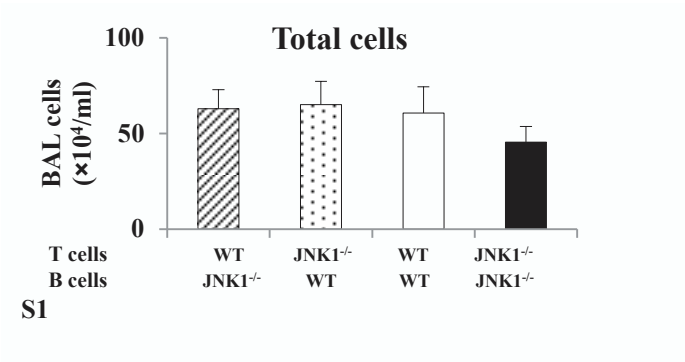


Figure 3. OVA-induced AHR in Rag2$^{-/-}$ mice receiving JNK1$^{-/-}$ or WT T cells in combination with JNK1$^{-/-}$ or WT B cells. Rag2$^{-/-}$ mice were injected with JNK1$^{-/-}$ or WT T cells together with JNK1$^{-/-}$ or WT B cells.

Thirty-six hours after the transfer, mice were sensitized with OVA, as described in Figure 1, followed by assay for AHR. Data are shown as means ± SE (n=8-10).



S1. Total inflammatory cells in the BAL fluids from Rag2$^{-/-}$ mice injected with JNK1$^{-/-}$ or WT T cells together with JNK1$^{-/-}$ or WT B cells, as described in Fig.3.

An Impaired AHR in irradiated JNK1$^{-/-}$ mice receiving unprimed WT T and B cells

To examine whether background components from JNK1$^{-/-}$ mice are responsible for reduced AHR in response to OVA, irradiated JNK1$^{-/-}$ and WT mice were injected with WT T and B cells. Thirty-six hours after the transfer, the mice were inoculated with OVA, followed by assay for AHR. The irradiated JNK1$^{-/-}$ mice receiving the T and B cells demonstrated reduced AHR relative to the control WT mice (Figure 4A), whereas comparable levels of inflammatory cell accumulation in the airways were found in JNK1$^{-/-}$ and control mice (Figure 4B). These results indicate that background components including smooth muscle are involved in the impaired AHR in JNK1$^{-/-}$ mice.

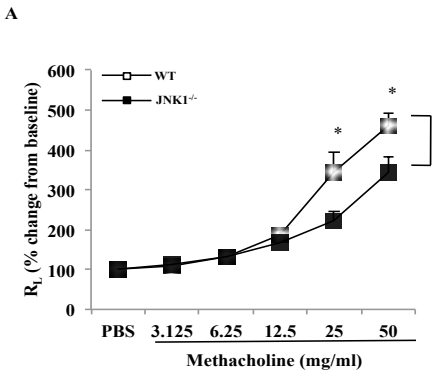
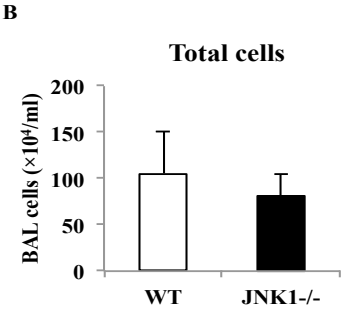


Figure 4. OVA-induced AHR in irradiated JNK1$^{-/-}$ and WT mice receiving both WT T plus B cells. Irradiated JNK1$^{-/-}$ and WT mice were injected with both WT T plus B cells. Thirty-six hours after the transfer, mice were sensitized with OVA, as described in Figure 1, followed by assays for AHR (A) and accumulation of inflammatory cells in BALF



(B). Data are shown as means ± SE (n=5-6). Experiments were performed twice, with essentially similar results.

Impaired AHR response in JNK1$^{-/-}$ mice following IL-13 administration

To examine whether JNK1$^{-/-}$ mice are resistant to induction of AHR in vivo by Th2 cytokines including IL-13 and IL-4, IL-13 or IL-4 was administered to unprimed JNK1$^{-/-}$ and WT mice. Intranasal administration of IL-13 to JNK1$^{-/-}$ mice produced a diminished AHR compared with that of the WT control (Figure 5A), whereas the levels of inflammatory cell accumulation were comparable in JNK1$^{-/-}$ and control mice (Figure 5B). Likewise, IL-4 showed a similar tendency, although statistically not significant (S2A & S2B). These results suggest that JNK1$^{-/-}$ mice are resistant to induction of AHR by the Th2 cytokine IL-13.

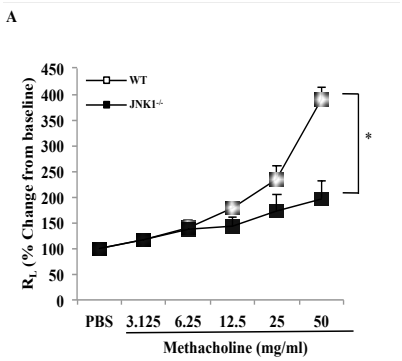


Figure 5. AHR induced by intranasal administration of IL-13 into JNK1$^{-/-}$ and control WT mice. JNK1$^{-/-}$ and control WT mice were administered IL-13 intranasally, followed by assays for AHR (A) and accumulation of inflammatory cells in BALFs (B). Data are shown as means ± SE (n=5).

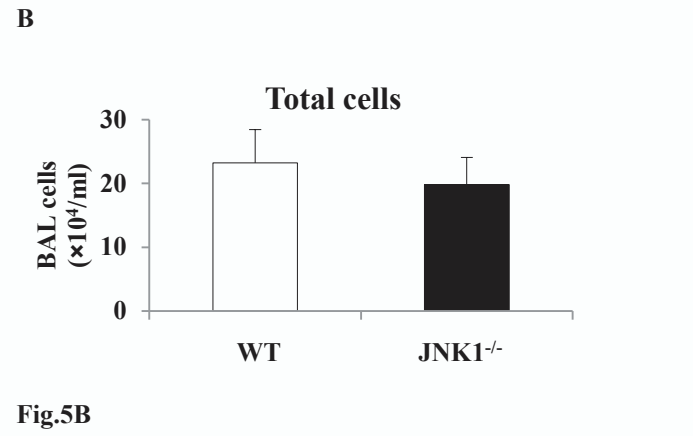
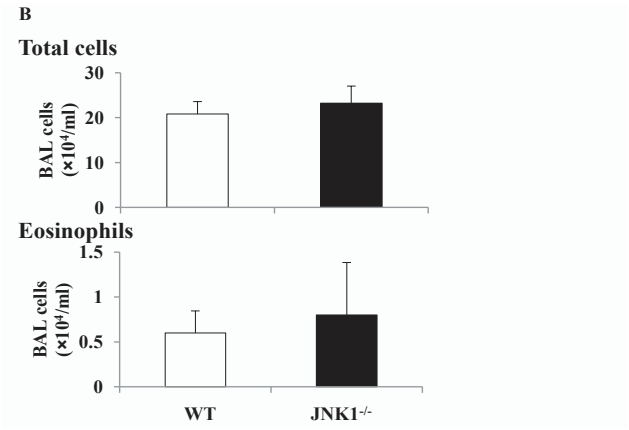


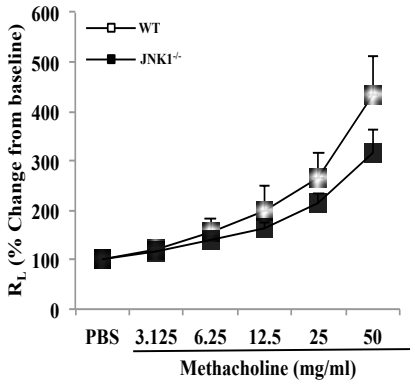
Fig.5B



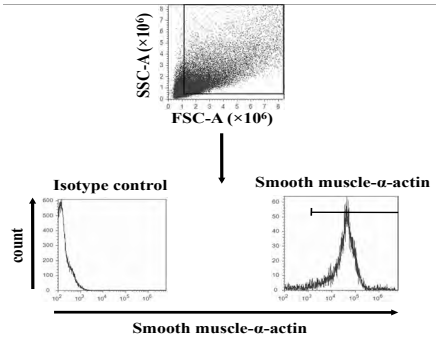
S2B

IL-4Rα expression on bronchial smooth muscle from JNK1$^{-/-}$ mice following OVA exposure

Because the interaction of IL-13 or IL-4 with IL-4Rα on smooth muscle induces AHR [20], we examined the expression levels of IL-4Rα on smooth muscle in the OVA- and control vehicle-treated JNK1$^{-/-}$ and WT mice. First, bronchial cells displayed a broad heterogeneity in shape and size (S3). Flow cytometric analysis of immunofluorescence for SM-α-actin, a marker for smooth muscle [21], revealed that around 90% of cells stained positively (S3). IL-4Rα expression on smooth muscle was increased in WT mice in response to OVA, whereas the OVA-induced increase in IL-4Rα expression in JNK1$^{-/-}$ mice was small relative to that of controls (Figures 6A and 6B). Thus, the OVA-stimulated increase in IL-4Rα expression was compromised in JNK1$^{-/-}$ mice, probably resulting in an inability of IL-13 to induce AHR.



S2. AHR and total inflammatory cells in BALFs from JNK1$^{-/-}$ and control WT mice after intranasal administration of IL-4. JNK1$^{-/-}$ and control WT mice were administered IL-4 intranasally, followed by assays for AHR (A) and accumulation of inflammatory cells in the airways (B). Data are shown as means ± SE (n=5-6). Experiments were performed twice, with essentially similar results.



S3

S3. Association of SM-α-actin expression with forward and side scatter distribution.

Cells were stained with anti-SM-α-actin or isotype-matched Abs. Cells were gated on forward and side scatter measurement (A), followed by analysis on a flow cytometer (B).

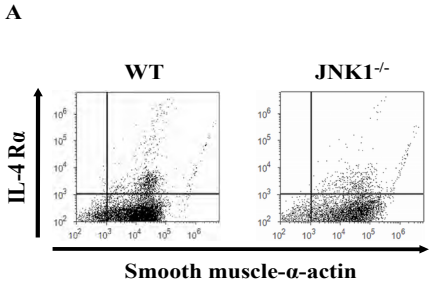


Figure 6. Expression of IL-4Rα on bronchial smooth muscle from JNK1$^{-/-}$ and WT mice following stimulation with OVA. JNK1$^{-/-}$ and WT mice sensitized with OVA, as described in Figure 1, were assayed for IL-4Rα expression on bronchial smooth muscle. Bronchial cells were stained with anti-IL-4Rα and anti-SM-α-actin, followed by flow cytometry assays. (A) Cells gated on FALS vs. SS were evaluated with SM-α-actin vs. IL-4Rα. (B) Percentage of IL-4Rα-positive cells within

the SM-α-actin-positive cells. Data are shown as means ± SE (n=5-7). Experiments were performed three times, with essentially similar results.

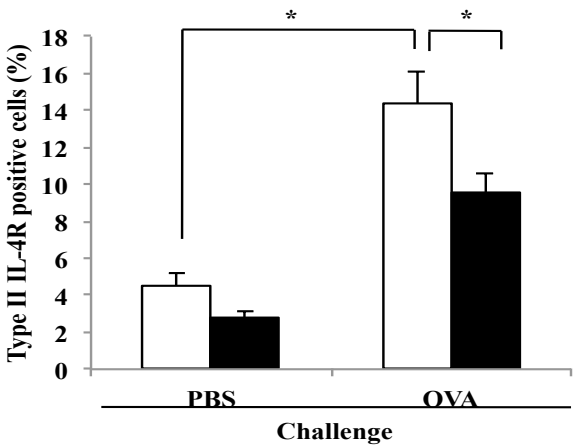


Fig 6B

Discussion

Bronchial asthma is characterized by the development of AHR and airway inflammation. The development of asthma has been shown to involve JNK activation, as revealed by a JNK inhibitor study [18] and ASK1-deficient mice [17]. Here, the role of JNK1 in the development of bronchial asthma using genetically deficient mice was examined, because immune cells harbor JNK1 and JNK2 [14]. A major observation of the present study is that exposure to OVA in the absence of alum induced a diminished AHR with comparable levels of inflammatory reaction in JNK1$^{-/-}$ mice, indicating that airway inflammation does not always result in an increase in AHR on allergen exposure.

Airway inflammation and mucus metaplasia of JNK1$^{-/-}$ and control mice were almost comparable following stimulation by OVA without alum, whereas levels of Th2 cytokines such as IL-4 and IL-5 in BALF were slightly higher in JNK1$^{-/-}$ mice. Consistent with our findings, Alcorn et al. reported that exposure to OVA/alum induced unaltered mucus metaplasia with increased production of IL-5, although they showed enhanced recruitment of inflammatory cells into the airway in JNK1$^{-/-}$ mice [22]. This subtle difference between their and our findings could be due to the adjuvant alum, because alum modifies the phenotype of bronchial asthma in response to an allergen in murine models [23]. Although the underlying mechanism for an apparent subtle difference in inflammatory reactions remains unclear, it is possible that the nature and/or levels of cytokines in BALF affects the recruitment of inflammatory cells into the airways.

Inflammatory cells or the Th2 cytokines IL-13 and IL-4 derived from them cause development of AHR [3,6]. JNK1$^{-/-}$ T and B cells induced AHR comparable to control cells when transferred into Rag2$^{-/-}$ mice, suggesting that JNK1 regulates AHR development downstream of the inflammatory cascade on OVA exposure. Moreover, JNK1$^{-/-}$ mice failed to develop AHR following intranasal administration of IL-13 compared with WT mice. However, the IL-13-mediated airway inflammation was unchanged in JNK1$^{-/-}$ and WT mice. Thus, downstream components including smooth muscle might be involved in the failure of AHR development in JNK1$^{-/-}$ mice.

Smooth muscle expressing type II IL-4R has been demonstrated to play a crucial role in the development of AHR [20]. OVA-induced upregulation of IL-4Rα was not found in JNK1$^{-/-}$ mice, compared with WT controls, suggesting that JNK1 in smooth muscle is necessary for development of AHR, at least through upregulation of IL-4Rα. Indeed, JNK1 plays a crucial role in the allergen-induced proliferation of rat smooth muscle [24]. Whether the induction of IL-4Rα on smooth muscle on OVA stimulation causes muscle contraction on IL-13 exposure remains to be determined.

Collectively, JNK1 is required for development of AHR downstream of the inflammatory cascade, but dispensable for the inflammatory reaction following allergen exposure in murine models. In contrast, JNK2 played an important role in the development of inflammatory reactions including Th2 cytokine production, resulting in AHR development (Takada et al. ms. in preparation). ASK1, an upstream kinase of the JNKs regulates both inflammatory reaction and AHR development [17]. Thus, JNKs have a specific role as well as an overall function in the development of allergic responses. These findings would be valuable for the design of JNK inhibitors for the treatment of bronchial asthma.

Acknowledgements

This work was supported by a grant from the Intractable Immune System Disease Center of Tokyo Medical University, which is supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Disclosure

We declare that we have no competing interests.

References

1. Cockcroft DW, Davis BE. Mechanisms of airway hyperresponsiveness. J Allergy Clin Immunol. 2006,118:551-559.

2. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol. 2008, 8(3):183-192.

3. Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. Nat Rev Immunol. 2010;10(12): 838-848.

4. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. Nat Rev

Immunol. 2008, 8(3):193-204.

5. Kasaian MT, Miller DK. IL-13 as a therapeutic target for respiratory disease. Biochem Pharmacol. 2008, 76(2):147-155.

6. Finkelman FD, Hogan SP, Hershey GK, Rothenberg ME, Wills-Karp M. Importance of cytokines in murine allergic airway disease and human asthma. J Immunol.2010,184(4):1663-1674.

7. Snapper CM, Finkelman FD, Paul WE. Regulation of IgG1 and IgE production by interleukin 4. Immunol Rev. 1988, 102:51-75.

8. Wills-Karp M, Finkelman FD. Untangling the complex web of IL-4- and IL-13-mediated signaling pathways. Sci Signal.2008, 1(51): pe55.

9. Eum SY, Maghni K, Tolloczko B, Eidelman DH, Martin JG. IL-13 may mediate allergen-induced hyperresponsiveness independently of IL-5 or eotaxin by effects on airway smooth muscle. Am J Physiol Lung Cell Mol Physiol. 2005, 288: L576-584.

10. Gao YD, Zou JJ, Zheng JW, Shang M, Chen X et al. Promoting effects of IL-13 on Ca2+ release and store-operated Ca2+ entry in airway smooth muscle cells. Pulm Pharmacol Ther. 2010, 23(3): 182-189.

11. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol. 2010, 10(4): 225-235.

12. Rincon M, Davis RJ. Regulation of the immune response by stress-activated protein kinases. Immunol Rev. 2009, 228(1): 212-224.

13. Zhang YL, Dong C. MAP kinases in immune responses. Cell Mol Immunol. 2005, 2(1): 20-27.

14. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. Annu Rev Immunol. 2002, 20: 55-72.

15. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001, 410(6824): 37-40.

16. Dong C, Yang DD, Wysk M, Whitmarsh AJ, Davis RJ et al. Defective T cell differentiation in the absence of Jnk1. Science. 1998, 282(5396): 2092-2095.

17. Takada E, Furuhata M, Nakae S, Ichijo H, Sudo K et al. Requirement of apoptosis-inducing kinase 1 for the induction of bronchial asthma following stimulation with ovalbumin. Int Arch Allergy Immunol. 2013, 162(2):104-114.

18. Nath P, Eynott P, Leung SY, Adcock IM, Bennett BL, Chung KF. Potential role of c-Jun NH2-terminal kinase in allergic airway inflammation and remodelling: effects of SP600125. Eur J Pharmacol. 2005, 506(3): 273-283.

19. Zhang CH, Li Y, Zhao W, Lifshitz LM, Li H et al. The transmembrane protein 16A Ca(2+)-activated Cl- channel in airway smooth muscle contributes to airway hyperresponsiveness. Am J Respir Crit Care Med. 2013, 187(4):374-381.

20. Perkins C, Yanase N, Smulian G, Gildea L, Orekov T et al. Selective stimulation of IL-4 receptor on smooth muscle induces airway hyperresponsiveness in mice. J Exp Med. 2011, 208(4): 853-867.

21. Halayko AJ, Solway J. Molecular mechanisms of phenotypic plasticity in smooth muscle cells. J Appl Physiol (1985). 2001, 90(1): 358-368.

22. Alcorn JF, van der Velden J, Brown AL, McElhinney B, Irvin CG et al. c-Jun N-terminal kinase 1 is required for the development of pulmonary fibrosis. Am J Respir Cell Mol Biol. 2009, 40(4): 422-432.

23. Nakae S, Lunderius C, Ho LH, Schafer B, Tsai M, Galli SJ. TNF can contribute to multiple features of ovalbumin-induced allergic inflammation of the airways in mice. J Allergy Clin Immunol.2007, 119(3): 680-686.

24. Eynott PR, Nath P, Leung SY, Adcock IM, Bennett BL et al. Allergen-induced inflammation and airway epithelial and smooth muscle cell proliferation: role of Jun N-terminal kinase. Br J Pharmacol. 2003,140(8): 1373-1380.