Pathophysiological Changes Induced by *Pseudomonas aeruginosa* Infection Are Involved in MMP-12 and MMP-13 Upregulation in Human Carcinoma Epithelial Cells and a Pneumonia Mouse Model

Ji-Won Park,[a,b] In-Sik Shin,[d] Un-Hwan Ha,[c] Sei-Ryang Oh,[a] Jae-Hong Kim,[b] Kyung-Seop Ahn[a]

Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Chungbuk, Republic of Korea[a]; College of Life Sciences and Biotechnology, Korea University, Seoul, Republic of Korea[b]; Department of Biotechnology and Bioinformatics, Korea University, Sejong, Republic of Korea[c]; College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea[d]

Pseudomonas aeruginosa infections persist in patients with cystic fibrosis (CF) and drive lung disease progression. *P. aeruginosa* potently activates the innate immune system mostly through the recognition of pathogen-associated molecular patterns, such as flagellin. Matrix metalloproteinases 12 and 13 (MMP-12 and MMP-13, respectively) exacerbate chronic lung infection and inflammation by promoting uncontrolled tissue rearrangements and fibrosis, yet the underlying molecular mechanisms by which this occurs remain largely unknown. In this study, we used quantitative bacteriology, histological examination, and proinflammatory cytokine levels to evaluate the effects of MMP-12 and MMP-13 on *P. aeruginosa* strain K-induced infection and pneumonia in H292 epithelial cells and mice, respectively. Under inflammatory stimulation, mRNA and protein expression levels of proinflammatory mediators were higher in strain K-infected mice and cells than in uninfected counterparts, in which MMP-12 and MMP-13 expression reached levels similar to those observed in epithelial cells. Moreover, we also found that the NF-κB pathway might be involved in the induction of cytokines in response to strain K infection. Taken together, these data suggest that MMP-12 and MMP-13 alter strain K infection in mice and play a role in inflammatory regulation by modulating cytokine levels.

*P*seudomonas aeruginosa is a Gram-negative, opportunistic pathogen that causes acute and chronic infections, particularly in immunocompromised patients (1, 2). Infection with *Pseudomonas* is associated with the development and progression of corneal damage, as well as pulmonary decline in patients with cystic fibrosis (CF) that is characterized by a massive accumulation of neutrophils (3, 4). Acute infections are major problems in immunocompromised patients, burn victims, and patients who are critically ill or require mechanical ventilation (5), whereas respiratory infections caused by *Pseudomonas* are a major clinical problem globally, especially in patients with chronic pulmonary disorders, including cystic fibrosis (CF), non-CF bronchiectasis (nCFB), and severe chronic obstructive pulmonary disease (COPD) (6–8). Unfortunately, eradication of *Pseudomonas* from hospital settings is considered impossible because of its intrinsic resistance to antibiotics and its ubiquitous presence in the environment (9).

The canonical NF-κB pathway has been defined primarily in response to signaling by tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), prototypical proinflammatory cytokines that have important roles in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), asthma, and chronic obstructive pulmonary disease (COPD) (10). NF-κB activation is also widely implicated in inflammatory diseases, and much attention has focused on the development of anti-inflammatory drugs targeting NF-κB (11). NF-κB binding sites found in the promoter regions of the genes encoding matrix metalloproteinase 12 (MMP-12) and MMP-13 are essential for the expression of these genes (12). However, the mechanisms through which *P. aeruginosa*-infected individuals increase the expression of MMP-12 and MMP-13 in inflammatory disease have not been fully deciphered.

Animal studies have evaluated the significance of matrix metalloproteinases (MMPs) in inflammatory diseases. MMPs are a family of zinc-dependent proteolytic enzymes that degrade various components of the extracellular matrix (ECM) and mediate its remodeling in both physiological and pathological processes. A major function of MMPs is to degrade the ECM to facilitate cell migration and invasion (13, 14). MMP-12 is able to degrade extracellular matrix components, such as elastin, and is involved in tissue remodeling processes (15). Moreover, it is an important pathogenic mediator of acute and chronic pulmonary inflammatory diseases (16); however, the transcriptional regulation of MMP-13, also known as collagenase 3, in *P. aeruginosa*-induced pneumonia is not well understood (17, 18). Pseudomonads possess a number of pathogenic virulence factors and secretory systems, but no studies to date have examined the roles of MMP-12 and MMP-13 in inducing the host's inflammatory responses.
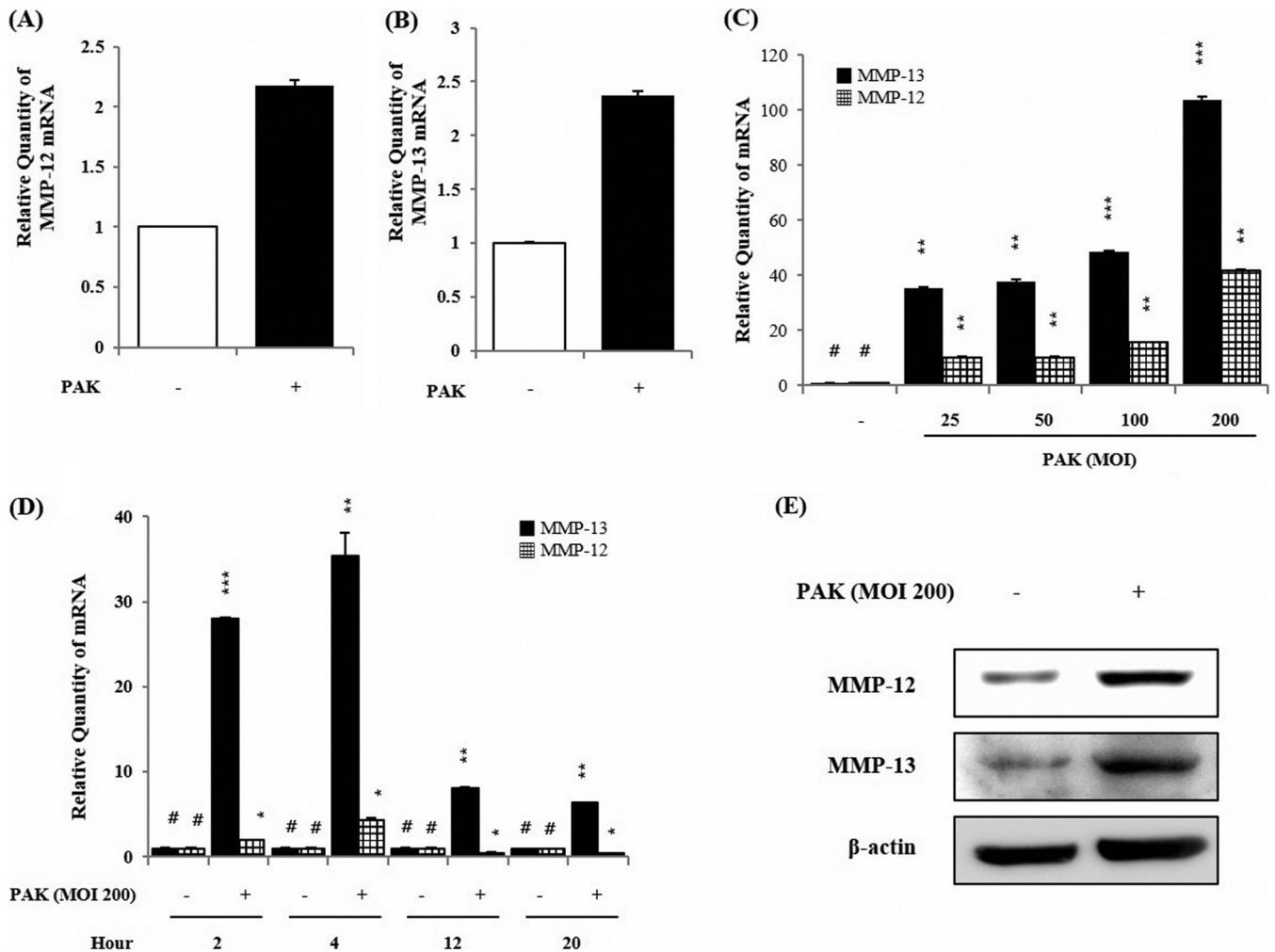
FIG 1 MMP-12 and MMP-13 expression in *P. aeruginosa* strain K (PAK)-infected H292 cells. (A, B, and E) MMP-12 mRNA (A) and MMP-13 mRNA (B) and MMP-12 and MMP-13 protein (E) expression was determined via real-time PCR and Western blotting, respectively. Additionally, the MOI dependence (C) and time dependence (D) of MMP-12 and MMP-13 expression were measured via real-time PCR. Data are presented as means ± standard deviations. #, significantly different from normal control group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Here, we showed that infection with *P. aeruginosa* strain K induced MMP-12 and MMP-13 expression in the host cells. Strain K infection was induced in mice to create the pneumonia model (here called pneumonia mice), and the results were examined. Notably, we found that proinflammatory cytokine expression was lower in cells transfected with small interfering RNAs (siRNAs) targeting MMP-12 or MMP-13. Moreover, we found that the induction of MMPs in response to strain K infection was dependent on NF-κB activation. Thus, the present report provides new insights into the roles of MMP-12 and MMP-13 in inducing proinflammatory cytokine expression during *Pseudomonas* infections.

MATERIALS AND METHODS

Human cell culture. NCI-H292 human airway epithelial cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in RPMI 1640 (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) in the presence of penicillin (100 U/ml), streptomycin (100 μg/ml; Sigma-Aldrich, St. Louis, MO, USA), and HEPES (25 mM) and incubated at 37°C in a 5% CO_2 atmosphere.

***In vitro* bacterial infection.** For direct bacterial challenge in H292 cells, *P. aeruginosa* strain K was grown in tryptic soy broth (Sigma-Aldrich) at 37°C until the optical density at 600 nm (OD_{600}) reached 0.5. The bacterial culture was then centrifuged at 6,000 × *g* for 10 min, washed in phosphate-buffered saline (PBS), and then resuspended for challenging H292 cells at a cell-to-bacterium ratio of 1:50. In some instances, cells were pretreated with the NF-κB inhibitor BAY-11-7082 (Calbiochem, Merck, Darmstadt, Germany) prior to infection.

Quantitative real-time reverse transcription-PCR (qRT-PCR) analysis. Total RNA was isolated using TRIzol reagent (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions and used to synthesize cDNA using the Omniscript reverse transcription (RT) kit (Qiagen, Hilden, Germany). Reactions were performed using SYBR green PCR master mix (Kapa Biosystems, Woburn, MA, USA) and the following primers: human MMP-12, 5′-TGG CCA AGA CCT AAG GAA TG-3′ and 5′-GAT GCA CAT TTC GAT GAG GA-3′; human MMP-13, 5′-ACC CTG GAG CAC TCA TGT TTC CTA-3′ and 5′-TGG CAT CAA GGG ATA AGG AAG GGT-3′; mouse MMP-12, 5′-TGG TAT TCA AGG AGA TGC ACA TTT-3′ and 5′-GGT TTG TGC CTT GAA AAC TTT TAG T-3′; mouse MMP-13, 5′-GCA ACA AAG TAG ATG CTG TCT ATG AGA-3′ and 5′-ATG CGA TTA CTC CAG ATA CTG TAT TCA A-3′. Reactions

were run in a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA) using the following thermal conditions: stage 1, 50°C for 2 min and 95°C for 10 min; stage 2, 95°C for 15 s and 60°C for 1 min. Stage 2 was repeated for 40 cycles. Relative mRNA levels were calculated using the comparative threshold cycle (C_T) method and normalized to that of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (5′-CCC TCC AAA ATC AAG TGG-3′ and 5′-CCA TCC ACA GTC TTC TGG-3′) or mouse GAPDH (5′-TGT GTC CGT CGG GAT CTG A-3′ and 5′-CCT GCT TCA CCA CCT TCT TGA T-3′).

Immunoblot analysis. Cells were lysed on ice for 10 min in 20 mM Tris-HCl (pH 7.4), 50 mM NaCl, 50 mM sodium pyrophosphate, 30 mM NaF, 5 μM zinc chloride, 2 mM iodoacetic acid, and 1% Triton X-100. The lysates were centrifuged at 15,000 × *g* for 15 min at 4°C, and protein concentrations were measured using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). Proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked in TBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl) containing 5% nonfat dry milk for 1 h and incubated with primary antibodies to NF-κB phospho-p65 (Cell Signaling, Danvers, MA, USA), NF-κB p65 (Santa Cruz, Dallas, TX, USA), phospho-IκB-α (Cell Signaling), IκB-α (Cell Signaling), MMP-12 (Enzo Life Sciences, Farmingdale, NY, USA), MMP-13 (Enzo Life Sciences), or β-actin (Cell Signaling, Danvers, MA, USA) for 16 h at 4°C. The immunoblots were washed and incubated with appropriate secondary antibodies and visualized using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA).

Luciferase assay. Nasal fibroblasts were transfected in A549 cells with pGL4.43 (luc2P/NF-κB-RE/Hygro) plasmid using Lipofectamine RNAiMAX transfection reagent (Invitrogen) according to the manufacturer's protocol. We used the human lung epithelial cell line A549, purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Twenty hours after transfection, cells were stimulated with *P. aeruginosa* strain K for 2 h, harvested, and then assessed for luciferase activity using the ONE-Glo luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions.

Immunohistochemistry (IHC). H292 cells were cultured on Permanox plastic chamber slides (Nunc, Rochester, NY) and fixed in methanol at 4°C for 20 min. Slides were washed three times with PBS and blocked with 3% (wt/vol) bovine serum albumin (BSA) in PBS for an additional 30 min. Next, the slides were incubated for 24 h at 4°C with anti-NF-κB p65 subunit (rabbit polyclonal IgG, 1:200 dilution; Santa Cruz) antibody. After washing to remove excess primary antibody, slides were further incubated with anti-rabbit Alexa Fluor 488-conjugated secondary antibody (Invitrogen) for 2 h at room temperature, washed with PBS, and then mounted using ProLong Gold antifade reagent containing 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) for 5 min prior to visualization by confocal laser scanning microscopy (LSM510m; Carl Zeiss, Germany). All samples were photographed under the same exposure conditions, and nuclei were quantified from the images obtained.

Mouse model of pneumonia induced by *P. aeruginosa* strain K infection. Four-week-old, specific-pathogen-free female C57BL/6 mice were purchased from the Orient Co. (Seoul, South Korea) and used after a week of quarantine and acclimatization. The mice were allowed sterilized tap water and standard rodent chow. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Korea Institute of Oriental Medicine Institutional Animal Care and Use Committee. Briefly, bacterial cultures were inoculated from glycerol stocks and cultured in LB medium overnight at 37°C until they were in log-phase growth. Bacteria were then centrifuged and resuspended to the appropriate number of CFU per milliliter in PBS, as determined by optical density and plating out a serial dilution on nutrient broth agar plates. Mice were slightly anesthetized by intraperitoneal injection of pentobarbital (Virbac, Fort Worth, TX, USA). Bacterial solutions at various concentrations (2.5×10^5, 2.5×10^6, or 2.5×10^7 CFU per mouse in 50 μl PBS) were then administered by
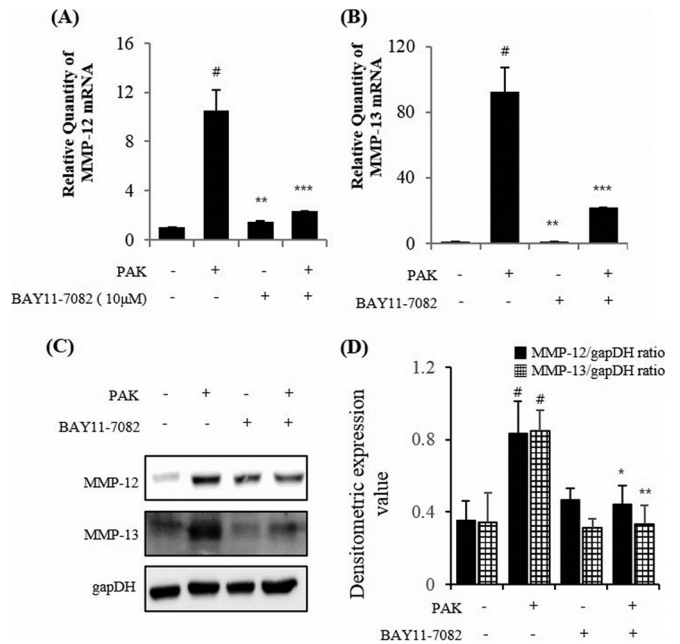
FIG 2 BAY-11-7082 inhibited MMP-12 and MMP-13 expression in *P. aeruginosa* strain K (PAK)-infected H292 cells. (A and B) MMP-12 (A) and MMP-13 (B) mRNA expression was determined via real-time PCR. (C and D) MMP-12 and MMP-13 protein expression was quantified using GAPDH as an internal control. #, significantly different from normal control group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

intranasal instillation with 25 μl per nostril. The rate of release was adjusted to allow mice to inhale the inoculum without trying to form bubbles. Mice were held in the hanging position until their breathing gradually returned to normal. The control mice were inoculated intranasally with 50 μl of PBS. Survival experiments and analysis of bronchoalveolar lavage fluid (BALF) were performed as previously described (19, 20).

Inflammatory cell counts in BALF. BALF samples were collected 20 h after infection. For this, ice-cold PBS (0.5 ml) was infused into the lungs three times and withdrawn each time using a tracheal cannula (a total volume of 1.5 ml). Total inflammatory cell numbers were then assessed by counting live cells in at least five squares of a hemocytometer by trypan blue staining. To determine differential cell counts, 100 μl of BALF was centrifuged onto slides using a Cytospin centrifuge (Hanil Science Industrial, Seoul, South Korea) (200 × *g*, 4°C, 10 min). After slides were dried, cells were fixed and stained using Diff-Quik staining reagent (B4132-1A; IMEB Inc., Deerfield, IL), according to the manufacturer's instructions. The remaining BALF was stored at −70°C for cytokine analysis.

Bacterial CFU determination in BALF. BALF samples from *P. aeruginosa* strain K-infected mice ($n = 5$ to 7 per group) were collected, and the numbers of viable bacteria were determined by the plate count method. Individual corneas were homogenized in sterile PBS, and aliquots (100 μl) of serial dilutions were plated onto *Pseudomonas* isolation agar (BD Biosciences, San Jose, CA, USA) plates in triplicate. Bacterial colonies were counted after an overnight incubation at 37°C, and the results were expressed as the mean number of CFU per milliliter of BALF.

Histology. After BALF samples were obtained, mice were sacrificed by intraperitoneal injection of pentobarbital (50 mg/kg of body weight; Hanlim Pharmaceutical Co., Seoul, South Korea), and lung tissue was fixed in 10% (vol/vol) neutral buffered formalin. Tissues were embedded in paraffin, sectioned at 4-μm thickness, and stained with hematoxylin and eosin (H&E) solution (hematoxylin from Sigma [MHS-16] and eosin from Sigma [HT110-1-32]). Quantitative analysis of inflammation and mucus production was performed in at least four squares per slide using an image analyzer (Molecular Devices Inc., Sunnyvale, CA, USA).
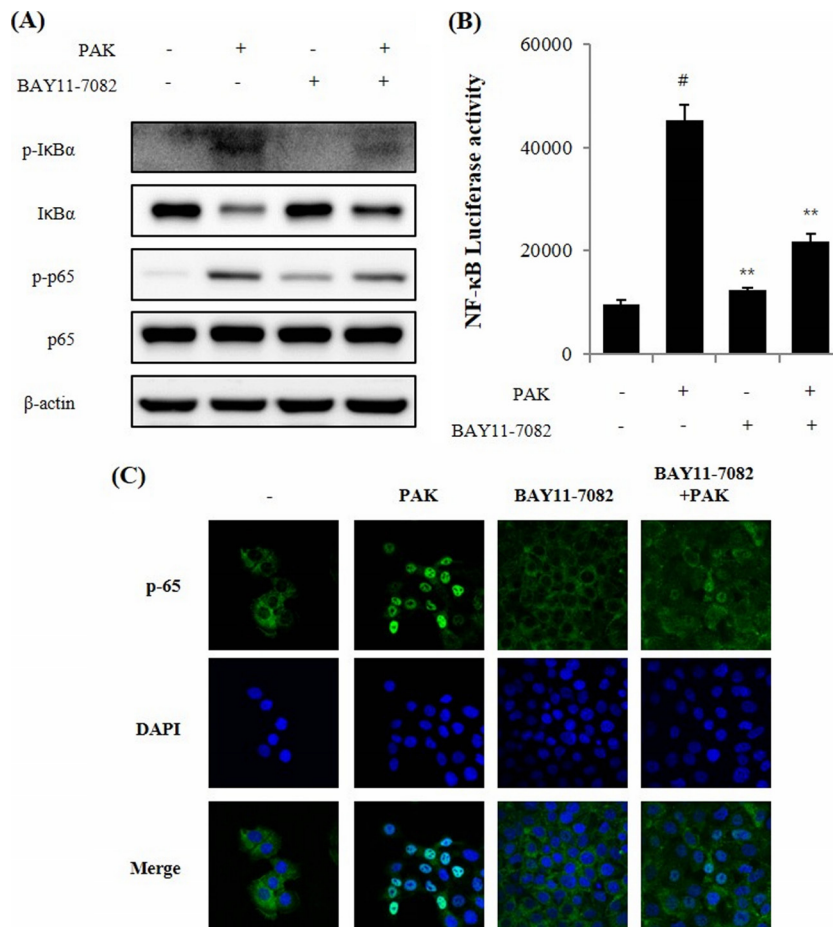
FIG 3 Inhibitory effects of BAY-11-7082 on the nuclear translocation of NF-κB in *P. aeruginosa* strain K (PAK)-infected H292 cells. (A) Nuclear extracts were from strain K-infected H292 cells and used for NF-κB p65 immunoblot assays. (B) NF-κB luciferase reporter activity in A549 cells pretreated with increasing concentrations of BAY-11-7082 prior to strain K infection (MOI, 200) for 30 min. Data are representative of three independent experiments. Data represent means ± standard deviations ($n = 3$). #, significantly different from normal control group; **, $P < 0.01$. (C) Intracellular NF-κB staining in infected H292 cells pretreated with BAY-11-7082. The nuclei of the corresponding cells were visualized using DAPI staining (magnification, ×400).

Proinflammatory cytokine ELISAs. The levels of IL-6, IL-1β, and TNF-α were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA) and performed according to the manufacturer's instructions. Cytokine concentrations were then determined using a microplate reader at 450 nm (Benchmark; Bio-Rad Laboratories, Hercules, CA).

Small interfering RNA (siRNA)-mediated knockdown. MMP-12 and MMP-13 Smartpool siRNAs (Dharmacon, Pittsburgh, PA, USA) were transfected into cells at a final concentration of 50 nM using Lipofectamine RNAiMAX reagent (Invitrogen).

Statistical analysis. The data represent the means ± standard errors of the means (SEMs). Statistical difference among groups was determined by one-way analysis of variance (ANOVA) with repeated measures followed by Newman-Keuls testing in SPSS 14.0 (IBM Software, Armonk, NY, USA). A *P* value of <0.05 was considered to be statistically significant.

RESULTS

Increased MMP-12 and MMP-13 expression in *P. aeruginosa* strain K-infected H292 cells. MMP-12 and MMP-13 mRNA expression was elevated in H292 cells in response to strain K infection (Fig. 1A and B). Moreover, MMP-12 and MMP-13 secretion increased following strain K infection at multiplicities of infection

(MOIs) of 25, 50, 100, and 200. All four doses of strain K elevated MMP-12 and MMP-13 expression (Fig. 1C). MMP-12 and MMP-13 were upregulated in strain K-infected H292 cells, peaked at 4 h, and started to decline after 12 h (Fig. 1D). In addition, MMP-12 and MMP-13 protein levels increased in a time-dependent manner in H292 cells following infection with strain K (Fig. 1E). These results show the increased expression of MMP-12 and MMP-13 induced by strain K in H292 cells.

Effect of the NF-κB inhibitor BAY-11-7082 on *P. aeruginosa* strain K-induced MMP-12 and MMP-13 expression and NF-κB p65 phosphorylation in H292 cells. We investigated the effects of NF-κB signaling on the production of MMP-12 and MMP-13 induced by strain K by using NF-κB inhibitors, including BAY-11-7082. BAY-11-7082 (Calbiochem, Merck, Darmstadt, Germany) is an inhibitor of IκB-α phosphorylation. BAY-11-7082, a potential anti-inflammatory agent, is an irreversible inhibitor of cytokine-inducible IκB-α phosphorylation (50% inhibitory concentration [IC$_{50}$], 10 μM) (21). The NF-κB inhibitor BAY-11-7082 has no effect on the growth of strain K (data not shown). BAY-11-7082 notably blocked the strain K-induced increase in MMP-12 and MMP-13 mRNA (Fig. 2A and B) and protein (Fig. 2C
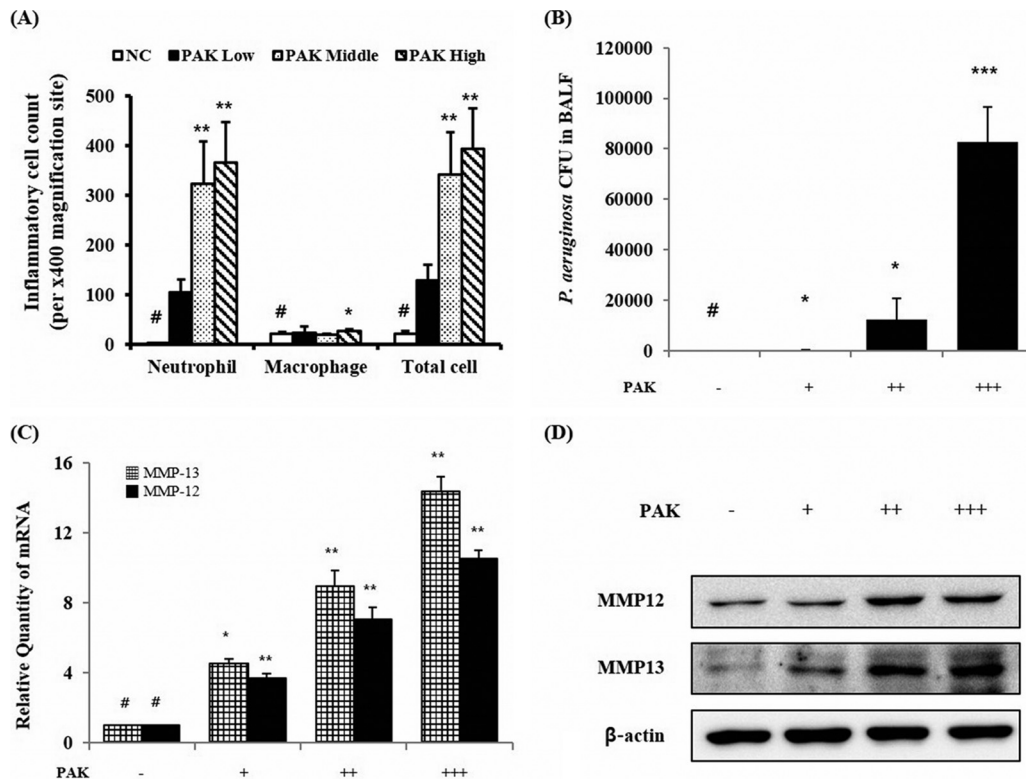
FIG 4 Effects of *P. aeruginosa* strain K (PAK) exposure on airway hyperresponsiveness and inflammatory cell counts. (A) Cell numbers in the bronchoalveolar lavage fluid (BALF) of strain K-infected mice were counted at ×400 magnification using a light microscope. (B) Inflammatory cell counts in BALF. (C and D) MMP-12 and MMP-13 mRNA (C) and protein (D) levels assessed by real-time PCR and Western blotting, respectively. NC (−), normal control mice treated with PBS only; PAK low (+), MOI of 2.5×10^5; PAK middle (++), MOI of 2.5×10^6; PAK high (+++), MOI of 2.5×10^7. #, significantly different from normal control group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

and D) expression. Moreover, BAY-11-7082 inhibited the phosphorylation of the NF-κB p65 subunit and IκB-α that occurs in response to strain K infection of H292 cells (Fig. 3A). This finding was also confirmed by luciferase assay (Fig. 3B) and IHC (Fig. 3C). Accordingly, pretreatment of the cells with BAY-11-7082 (10 μM, 1 h), a specific IκB-α inhibitor, blocked NF-κB p65 activation induced by strain K infection.

Effect of *P. aeruginosa* strain K infection in pneumonia mice. Respiratory epithelial cells secrete surfactant proteins, antimicrobial peptides, and complement; all these secreted proteins are important in innate mucosal immunity (22, 23). In addition, alveolar macrophages, neutrophils, lymphocytes, and circulating antibodies participate in the clearance of microorganisms from the lung, usually at the cost of some degree of inflammation (24). A loss of some of these barriers frequently results in lung infection. Based on these, we assessed the number of inflammatory cells in the BALF from mice with strain K lung infection. Infected mice exhibited a significant increase in the number of inflammatory cells in the BALF compared to mock-infected and normal controls (Fig. 4A). The strain K-infected mice also exhibited a dose-dependent induction of inflammatory cells, particularly neutrophils, compared with the normal control mice. Mice were infected with strain K (approximately 2.5×10^5 CFU/mouse). The immunomodulation of the NF-κB pathway seems to affect host defense against infection by strain K in murine airway epithelia (25). We therefore wanted to determine whether the induction of proinflammatory cytokines MMP-12 and MMP-13 by strain K in murine lungs is mediated by NF-κB signaling. Lungs were harvested 24 h after infection, and quantitative cultures of lung homogenates were performed. Mice exposed to strain K showed about 2,000-times-higher CFU counts of strain K in the lung homogenates than did normal mice without strain K (median, 2.5×10^5 CFU/ml lung homogenate versus 6.0×10^4 CFU/ml in infected mice; $P < 0.01$) (Fig. 4B). The strain K-infected pneumonia mice also displayed an MOI-dependent increase in MMP-12 and MMP-13 mRNA and protein expression (Fig. 4C and D). As shown in Fig. 5A and B, strain K-infected pneumonia mice displayed elevated levels of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α, compared with the normal controls.

Effects of *P. aeruginosa* strain K infection on NF-κB activation in mice. NF-κB has been proposed as a critical link between the interaction of strain K with airway epithelial cells and the innate immune response that ensues (26). We first determined whether the binding of strain K aggregates was associated with NF-κB activation, as assessed by phosphorylation of the NF-κB subunit p65 in mouse lungs. Strain K-infected pneumonia mice displayed elevated NF-κB activation in lung tissue, as demonstrated by increased phosphorylation of the NF-κB p65 subunit and IκB-α compared to control counterparts (Fig. 6A and B).

Inflammatory cytokine expression is enhanced in *P. aeruginosa* strain K-infected pneumonia mice. In order to study the histopathological changes associated with strain K infection in the human lung, lungs isolated from strain K-infected mice were sectioned and stained with hematoxylin and eosin (H&E). Histo-
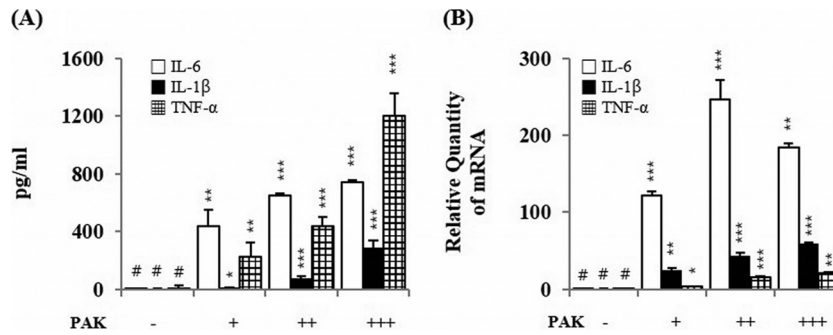
FIG 5 Effects of *P. aeruginosa* strain K infection on cytokine secretion into BALF. IL-6, IL-1β, and TNF-α protein (A) and mRNA (B) levels. See Fig. 4 legend for definitions of plus and minus signs. #, significantly different from normal control group; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

pathological changes in human lung tissues following strain K infection are independent of the route of infection (intragraft, indirect, or intraperitoneal) and present in small focal areas with syncytia and necrosis as early as day 1 postinfection (27, 28). Examination of the lungs from strain K-infected mice showed a similar severity of inflammation in the lung parenchyma. The inflammation was characterized by perivascular and alveolar inflammatory cell infiltration. In contrast, the lungs of control mice receiving saline did not show substantial inflammatory changes in the lung parenchyma. Moreover, the bronchial epithelial cells of these mice showed a high degree of vacuolization and sloughing in nonciliated epithelial cells, as well as damage to ciliated epithelial cells, which was more pronounced in the bronchioles (Fig. 7).

MMP-12 and MMP-13 are necessary for proinflammatory cytokine expression in response to *P. aeruginosa* strain K infection. To determine whether MMP-12 and MMP-13 are necessary for strain K-induced proinflammatory cytokine expression, we transfected cells with siRNAs targeting either MMP-12 or MMP-13 and saw a detectable reduction in protein 24 h after transfection. We have found effects on the expression of MMP-12 and MMP-13 (data not shown). Notably, the induction of proinflammatory cytokines previously observed in response to strain K infection in normal H292 cells was absent in siRNA-transfected cells. Using an ELISA, proinflammatory cytokine expression in response to the IL-1β and TNF-α consensus sequence was also significantly reduced in the presence of proinflammatory cytokine-specific siRNAs in H292 cells (Fig. 8A and B). These data

indicate that MMP-12 and MMP-13 are necessary for proinflammatory cytokine expression.

DISCUSSION

MMPs are secreted endopeptidases with major functions in cell growth, migration, ECM degradation and remodeling, and the pathogenesis of inflammatory diseases of the connective tissues (29, 30). MMP-13 plays an important role in collagen degradation, particularly that of collagen type II, but also those of collagen types I, III, and X, which are the major components of cartilage and bone (31). MMP-13 is also detected at higher levels in the synovial fluid of patients with osteoarthritis and rheumatoid arthritis (32, 33), where it is thought to be expressed mainly by chondrocytes and synovial cells. Moreover, MMP-13 plays an important role in tumor invasion and metastasis, especially in breast carcinoma, head-neck carcinoma, chondrosarcoma, basal cell carcinoma, melanoma, and vulvar squamous cell carcinoma (34–36). Additionally, recent studies highlight new ways in which the macrophage-derived metalloproteinase MMP-12 (also known as macrophage metalloelastase) regulates the abrogation of the acute immune response (2). Thus, MMP-12 can elicit both proinflammatory and anti-inflammatory activity in a tissue- or disease-dependent manner.

Various bacterial pathogens—including *P. aeruginosa* strain K—cause acute and chronic infections in humans. During infections, pathogens produce different sets of virulence factors to enhance their colonization and dissemination, as well as to counteract the host immune response. Thus, we investigated the effect of
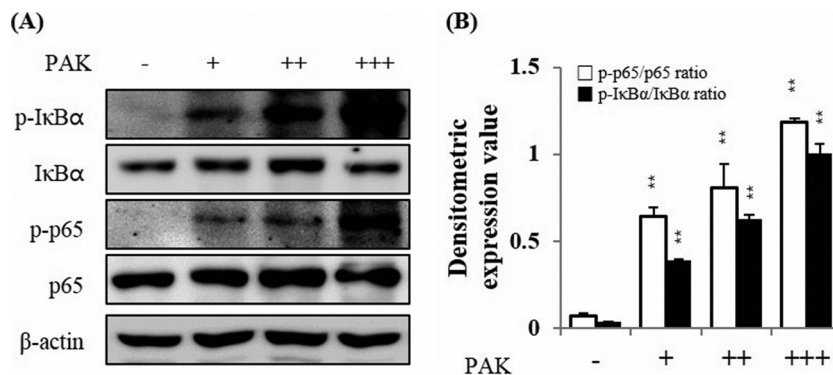

FIG 6 Effects of *P. aeruginosa* strain K (PAK) infection on NF-κB p65 phosphorylation in lung tissue. (A and B) NF-κB p65 phosphorylation in the lung tissue of infected or mock-infected mice. See Fig. 4 legend for definitions of plus and minus signs. **, $P < 0.01$.
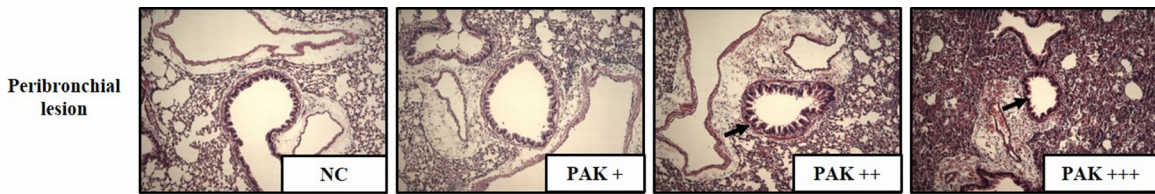
FIG 7 Histopathology of mouse lungs 20 h after inoculation with *P. aeruginosa* strain K (PAK). Magnification, ×250. NC, normal control mice treated with PBS only. See Fig. 4 legend for definitions of plus signs.

NF-κB activity on MMP-12 and MMP-13 expression in strain K-infected pneumonia mice. Consistent with these observations, expression and delivery of MMP-12 and MMP-13 were impaired when mice were infected by strain K. Examination of bacterial and host cells in the bronchoalveolar lavage fluids from infected mice revealed that the induced expression of MMP-12 and MMP-13 was dependent on NF-κB p65 phosphorylation. Notably, overexpression of the proinflammatory cytokines IL-6, IL-8, and TNF-α (37) in response to strain K-induced NF-κB activation has been implicated in disease pathogenesis in rodent models of pneumonia. Further studies demonstrate that strain K led to the upregulation of proinflammatory cytokine expression while enhancing that of MMP-12 and MMP-13. Our results demonstrate that strain K infection regulates the expression of genes associated with acute and chronic infections in an NF-κB-dependent manner and therefore plays an essential role in the pathogenesis of pneumonia (Fig. 5).

P. aeruginosa is known to have a significant effect on NF-κB activation in lung disease (38) and is known to regulate the production of cytokines and MMPs, including MMP-12 and MMP-13 (39–41). While cytokines can act as the substrates for MMPs, they can also regulate MMP expression under pathological conditions (14, 42). Investigators have shown that macrophage MMP-12 or MMP-13 gene and protein expression are promoted by some cytokines, including TNF-α and IL-1β. We have shown here that IL-6, TNF-α, and IL-8—all of which are known to be secreted from lung lymphocytes—specifically upregulate MMP-12 and thus favor a proteolytic microenvironment that facilitates lung destruction. siRNA experiments with MMP-12 and MMP-13 were previously performed (43). In addition, H292 cells were treated with short interfering MMP-12 (siMMP-12) and siMMP-13, and as a result of strain K infection, MMP-12 and MMP-13 were affected in the knockout of the target. Similarly, IL-1β and TNF-α expression was significantly attenuated in H292 cells transfected with siRNAs targeting MMP-12 and MMP-13; this indicates that MMP-12 and MMP-13 are necessary for proinflammatory cytokine expression (Fig. 8). Since MMP expression is known to enhance respiratory irritation in lung infection, these results expand this association to include cytokine-induced pathogenesis, as observed in lung biopsy specimens.

Most importantly, the present study found that *P. aeruginosa* strain K is capable of triggering MMP-12 and MMP-13 expression in lung by inducing NF-κB activation. MMP-12 and MMP-13 are considered important sources of the inflammatory mediators in respiratory airway inflammation, although their role in the inflammation of airway inflammation has not yet been thoroughly studied. Therapies for chronic respiratory inflammation are currently limited to pharmacologic bronchodilation to relieve dyspnea, antibiotics for intercurrent respiratory tract infection, and vaccination against prominent respiratory pathogens (44); thus, our findings provide genuine hope that future therapies may be capable of preventing or halting lung immunopathology by providing insight into the molecular pathogenesis of pneumonia.

Conclusion. NF-κB activation influences the expression of MMP-12 and MMP-13 in the mouse pneumonia model, suggesting that MMP expression may be important in respiratory disease pathogenesis and a potential target for anti-inflammatory therapies.
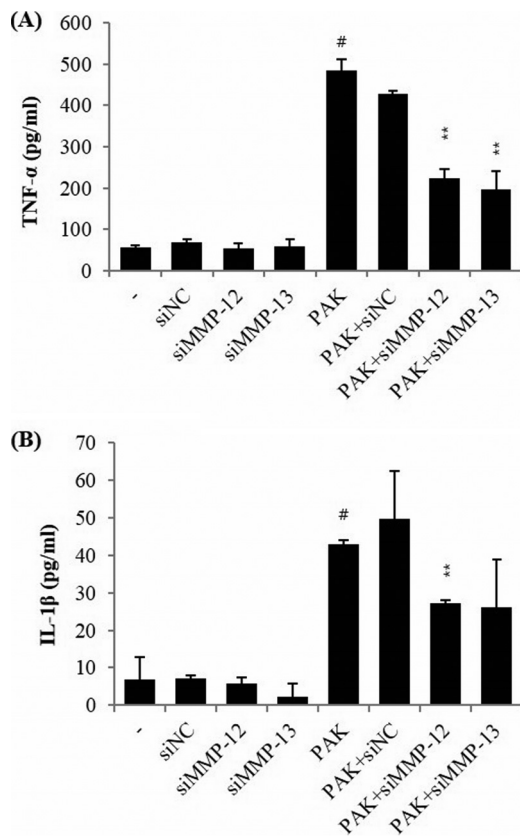

FIG 8 Proinflammatory cytokine expression in MMP-12 and MMP-13 siRNA-transfected H292 cells after *P. aeruginosa* strain K (PAK) infection. TNF-α (A) and IL-1β (B) protein levels were assessed by qRT-PCR and ELISA, respectively. Bars indicate the mean ± standard deviation from three independent experiments performed in duplicate. −, normal control H292 cells treated with PBS only; siNC, H292 cells treated with control siRNA; siMMP-12, H292 cells treated with MMP-12 siRNA only; siMMP-13, H292 cells treated with MMP-13 siRNA only; PAK + siNC, H292 cells treated with control siRNA plus strain K (MOI of 200); PAK + siMMP-12, H292 cells treated with MMP-12 siRNA plus strain K (MOI of 200); PAK + siMMP-13, H292 cells treated with MMP-13 siRNA plus strain K (MOI of 200). #, significantly different from normal control group; **, $P < 0.01$.

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