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Small Molecule Mimetics of α -Helical Domain of IRAK2 Attenuate the Proinflammatory Effects of IL-33 in Asthma-like Mouse Models

Jinghong Li,^{*,†} Kunio Saruta,[‡] Justin P. Dumouchel,^{*,†} Jenna M. Magat,^{*,†} Joanna L. Thomas,[†] Dariush Ajami,[‡] Mitra Rebek,[‡] Julius Rebek, Jr.,[‡] and Timothy D. Bigby^{*,†,1}

IL-33 and its receptor ST2 play important roles in airway inflammation and contribute to asthma onset and exacerbation. The IL-33/ST2 signaling pathway recruits adapter protein myeloid differentiation primary response 88 (MyD88) to transduce intracellular signaling. MyD88 forms a complex with IL-R-associated kinases (IRAKs), IRAK4 and IRAK2, called the Myddosome (MyD88–IRAK4–IRAK2). The myddosome subsequently activates downstream NF- κ B and MAPKs p38 and JNK. We established an asthma-like mouse model by intratracheal administration of IL-33. The IL-33 model has a very similar phenotype compared with the OVA-induced mouse asthma model. The importance of MyD88 in the IL-33/ST2 signaling transduction was demonstrated by the MyD88 knockout mice, which were protected from the IL-33-induced asthma. We synthesized small molecule mimetics of the α -helical domain of IRAK2 with drug-like characteristics based on the recent advances in the designing of α -helix compounds. The mimetics can competitively interfere in the protein–protein interaction between IRAK2 and IRAK4, leading to disruption of Myddosome formation. A series of small molecules were screened using an NF- κ B promoter assay in vitro. The lead compound, 7004, was further studied in the IL-33-induced and OVA-induced asthma mouse models in vivo. Compound 7004 can inhibit the IL-33-induced NF- κ B activity, disrupt Myddosome formation, and attenuate the proinflammatory effects in asthma-like models. Our data indicate that the Myddosome may represent a novel intracellular therapeutic target for diseases in which IL-33/ST2 plays important roles, such as asthma and other inflammatory diseases. *The Journal of Immunology*, 2018, 200: 4036–4043.

Interleukin-33 was identified as the functional ligand for the IL-1R family member ST2 (1). IL-33 is constitutively expressed in structural cells, such as bronchial and alveolar epithelial cells, endothelium, bronchial smooth muscle cells, and fibroblast cells. IL-33 is also expressed in dendritic cells, macrophages, and mast cells (1). ST2 belongs to the TLR/IL-1R superfamily (2). The ST2 gene also encodes a soluble molecule by alternative splicing (3). Soluble ST2 (sST2) is not thought to induce signaling, and instead, it acts as a decoy receptor for IL-33 (4). Polymorphisms of human IL-33 and ST2 genes are associated with asthma (5–7) and increased numbers of eosinophils (6).

The important roles for IL-33 and ST2 in asthma are further supported by recent studies that reveal IL-33 is a contributing factor to airway inflammation and asthma exacerbation (8, 9). IL-33 increases

ST2 expression and IL-13 production in eosinophils (10). IL-33 dysregulates the regulatory T cells, leading to impaired immunologic tolerance and promotes innate and adaptive type 2 immunity in the lung (11). In addition, IL-33 loss-of-function mutation reduces blood eosinophil counts, and people with this mutation are protected from asthma (12). ST2 knockout mice are partially protected from house dust mite-induced asthma, especially in the peripheral lung (13). The serum concentrations of sST2 are increased during asthma acute exacerbation, suggesting that the host tries to attenuate IL-33 signaling (14). These findings suggest the IL-33/ST2 signaling pathway is involved in both the onset and the acute exacerbations of asthma (15). Therefore, blocking the IL-33/ST2 signaling pathway may have therapeutic potential for asthma (16, 17).

On a molecular level, IL-33/ST2 signaling pathway recruits adapter protein myeloid differentiation primary response 88 (MyD88) to transduce intracellular signaling. In animal allergic asthma models, IL-1R/MyD88 signaling is critical for the initiation of inflammatory response in the lung (18). MyD88 forms a complex with IL-R-associated kinases (IRAKs), IRAK4 and IRAK2, called the Myddosome (MyD88–IRAK4–IRAK2) (19). X-ray crystallography showed that the complete Myddosome structure is formed with six MyD88s, four IRAK4s, and four IRAK2s (19). After the formation of the Myddosome, phosphorylated IRAK-1 dissociates from MyD88 and then associates with TRAF-6, which in turn causes downstream activation of NF- κ B and MAPKs p38 and JNK (20). The Myddosome structure has made it a possible target for blocking IL-33/ST2 signaling. In order for the Myddosome to signal, the MyD88–IRAK4 complex must bind IRAK2. MyD88–IRAK4 and IRAK4–IRAK2 protein–protein interactions have different binding properties, which allow for specific configurations of the Myddosome (21).

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Abbreviations used in this article: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; IRAK, IL-R-associated kinase; MyD88, myeloid differentiation primary response 88; sST2, soluble ST2.

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It was reported that systemic and intranasal administration with IL-33 leads to eosinophilia, epithelial cell hypertrophy, and increased mucus secretion in mice (22). IL-33 augments the production of Th2 cytokines IL-4, IL-5, and IL-13 (1, 23). In this study, we demonstrated that intratracheal administration of IL-33 for 4 d at a much lower dose (500 ng) induces an asthma-like phenotype mouse model. The importance of MyD88 in the IL-33/ST2 signaling transduction was demonstrated by the MyD88 knockout mice, which were protected from the IL-33-induced asthma. To disrupt the Myddosome formation, we synthesized a series of small molecule mimetics of the α -helical domain of IRAK2. The mimetics can competitively bind with IRAK4, therefore interfering in the protein–protein interaction between IRAK2 and IRAK4. The design of the mimetics involves the scaffolds with mimicry side-chain residues on the α -helical domain. These residues play a key role in mediating IRAK4–IRAK2 protein–protein interactions and signaling transduction. The new generation of the scaffolds have more drug-like characteristics than the old generations (24). We first screened the effects of the small molecule mimetics on IL-33-induced NF- κ B transcriptional activity in T cells. We further studied the lead compound 7004 in the IL-33-induced and OVA-induced asthma-like mouse models. We found compound 7004 can attenuate the proinflammatory effects of IL-33 in vitro and in vivo.

Materials and Methods

Reagents, cell culture, and reporter assays

EL4 murine thymoma cells were purchased from ATCC (Manassas, VA). Cells were grown in RPMI 1640 medium supplemented with 10% FBS. For promoter assay, EL4 cells were stably transfected with a NF- κ B promoter–luciferase reporter, pGL4.32 (Promega, Sunnyvale, CA). The cells were exposed to varying concentrations of IL-33 (Cell Sciences, Canton, MA) to test its effect on NF- κ B transcriptional activity. The cells were also preincubated with compound 7000 series for 30 min before IL-33 (10 nM) treatment of 4 h. The effect of compound 7000 series on IL-33-induced NF- κ B transcriptional activity was measured by the luciferase activity (Promega).

Coimmunoprecipitation and Western blot analysis

For coimmunoprecipitation experiments, 100 μ l of total cell lysate were incubated with 50 μ l of anti-IRAK2-conjugated (Abcam, Cambridge, MA) Dynabeads (Life Technologies, Grand Island, NY) for 1 h at room temperature, according to the manufacturer's manual. Proteins were separated in 10% SDS/PAGE and transferred to nitrocellulose membrane. The membrane was blocked for 1 h in blocking buffer for fluorescent Western blot (Rockland, Gilbertsville, PA). Proteins were detected by Western blot by using anti-IRAK4 Ab (Abcam). Fluorescent-labeled secondary Ab (LI-COR Biosciences, Lincoln, NE) was used to detect the protein bands. An Odyssey imaging system imager was used to capture the images (LI-COR Biosciences).

Isolation of mouse primary spleen CD4⁺ cells

We isolated mouse primary spleen CD4⁺ cells using negative selection by MACS system (Miltenyi Biotec, Auburn, CA). The CD4⁺ T cells were grown in RPMI 1640 medium supplemented with 10% FBS. CD4⁺ T cells were stimulated with PMA (100 ng/ml) for 12 h and then were preincubated for 30 min with vehicle or 7004. The cells were then treated with IL-33 (10 nM) for 4 h. Cells culture supernatant was collected, and IL-5 and IL-13 were measured by ELISA.

IL-33-induced and OVA-induced asthma-like phenotype in mouse models

All studies were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Veterans Affairs San Diego Healthcare System. Mouse strains studied included C57BL/6 from The Jackson Laboratory (Bar Harbor, ME) and MyD88 knockout from Dr. S. Akira (Research Institute for Microbial Disease, Osaka University, Osaka, Japan). IL-33 model is 6–8-wk-old mice that were intratracheally administrated with 500 ng of IL-33 per day for four consecutive days.

OVA model is 6–8-wk-old mice that were immunized and sensitized to OVA using a 21 d protocol. Mice were immunized by i.p. injection of 50 μ g of OVA and 1 mg of alum in PBS on day 0 and day 7, followed by four intratracheal injections of 20 μ g of OVA in 50 μ l of PBS on days 17, 18, 19, and 20. On day 5 for the IL-33 model or on day 21 for the OVA model, the mice were intubated with a 20 gauge i.v. catheter and placed on a computer-controlled small animal ventilator (flexiVent; SCIREQ, Montreal, Canada) delivering 2% isoflurane continuously (25). Baseline airway resistance was measured. The animals were then challenged with an ultrasonic aerosol of 1.5, 3, 6, 12, and 24 mg/ml methacholine. The peak airway resistance with each dose was obtained. Following the methacholine challenges, the animals were harvested. Bronchoalveolar lavage fluid (BALF) was obtained with 0.5 ml of PBS repeated for three times. The total cell counts and differentiation of the cells in BALF were studied. BALF was collected, and IL-5 and IL-13 levels were measured by ELISA.

Synthesis of small molecule mimetics of α -helical domain of IRAK2

In the MyD88–IRAK4–IRAK2 complex, W62, W63, and M66 in α helix of IRAK2 were confirmed to interact with IRAK4 via x-ray crystallography study, and furthermore, mutagenesis showed that two of these residues (W62 and M66) were critical for signal transduction (19). To mimic these three essential residues and inhibit signal transduction, we devised a pyrazinyl piperazine scaffold that resembles the IRAK2 interface in IRAK4–IRAK2 interactions. The scaffold allows for the presentation of side-chains in a similar orientation to the i, i+1, and i+4 of the α helix of IRAK2. A series of the small molecule mimetics of the α -helical domain of IRAK2 were created based on the pyrazinyl piperazine scaffold by adding a variety hydrophobic aromatic and alkyl groups (see *Results*).

In vivo osmotic minipump delivery of compound 7004

To reduce the toxicity because of vehicle, different solvents were tested to dissolve 7004. A solution of 50% DMSO, 30% polyethylene glycol, 15% ethanol, and 5% PBS was found to be the least toxic vehicle without reducing the solubility of compound 7004. The Alzet model 2001 osmotic minipump (Alzet, Palo Alto, CA) has a reservoir of 200 μ l capacity with a mean pumping rate of 1.0 μ l/h for 1 wk duration. The minipump device was implanted s.c. in mice on the back. Treatment with compound 7004 started 1 d before the first dose of IL-33 or on day 16 of the OVA protocol (the day before starting intratracheal challenges). The minipump delivered 1 μ l/h of 7004 at 20 mM (9.5 μ g/h) or vehicle. This dose is equivalent to 0.8 μ mol/kg/hr by infusion therapy. In all cases, vehicle solution was used as control. Compound 7004 or vehicle was continuously delivered s.c. until harvest.

Statistical analysis

ANOVA was performed using GraphPad Prism software (La Jolla, CA). Statistical analyses of data were performed using paired and unpaired Student *t* tests or using one-way ANOVA. Statistical significance is defined by *p* < 0.05.

Results

IL-33 induces mouse asthma-like phenotype, and MyD88 is necessary to mediate IL-33 signaling

The comparison of the IL-33-induced and OVA-induced mouse asthma models is in Fig. 1A. It takes 21 d to establish the OVA model. It takes 4 d to establish the IL-33 model. IL-33-induced models have airway hyperresponsiveness (AHR) similar to OVA models (Fig. 1B). We found that IL-33-induced models had high cell counts in their BALF, indicating a large inflammatory cell infiltrate with eosinophilia infiltration (Fig. 1C). Next, we looked for Th2 cytokine expression in the BALF. Increases in the Th2 cytokines, IL-5 and IL-13, were more robust in IL-33-induced models compared with OVA models (Fig. 1D). This is likely mediated by the type 2 innate lymphoid cells. Type 2 innate lymphoid cells are known to produce high levels of IL-5 and IL-13 in response to IL-33 treatment (26). MyD88 knockout mice given IL-33 did not develop an asthma-like phenotype. They had no significant differences in airway resistance (Fig. 1E), BALF total cell counts, and cell differentials compared with the saline controls (Fig. 1F).

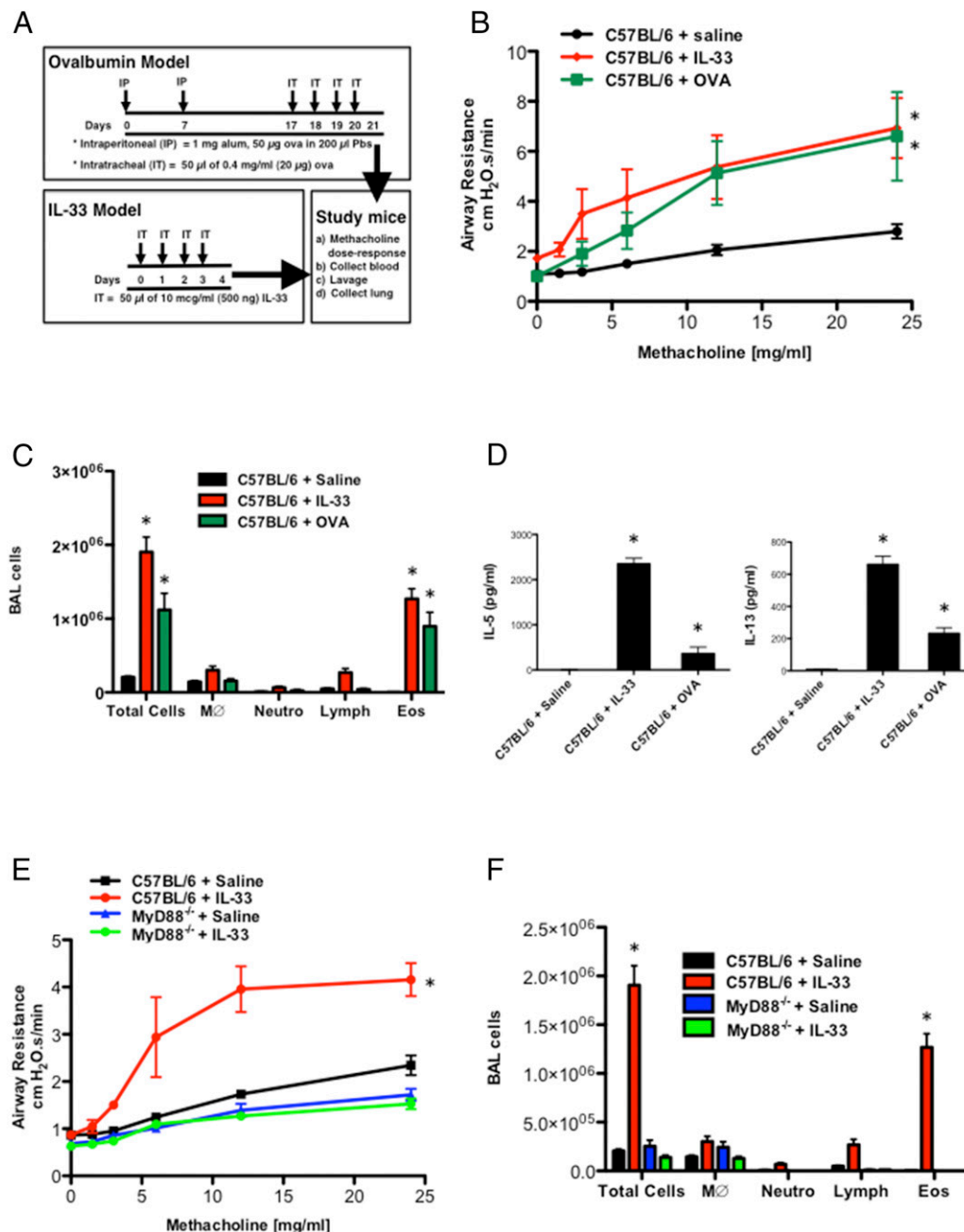


FIGURE 1. IL-33 induces mouse asthma-like phenotype, and MyD88 is necessary to mediate IL-33 signaling. **(A)** Comparison of IL-33- and OVA-induced asthma-like phenotype models. In the OVA model, mice are immunized on day 0 and day 7, then undergo intratracheal challenge with OVA on day 17, 18, 19, and 20. The mice are studied on day 21. In the IL-33 model, mice undergo intratracheal challenge with IL-33 on day 0, 1, 2, and 3. The mice are studied on day 4. **(B)** IL-33-induced AHR is similar to OVA-induced AHR. The peak airway resistance with series of methacholine was obtained. * $p < 0.01$ compared with saline control ($n = 8$). **(C)** The BALF total cell counts and differentials of macrophages, neutrophils, lymphocytes, and eosinophils were obtained. IL-33 increases the inflammatory cell infiltrate in the lung, specifically causing eosinophilia, comparable to the OVA-induced model. * $p < 0.01$ compared with saline control ($n = 8$). **(D)** IL-33 induces a more robust Th2 cytokine response in BALF. IL-5 and IL-13 are both elevated. * $p < 0.01$ compared with saline control ($n = 8$). **(E)** Wild type C57BL/6 or MyD88 knockout mice were intratracheally administrated with IL-33. In the wild type mice, IL-33 group has increased airway resistance compared with saline group ($n = 8$). * $p < 0.01$ compared with saline control. In the MyD88 knockout mice, there were no significant differences of airway resistance between IL-33 and saline groups ($n = 8$). **(F)** The BALF total cell counts and differentials of macrophages, neutrophils, lymphocytes, and eosinophils were obtained. In the wild type mice, IL-33 increased total cell counts and eosinophils compared with saline group ($n = 8$). * $p < 0.01$ compared with saline control. In the MyD88 knockout mice, there were no significant differences of cell infiltration or eosinophilia between IL-33 and saline groups ($n = 8$).

Design and synthesis of small molecule mimetics of α -helical domain of IRAK2

For IL-33/ST2 signaling, MyD88 first binds to IRAK4 via V43, A44, E52, Y58, I61, and R62 residues to form a binary complex. The interacting amino acids of IRAK4 are R12, V16, R20, E69, T76,

and N78 (19). The MyD88–IRAK4 complex recruits IRAK2 to form a ternary Myddosome complex (19). Structure-based mutagenesis indicates that E25, Q50, F51, R54, and A94 residues in IRAK4 and Y6, W62, M66, and R67 in IRAK2 are critical residues for IRAK4–IRAK2 engagement. The protein–protein interaction at

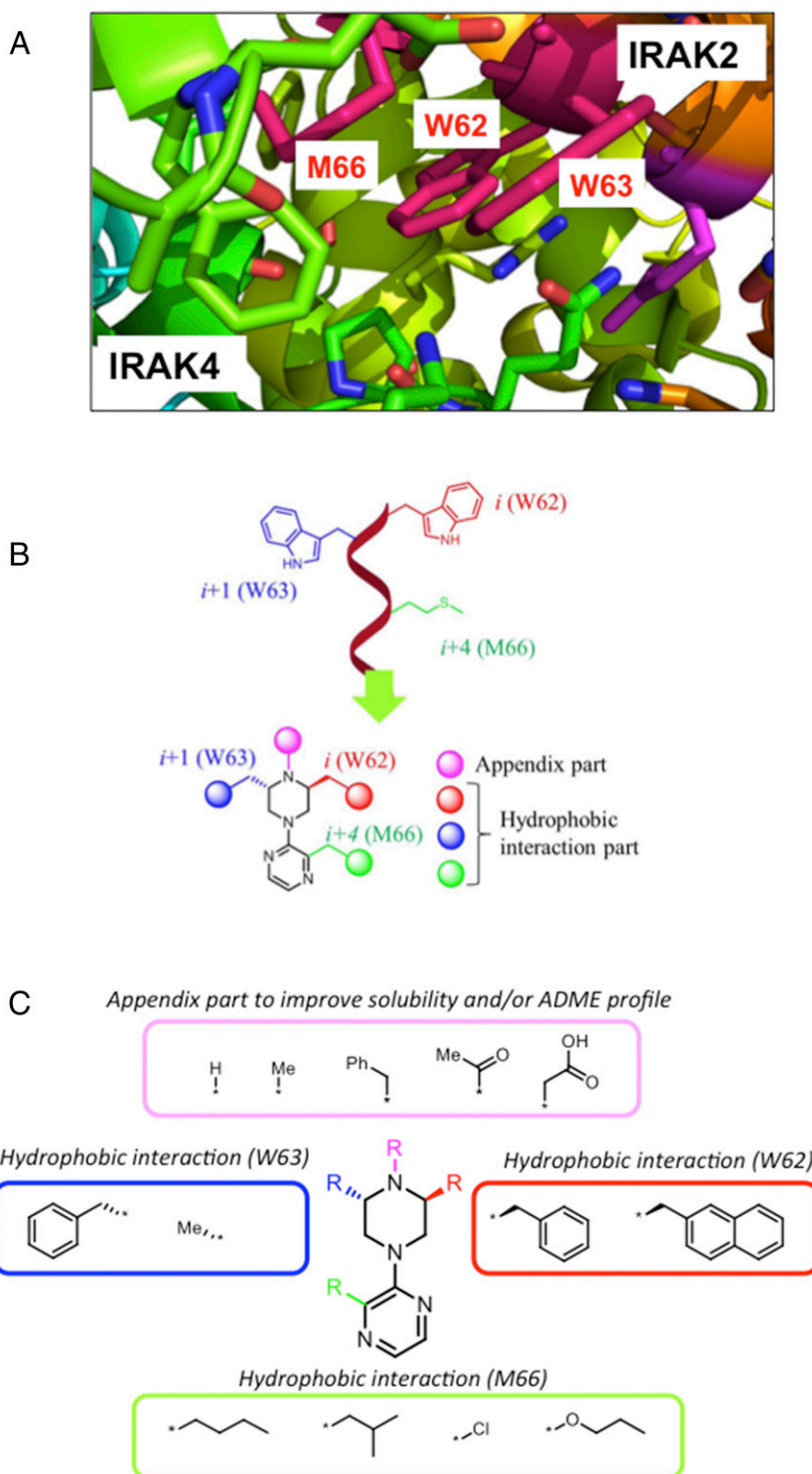


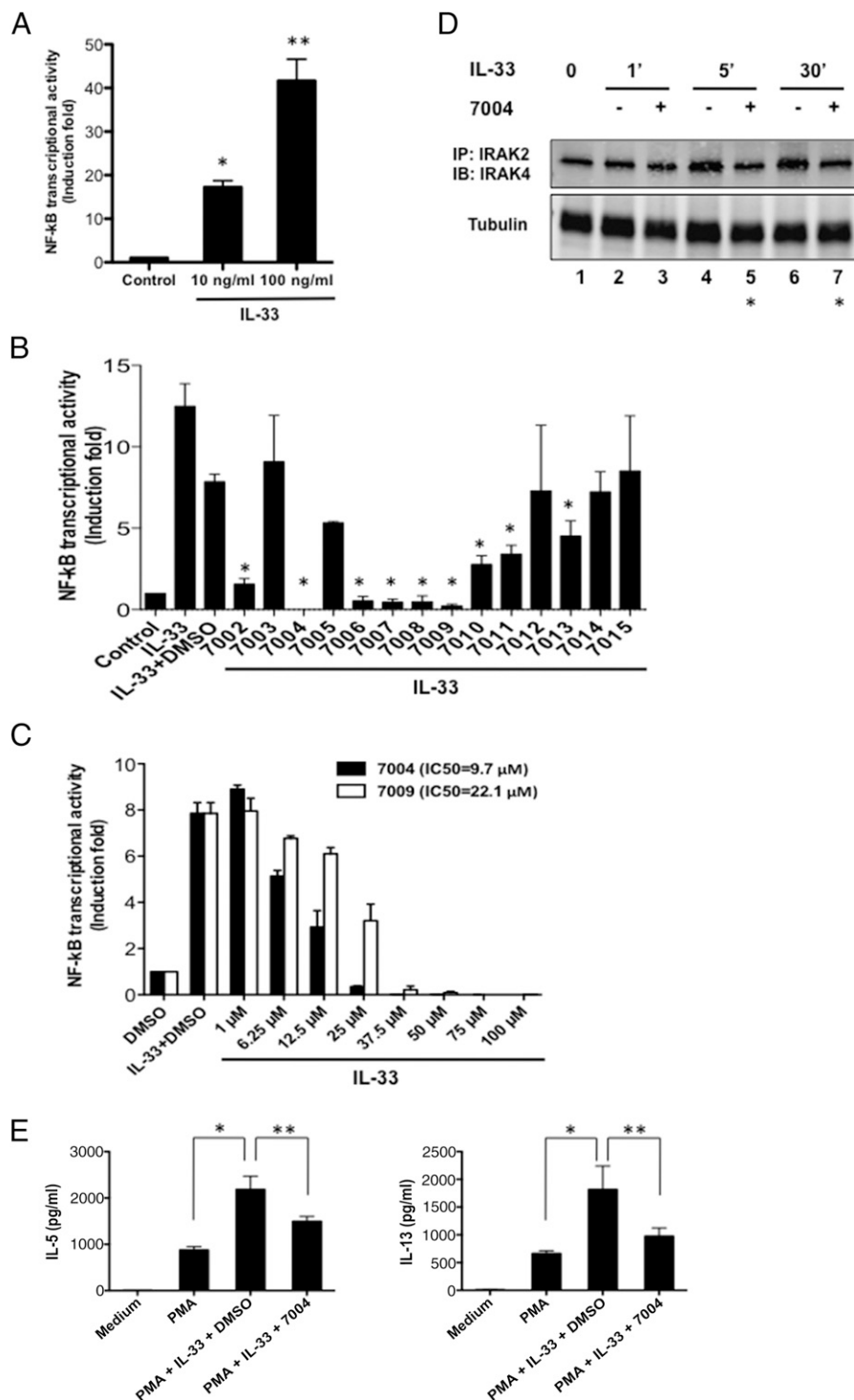
FIGURE 2. Design and synthesis of small molecule mimetics of α -helical domain of IRAK2. **(A)** X-ray structure of MyD88-IRAK4-IRAK2 complex (PDB: 3MOP) indicated that W62, W63, and M66 in α helix of IRAK2 (shown in magenta) interacted with IRAK4. **(B)** Pyrazinyl piperazine as a novel α -helical mimetic scaffold to allow for the presentation of side-chains in a similar orientation to the i , $i+1$, and $i+4$ on α helix. **(C)** α -helical domains of IRAK2 mimetics (7000 series) were synthesized based on diversity of hydrophobicity and improvement of solubility and absorption, distribution, metabolism, and excretion profile.

the IRAK4–IRAK2 interface involves α helices. Three amino acids in the α -helical domain of IRAK2, including W62, W63, and M66, are essential for the IRAK2–IRAK4 protein–protein interaction (Fig. 2A). Furthermore, mutagenesis showed that two of these residues (W62 and M66) were critical for signal transduction (Fig. 2A). A pyrazinyl piperazine scaffold that resembles the IRAK2 interface involved in the IRAK2–IRAK4 interaction was synthesized (Fig. 2B). A series of the small molecule mimetics of the α -helical domain of IRAK2 was created based on the pyrazinyl piperazine scaffold by adding side-chains to improve solubility, absorption, distribution, metabolism, and excretion profile. (Fig. 2C).

Compound 7004 and 7009 inhibit IL-33–induced NF- κ B transcriptional activity in a dose-dependent manner by disrupting Myddosome formation

To examine if IL-33 can induce NF- κ B transcriptional activity in vitro, we analyzed a dose-dependent curve of IL-33 in an NF- κ B promoter reporter assay. EL4 T cells stably transfected with an NF- κ B promoter luciferase reporter, pGL4.32, were used for the in vitro studies. As shown in Fig. 3A, IL-33–induced NF- κ B transcriptional activity in a dose-dependent manner. We then studied our small molecule mimetics of α -helical domain of IRAK2 for their effects on IL-33–induced NF- κ B transcriptional

FIGURE 3. Compound 7004 and 7009 inhibit IL-33-induced NF- κ B transcriptional activity in a dose-dependent manner by disrupting Myddosome formation. **(A)** IL-33 induced NF- κ B transcriptional activity in dose-dependent manner. EL4 cells stably transfected with a NF- κ B promoter-luciferase reporter, pGL4.32, were exposed to IL-33 (0, 10, 100 nM) for 4 h. Cells were lysed, and NF- κ B transcriptional activity was measured by the luciferase activity ($n = 6$). * $p < 0.01$ compared with control. ** $p < 0.01$ compared with lower concentrations. **(B)** Screening of the small molecule mimetics of α -helical domain of IRAK2 (7000 series). All compounds were screened at 100 μ M concentrations. EL4 cells were preincubated for 30 min with individual compound and then were exposed to IL-33 (10 nM) for 4 h. Cells were lysed, and NF- κ B transcriptional activity was measured by the luciferase activity ($n = 6$). * $p < 0.01$ compared with IL-33 or IL-33 + DMSO controls. **(C)** Dose-dependent curves and IC_{50} of compounds 7004 and 7009. EL4 cells were preincubated for 30 min with the varying concentrations of 7004 and 7009 (0–100 μ M) and then were exposed to IL-33 (10 nM) for 4 h. The IC_{50} values for compounds 7004 and 7009 were 9.7 and 22.1 μ M, respectively ($n = 6$). **(D)** Compound 7004 disrupted the Myddosome formation. The EL4 cells were preincubated for 30 min with vehicle or 7004 and then were treated with IL-33 for 1, 5, and 30 min. IL-33 exposure of EL4 T cells induced a significant increase in IRAK2–IRAK4 association at 5 and 30 min (lane 4, 6). Preincubation of the cells with compound 7004 decreased the IRAK2–IRAK4 association (lane 5, 7) ($n = 6$). * $p < 0.05$ compared with vehicle control. **(E)** Isolated mouse primary spleen $CD4^+$ cells were preincubated for 30 min with vehicle or compound 7004. The cells were then treated with IL-33 (10 nM) for 4 h. Cells culture supernatant was collected, and IL-5 and IL-13 were measured by ELISA. Compound 7004 partially decreases the IL-5 and IL-13 levels in culture supernatant ($n = 6$). * $p < 0.01$ between control and IL-33 groups. ** $p < 0.05$ between IL-33 + DMSO and IL-33 + 7004 groups.



activity. The cells were preincubated with each small molecule compound for 30 min before IL-33 treatment (Fig. 3B). Dose response curves of the most effective compounds, 7004 and 7009, were generated, and IC_{50} s were calculated. Lipophilic compounds 7004 and 7009 inhibited IL-33-induced NF- κ B reporter activity with IC_{50} s of 9.7 and 22 μ M, respectively (Fig. 3C). Water-soluble compounds 7002, 7005, and 7011 had modest inhibitory activities and higher IC_{50} s (data not shown).

To analyze the specificity of compound 7004 in disrupting Myddosome formation, we performed coimmunoprecipitation by using Abs specific for IRAK2 and IRAK4. Treatment of IL-33

results in a significant increase in IRAK2–IRAK4 association in EL4 T cells, demonstrated by coimmunoprecipitation studies. The IL-33-induced increase of IRAK2–IRAK4 association can be seen 5 min after receiving IL-33, and the effect remained after 30 min (Fig. 3D, lane 4, 6). Preincubation of the cells with compound 7004 for 30 min decreases the IRAK2–IRAK4 association (Fig. 3D, lane 5, 7). We further isolated mouse primary spleen $CD4^+$ cells. The cells were preincubated for 30 min with compound 7004 before treatment with IL-33. In the cell culture supernatants, preincubation with compound 7004 decreases the IL-5 and IL-13 production (Fig. 3E).

Compound 7004 attenuates the proinflammatory effects in vivo in IL-33-induced and OVA-induced asthma-like mouse models

Next, we analyzed the effects of compound 7004 in asthma-like mouse models. Compound 7004 was continuously delivered by osmotic minipump s.c. We found that compound 7004 partially blocked the IL-33-induced and OVA-induced AHR (Fig. 4A). Additionally, inflammatory cell infiltration, including eosinophils, was reduced in BALF (Fig. 4B). Compound 7004 also partially decreased IL-5 and IL-13 in BALF (Fig. 4C).

Discussion

In the United States, the burden of asthma continues to grow with an increase in the prevalence from 7.3% in 2001 to 8.4% in 2010 (27). During the last decade, anti-IgE Ab has been the biologic treatment for severe asthmatic patients with elevated IgE levels (28). Anti-IL-5 Ab and anti-IL-5 receptor α Ab became available on the market recently. The anti-IL-5 biologics treatment is effective in highly selected patient populations with specific phenotypes of severe asthma with persistent, glucocorticosteroid-resistant eosinophilia (28). Abs against IL-13, IL-4, and IL-17 are still under evaluation (28). There are varieties of inflammatory cells and cytokines that contribute to the onset and exacerbation of asthma. Therefore, the therapies targeting single cytokines are only effective in selected patient populations but not general asthmatic patients. Recent understanding of the innate immune response in the pathogenesis of asthma, such as IL-33, IL-25, and thymic stromal lymphopoietin pathways, provide potential for therapeutic targets other than Th2 cytokines.

Interrupting the IL-33/ST2 signaling pathway has been previously demonstrated to be effective in mouse asthma-like models (29). Overexpression of sST2, which functions as a decoy receptor for IL-33, in an OVA-induced mouse asthma model results in decreased eosinophils, IL-4, and IL-5 in the BALF (30). i.p. injection of an ST2 Ab on day 25, 27, and 29 of an OVA-induced asthma model can decrease AHR (31). Treatment with anti-IL-33 Ab before the sensitization and challenge of OVA can prevent the development of asthma in a mouse model (32). So far, no attempt has been made to evaluate Myddosome as a potential therapeutic target for asthma. We anticipate that disrupting the Myddosome complex formation to block the IL-33/ST2 signaling pathway may provide more effective treatment options compared with the Abs against IL-33 or ST2. In addition, because many TLR and IL-1R signaling use MyD88 as the adapter protein, disrupting the Myddosome complex formation is expected to have widespread anti-inflammatory properties that include other signaling pathways using IRAK4 and IRAK2.

In general, Abs interfere with protein–protein interactions in the extracellular region, but they cannot reach the intracellular region because of their poor cell membrane permeability. Small molecular compounds, in contrast, can penetrate cell membranes and can function intracellularly where most of the signaling transduction occurs. Our previous work showed that a synthetic small molecule mimetic of the BB-loop of the TLR/IL-1R domain of MyD88 could disrupt MyD88 and IL-1R association and reduce fever and inflammation caused by IL-1 in mice (33). Synthetic small molecules that can inhibit protein–protein interaction have proved effective in several disease models (34, 35).

In this study, we focused on disrupting the Myddosome formation by interfering in the IRAK2–IRAK4 protein–protein interaction. The roles of IRAKs have been implicated in certain disease models, such as human immunodeficiencies and inflammatory diseases (36, 37). IRAK4 was studied as the therapeutic

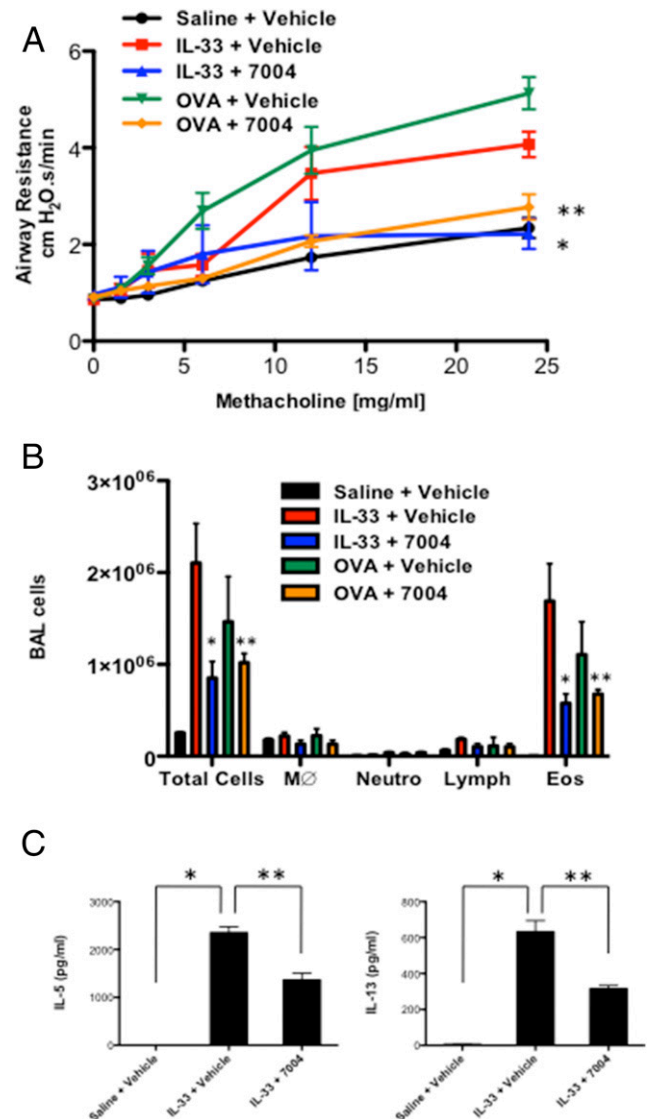


FIGURE 4. Compound 7004 attenuates the proinflammatory effects in vivo in IL-33-induced and OVA-induced asthma-like mouse models. **(A)** In the IL-33 + vehicle group, the mice have increased airway resistance compared with the saline + vehicle group ($n = 6$). In the IL-33 + 7004 group, the mice have mildly increased airway resistance compared with the saline + vehicle group at low doses of methacholine but have no significant differences at high doses of methacholine (12, 24 mg/ml). At high doses of methacholine, the IL-33 + 7004 group has significantly lower airway resistance compared with the IL-33 + vehicle group ($n = 6$). $*p < 0.05$ between IL-33 + vehicle and IL-33 + 7004 groups. In the OVA + vehicle group, the mice have increased airway resistance compared with the saline + vehicle group ($n = 6$). The OVA + 7004 group has significantly lower airway resistance compared with the OVA + vehicle group ($n = 6$). $**p < 0.05$ between OVA + vehicle and OVA + 7004 groups. **(B)** The BALF total cell counts and differentials of macrophages, neutrophils, lymphocytes, and eosinophils were obtained. The IL-33 + vehicle group has increased total cell counts and eosinophils compared with the saline + vehicle group ($n = 6$). The IL-33 + 7004 group has increased total cell counts and eosinophils compared with the saline + vehicle group, but the numbers are significantly lower than the IL-33 + vehicle group ($n = 6$). The OVA + vehicle group has increased total cell counts and eosinophils compared with the saline + vehicle group ($n = 6$). The OVA + 7004 group has increased total cell counts and eosinophils compared with the saline + vehicle group, but the numbers are significantly lower than the OVA + vehicle group ($n = 6$). $*p < 0.05$ between IL-33 + vehicle and IL-33 + 7004 groups. $**p < 0.05$ between OVA + vehicle and OVA + 7004 groups. **(C)** Compound 7004 partially decreased IL-5 and IL-13 levels in BALF. $*p < 0.01$ between saline + vehicle and IL-33 + vehicle groups. $**p < 0.05$ between IL-33 + vehicle and IL-33 + 7004 groups.

target in LPS-induced shock, autoimmune diseases, and malignancy (38–41). There are studies targeting the Myddosome formation to decrease inflammation in LPS-induced mouse septic shock model (42). A small molecule analog of brazilin (linQ) was identified as a potent inhibitor of IRAK1-dependent NF- κ B activation by disrupting the MyD88–IRAK1–TRAF6 complex formation (42).

We found that small molecule mimetics of the α -helical domain of IRAK2 were capable of inhibiting the IL-33-induced NF- κ B activation in vitro and attenuating the proinflammatory responses in asthma-like mouse models in vivo. The small molecule compound 7004 specifically reduces the IRAK2–IRAK4 protein–protein interaction. The therapeutic effects of compound 7004 are observed not only in the IL-33-induced model, but also in the OVA-induced model. We have examined the IL-33 and ST2 expression levels in the OVA-induced model and found they were both elevated (data not shown). This explains the effectiveness of compound 7004 in the OVA-induced model and sheds light on the interaction between innate and adaptive immunities in asthma. Our data indicate that the Myddosome may represent a novel intracellular therapeutic target for diseases in which IL-33/ST2 plays important roles, such as asthma and other inflammatory diseases. Efforts are underway to refine the small molecule compounds to increase the potency and drug-like characteristics.

Disclosures

The authors have no financial conflicts of interest.

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