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Bradykinin inducible receptor is essential to lipopolysaccharide-induced acute lung injury in mice

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ABSTRACT

Lipopolysaccharides from gram-negative bacteria are amongst the most common causative agents of acute lung injury, which is characterized by an inflammatory response, with cellular infiltration and the release of mediators/cytokines. There is evidence that bradykinin plays a role in lung inflammation in asthma but in other types of lung inflammation its role is less clear. In the present study we evaluated the role of the bradykinin B_1 receptor in acute lung injury caused by lipopolysaccharide inhalation and the mechanisms behind bradykinin actions participating in the inflammatory response. We found that in C57Bl/6 mice, the bradykinin B_1 receptor expression was up-regulated 24 h after lipopolysaccharide inhalation. At this time, the number of cells and protein concentration were significantly increased in the bronchoalveolar lavage fluid and the mice developed airway hyperreactivity to methacholine. In addition, there was an increased expression of tumor necrosis factor-alpha, interleukin-1 beta and interferon-gamma and chemokines (monocytes chemotactic protein-1 and KC) in the bronchoalveolar lavage fluid and in the lung tissue. We then treated the mice with a bradykinin B_1 receptor antagonist, R-954 (Ac-Orn-[Oic^2, α-MePhe^5, D-βNal^7, Ile^8]desArg^9-bradykinin), 30 min after lipopolysaccharide administration. We observed that this treatment prevented the airway hyperreactivity as well as the increased cellular infiltration and protein content in the bronchoalveolar lavage fluid. Moreover, R-954 inhibited the expression of cytokines/chemokines. These results implicate bradykinin, acting through B_1 receptor, in the development of acute lung injury caused by lipopolysaccharide inhalation.

1. Introduction

Acute lung injury and its severe form, acute respiratory distress syndrome, are leading causes of mortality in humans and are characterized by neutrophil recruitment, an increase in pro-inflammatory mediators, bronchoconstriction, and injury of the alveolar epithelium and endothelium with protein leakage in the alveolar space (Weiland et al., 1986; Sibille and Reynolds, 1990; Goodman et al., 1996; Bhatia and Moochhala, 2004;). Experimental acute lung injury induced by the intratracheal instillation of lipopolysaccharide, a component of the gram-negative bacterial cell wall, shares similarities with acute respiratory distress syndrome (Brigham and Meyrick, 1986; Kabir et al., 2002). Lipopolysaccharides are recognized by host cells through the Toll-like receptor 4. This signaling is critical for the activation of nuclear factor kappa B and, consequently, the release of pro-inflammatory cytokines such as interleukin-1 beta and tumor necrosis factor-alpha (Dauphinee and Karsan, 2006; Cook et al., 2004).

These inflammatory cytokines can induce the expression of the inducible receptor of bradykinin, the B_1 receptor, which is weakly expressed under physiological conditions but strongly up-regulated after inflammatory stimuli (Marceau et al., 1998). Activation of the bradykinin B_1 receptor by its natural agonist, Des-Arg-Bradykinin promotes multiple pro-inflammatory effects such as cellular migration, release of cytokines (interleukin-1 beta), pain and edema (Perretti et al., 1993; Marceau and Bachvarov, 1998; Campos et al., 2001). Moreover, bradykinin can act on its receptor (bradykinin B_2 receptor) under physiological conditions, which is responsible for most effects of kinin, such as the control of blood pressure (Regoli et al., 1998). We and other groups have previously shown that the bradykinin B_1 receptor is involved in kidney and intestinal damages after ischemia and reperfusion injury of these tissues (Souza et al., 2004; Wang et al., 2008). In a mouse model of asthma, the bradykinin B_1 receptor was shown to modulate cellular infiltration, airway reactivity and mucus production (Gama Landgraf et al., 2003).

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We believe that the activation of the bradykinin B_1 receptor contributes to the inflammation and might be involved in an acute lung injury caused by lipopolysaccharide. Then, in the present study we evaluated the expression of the constitutive (B_2) and inducible (B_1) bradykinin receptors in the acute lung injury caused by lipopolysaccharide inhalation and studied in further detail the role of the bradykinin B_1 receptor in some parameters of lung inflammation.

2. Material and methods

2.1. Animals

Male C57Bl/6 mice, age 6–8 weeks (25–28 g) from our own facilities, were housed in individual and standard cages, with free access to water and food. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Sciences Institute/USP — Ethical Committee for Animal Research (CEEA).

2.2. Experimental model of acute lung injury

Mice were anesthetized with Ketamine–Xylazine (Agribrands do Brazil, São Paulo, Brazil). Holding the mice in an upright position, 20 μL of lipopolysaccharide from *Escherichia coli* (O111:B4 Sigma-Aldrich) at a dose of 20 μg/animal was diluted in saline and applied intranasally using an ultra-fine pipette tip. Control animals received only saline. Airway hyperreactivity to methacholine was measured at 6 or 24 h after treatment with lipopolysaccharide. After this, mice were killed to collect the bronchoalveolar lavage fluid and the lungs.

2.3. Antagonism of the bradykinin B_1 receptor

Mice were treated intranasally with 20 μL (200 μg/kg) of the bradykinin B_1 receptor antagonist R-954 (Ac-Orn-[Oic^2, α-MePhe^5, D-βNal^7, Ile^8]desArg^9-bradykinin) 30 min after the administration of lipopolysaccharide. R-954 was synthesized in the Department of Pharmacology, Faculty of Medicine, University of Sherbrooke, Quebec, Canada and kindly donated by Dr. Pierre Sirois.

2.4. Determination of airway responsiveness

Unrestrained conscious mice were placed in a whole body plethysmographic chamber (Buxco Electronics Inc., Wilmington, NC, USA) to analyze respiratory waveforms, as previously described (Keller et al., 2006). After each nebulization with increasing doses of aerosol methacholine (3, 6, 12 and 25 mg/ml), recordings were taken for 5 min. The Penh values measured during each 5 min sequence were averaged and recorded for each methacholine concentration. These same values were then used to construct a curve for each group of mice using a standard computer program, and the results were expressed as the area under the curve of Penh values (Fernvik et al., 2002).

2.5. Bronchoalveolar lavage fluid

The bronchoalveolar lavage fluid was performed to evaluate cell infiltration and protein leakage in the alveolar space. Animals were euthanized by an intraperitoneal injection of ketamine/xylazine (50 μL of a 100 mg/ml solution) at 6 or 24 h after lipopolysaccharide treatment. A tracheal cannula was inserted via a mid-cervical incision and the airways were washed two times with 1 ml of phosphate-buffered saline (PBS, pH 7.4 at 4 °C). The bronchoalveolar lavage fluid was centrifuged at 170×*g* for 10 min at 4 °C. The supernatant was removed for measurement of the total protein concentration using the DC-protein kit (Bio-Rad Laboratories, Inc, Hercules, CA, USA). The cell pellet was re-suspended in 0.5 ml of PBS. One volume of a solution containing 0.5% crystal violet dissolved in 30% acetic acid was added to nine volumes of the cell suspension. The total number of cells was determined by counting in a hematocytometer. Differential cell counts were performed after cytocentrifugation and staining with hematoxylin–eosin (Hema 3 System).

2.6. Histology

After the bronchoalveolar lavage fluid performance, the lungs were flushed by injecting 10 ml of PBS through the right ventricle to remove residual blood, fixed in 10% phosphate-buffered formalin for 24 h and then in 70% ethanol until embedding in paraffin. Tissues were sliced in 5 μ sections, stained with hematoxilin/eosin and examined microscopically. The analyses were performed by a blind pathologist.

2.7. Quantification of messenger RNA (mRNA) of cytokines

Lung samples, including bronchi, bronchioles and parenchyma, were quickly frozen in liquid nitrogen. Total RNA was isolated from the lung tissue using the TRizol Reagent (Invitrogen, USA), and the RNA concentration and the purity of the samples were determined by spectrophotometer readings at 260 nm and 280 nm. First-strand cDNA was synthesized using the MML-V reverse transcriptase (Promega, USA). All experimental real time PCR protocols were based on the manufacturer's recommendation using the TaqMan gold RT-PCR Core Reagents Kit (PerkinElmer/Applied Biosystems). Primers and probes to hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Mm01324427_m1), bradykinin B_1 receptor (Mm00432059_s1), bradykinin B_2 receptor (Mm01339907_m1), interleukin-1 beta (Mm01336189_m1), tumor necrosis factor-alpha (Mm99999068_m1), interferon-gamma (Mm00801778_m1), interleukin-6 (Mm99999064_m1) and chemokines KC (Mm00433859_m1) were purchased from Applied Biosystems. Cycling conditions were as follows: 10 min at 95 °C, then 45 cycles of 15 s at 95 °C and 1 min at 60 °C. The amount of the target gene was normalized first to an endogenous reference (hypoxanthine-guanine phosphoribosyltransferase) and then relative to a calibrator (sample with the lowest expression — saline animal), using the $2^{-\Delta\Delta Ct}$ method. Hence, steady-state mRNA levels were expressed as an n-fold difference relative to the calibrator. Analyses were performed with the Sequence Detection Software 1.9 (SDS).

2.8. Cytokine assay

A bioplex (Millipore — Billerica, MA, USA) mouse cytokine assay kit and the Bioplex 200 Suspension Array System/Luminex (Bio-Rad, Hercules, CA, USA) were used to measure tumor necrosis factor-alpha, interferon-gamma, interleukin 1-beta and monocyte chemotactic protein-1 in the bronchoalveolar lavage fluid supernatant. The kit was used according to the manufacturer's instructions. The data were analyzed using the BioPlex Manager software version 4.0. Standard curves ranged from 1.95 to 32.000 pg/ml. The lower limit of detection for each cytokine was as follows: 3.36 pg/ml for tumor necrosis factor-alpha, 5.53 pg/ml for interferon-gama, 5.56 pg/ml for interleukin 1-beta, and 20.6 pg/ml for monocyte chemotactic protein-1.

2.9. Statistical analysis

All data were described as mean ± S.E.M. Statistical evaluation of the data was carried out using the *t*-test or the One Way Analysis of Variance (ANOVA) followed by Tukey's post test. A *P* value lower than 0.05 was considered to be significant. All statistical analyses were performed with the aid of Sigma Stat Software 2.0 (Jandel Corporation, TX, USA).

3. Results

3.1. Characterization of lipopolysaccharide-induced acute lung injury

C57Bl/6 mice received 20 μg of lipopolysaccharide intranasally. After 6 and 24 h, airway hyperreactivity was evaluated and the bronchoalveolar lavage fluid was collected. A significant increase in the total number of cells in the bronchoalveolar lavage fluid was observed after administration of lipopolysaccharide in both the 6 and 24 h lipopolysaccharide-treated groups (Fig. 1A). The differential cell counts in the bronchoalveolar lavage fluid were increased due to neutrophils infiltration. Neutrophils infiltration after 6 h was 0.9 ± 0.8 *versus* 82.3 ± 29.2 cells $\times 10^4$/ml and after 24 h was 0.8 ± 0.3 *versus* 33.6 ± 7.6 cells $\times 10^4$/ml, considering the control *versus* the lipopolysaccharide group. Our histological finds showed neutrophil infiltration in lung parenchyma 24 h after lipopolysaccharide administration (data not shown). Additionally, mice developed airway hyperreactivity at 6 and 24 h after lipopolysaccharides administration (Fig. 1B). The concentration of the total protein in the bronchoalveolar lavage fluid was significantly higher at 24 h in lipopolysaccharide-treated group as compared to the control (Fig. 1C). Altogether, these results indicate that this model reproduces some features of acute lung injury.

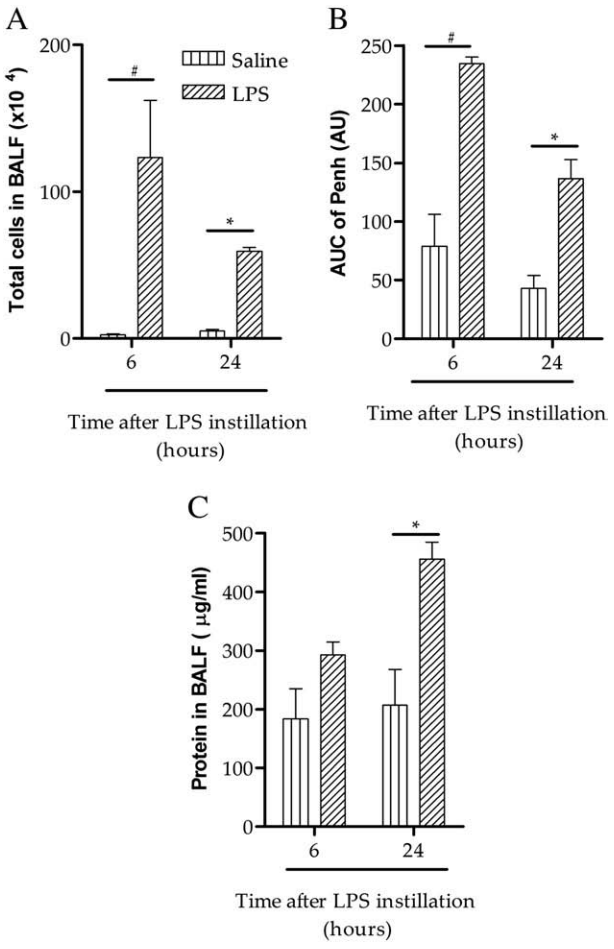

Fig. 1. Acute lung injury after lipopolysaccharide administration. Mice received 20 μg of lipopolysaccharide intranasally. After 24 h, airway reactivity was analyzed and the bronchoalveolar lavage fluid was collected. (A) Total number of cells in the bronchoalveolar lavage fluid. (B) Airway reactivity to increasing doses of methacholine expressed as the area under the curve of Penh values. (C) Total protein concentration in the bronchoalveolar lavage fluid. Statistical analyses were performed by ANOVA followed by Tukey's post test, $^{*\#}P<0.05$; $n=5$.

3.2. Lipopolysaccharide induces the bradykinin B1 receptor expression in the lung

The expression of the bradykinin receptors in the lungs was determined 6 h and 24 h after lipopolysaccharide inhalation. We observed that, 24 h after the lipopolysaccharide administration, the bradykinin B_1 receptor expression was up-regulated in comparison to the control (Fig. 2B). In contrast, at 6 h after the lipopolysaccharide inhalation, no significant difference in the bradykinin B_1 receptor and B_2 receptor expressions was observed in comparison to the control (Fig. 2A). Also, there was no significant difference in bradykinin B_2 receptor expression between lipopolysaccharide and control groups.

3.3. Blockage of the bradykinin B_1 receptor prevents acute lung injury

To assess whether the bradykinin B_1 receptor is involved in acute lung injury induced by lipopolysaccharide inhalation, mice were treated intranasally with the bradykinin B_1 receptor antagonist (R-954) 30 min after lipopolysaccharide administration. At 24 h after lipopolysaccharide administration, we observed that treatment with R-954 significantly prevented the cellular increase in the bronchoalveolar lavage fluid and the airway hyperreactivity observed in the lipopolysaccharide group (Fig. 3A and 3B). Moreover, treatment with R-954 reduced the increase in the total protein concentration in the bronchoalveolar lavage fluid when compared to lipopolysaccharide alone (Fig. 3C). In addition to that, the histological assessment showed that treatment with R-954 significantly reduced cellular infiltration in lung parenchyma around bronchi as compared to lipopolysaccharide-treated animals (Fig. 4).

3.4. Bradykinin B_1 receptor antagonist inhibits pro-inflammatory cytokines release

To better understand the role of the bradykinin B_1 receptor in acute lung injury, we measured the expression of pro-inflammatory cytokines and a chemokine in the lung tissue homogenates. We observed that intranasal instillation of lipopolysaccharide induced the expression of tumor necrosis factor-alpha, interleukin 1-beta, interleukin-6 and interferon-gamma, and of the chemokine KC. The expression of these cytokines was prevented by treatment with R-954 (Fig. 5). In addition, we also quantified cytokines in the bronchoalveolar lavage fluid. We observed high levels of tumor necrosis factor-alpha, interleukin 1-beta and interferon-gamma, and the monocyte chemotactic protein-1 in the

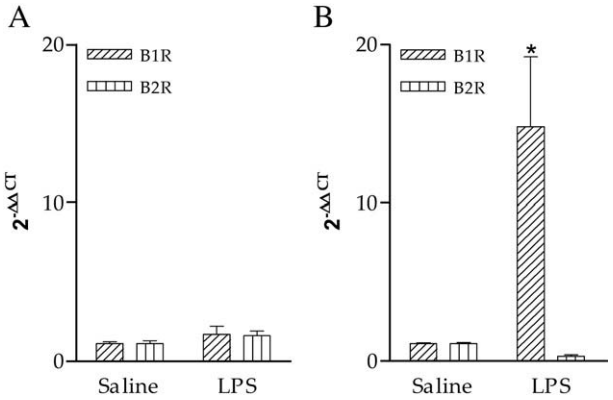

Fig. 2. Bradykinin receptor expression after lipopolysaccharide administration. Bradykinin receptor expression in the lung was analyzed by real time PCR. No difference was observed in the 6 h group. 24 h after lipopolysaccharide administration, the bradykinin B_1 receptor was up-regulated in comparison to control. Statistical analyses were performed by ANOVA followed by Tukey's post test. *LPS B1R *versus* Saline B1R, $P<0.05$; $n=5$.

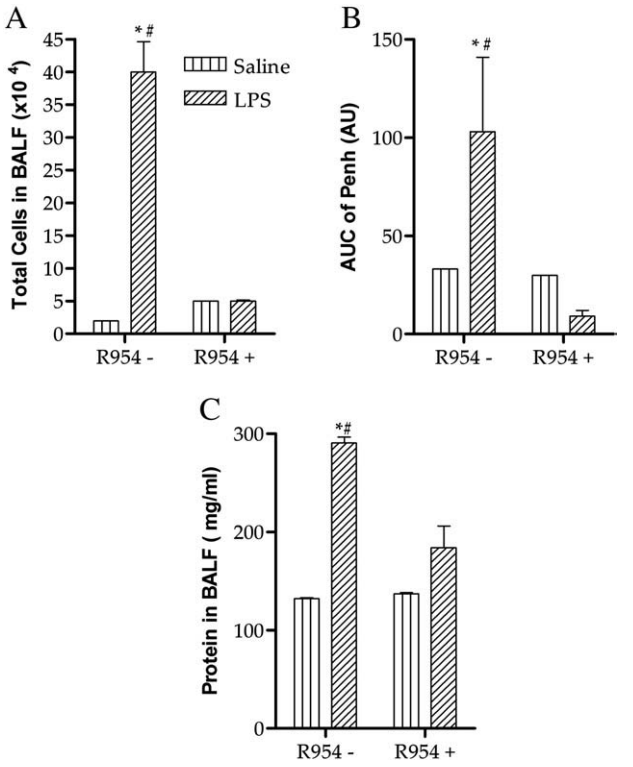

Fig. 3. Treatment with the bradykinin B1 receptor antagonist prevents acute lung injury. Mice were treated with 20 ug of lipopolysaccharide and, 30 min later, with 200 μg/kg of the bradykinin B_1 receptor antagonist (R-954). After 24 h, airway reactivity was analyzed and the bronchoalveolar lavage fluid was collected. (A) Total number of cells in the bronchoalveolar lavage fluid. (B) Airway reactivity to increasing doses of methacholine expressed by area under curve of Penh values. (C) Total protein concentration in the bronchoalveolar lavage fluid. Statistical analyses were performed by ANOVA followed by Tukey's post test. *LPS *versus* saline; #LPS *versus* LPS + R954, $P<0.05$; $n=5$.

bronchoalveolar lavage fluid after administration of lipopolysaccharide, whereas in control mice these mediators were not detected. Treatment with R-954 was able to significantly reduce the levels of monocyte chemotactic protein-1 and interferon-gama and to abolish tumor necrosis factor-alpha and interleukin 1-beta in bronchoalveolar lavage fluid (Fig. 6).

4. Discussion

The presence of Gram-negative bacteria in the lungs is one of the most common causes of acute respiratory distress syndrome. Acute respiratory distress syndrome is the severe form of acute lung injury and is a leading cause of mortality in humans (Brigham and Meyrick, 1986). Thus, many efforts have been made to better understand the mechanism of this disease in order to develop new therapies. In the present work, we have demonstrated a new possible target for therapy; we found that treatment with a bradykinin B_1 receptor antagonist is able to prevent acute lung injury caused by lipopolysaccharide inhalation.

Following intranasal instillation, lipopolysaccharide would first activate the alveolar macrophages and endothelial cells, and later the infiltrating neutrophils. Binding of lipopolysaccharide to Toll-like receptors presented in these cells triggers the activation of intracellular signaling cascades. In rat lung macrophages, lipopolysaccharide was shown to induce the activation of nuclear factor kappa B and mitogen-activated protein kinases pathways, release tumor necrosis factor-alpha, and expression of the inducible enzymes nitric oxide synthase and ciclooxigenase-2 (Martins et al., 2008a,b).

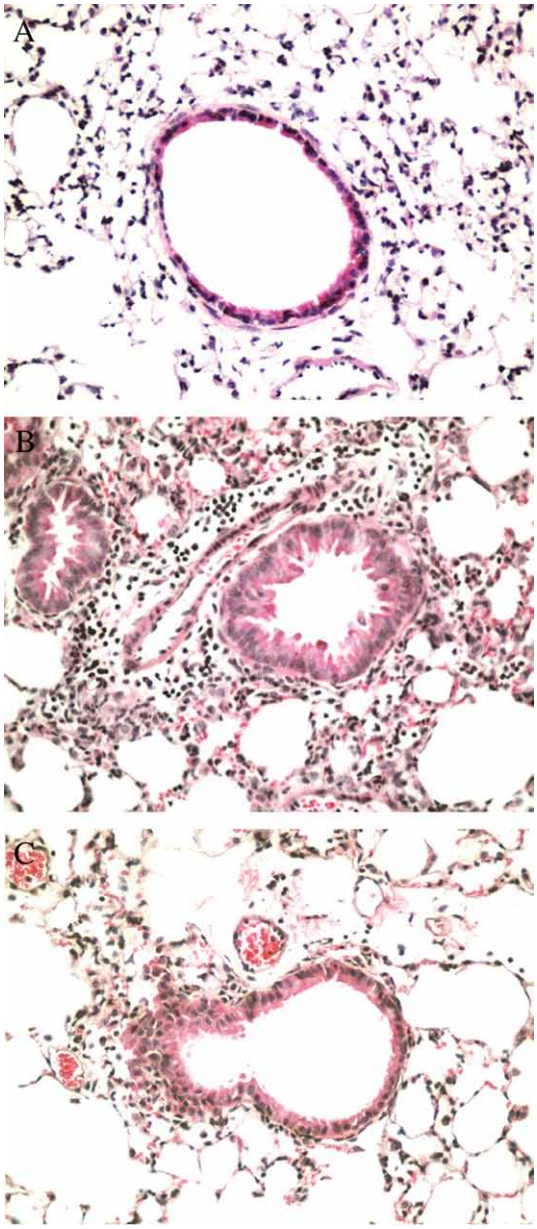

Fig. 4. Histological assessment after lipopolysaccharide and R-954 treatment. Lungs were harvested 24 h after lipopolysaccharide instillation, formalin-fixed and sections stained with hematoxylim–eosin. (A) Histological appearance from control animals. (B) Lipopolysaccharide-treated mice showing peribronchial infiltration of polymorphonuclear cells. (C) R-954 + lipopolysaccharide-treated mice showing reduced cellular infiltration as compared to lipopolysaccharide mice. Images are shown as representative fields of one of five mice at each condition at ×400 magnification.

Lefort et al. (2001), have previously described that the instillation of lipopolysaccharide from *E. coli* in the airways of C57Bl/6 mice induces lung inflammation with an increase in neutrophils within the lungs and in the bronchoalveolar lavage fluid, enhancement of vascular permeability evidenced by protein leakage in the bronchoalveolar lavage fluid, and finally, the development of airway hyperreactivity to methacholine. In our model of acute lung injury, we administered lipopolysaccharide from *E. coli* intranasally to C57bl/6 mice. After 6 and 24 h, we observed an increase in neutrophils in the bronchoalveolar lavage fluid and lungs and airway hyperreactivity to methacholine, corroborating Lefort's study. We also observed a significant increase in protein leakage in the bronchoalveolar lavage fluid.

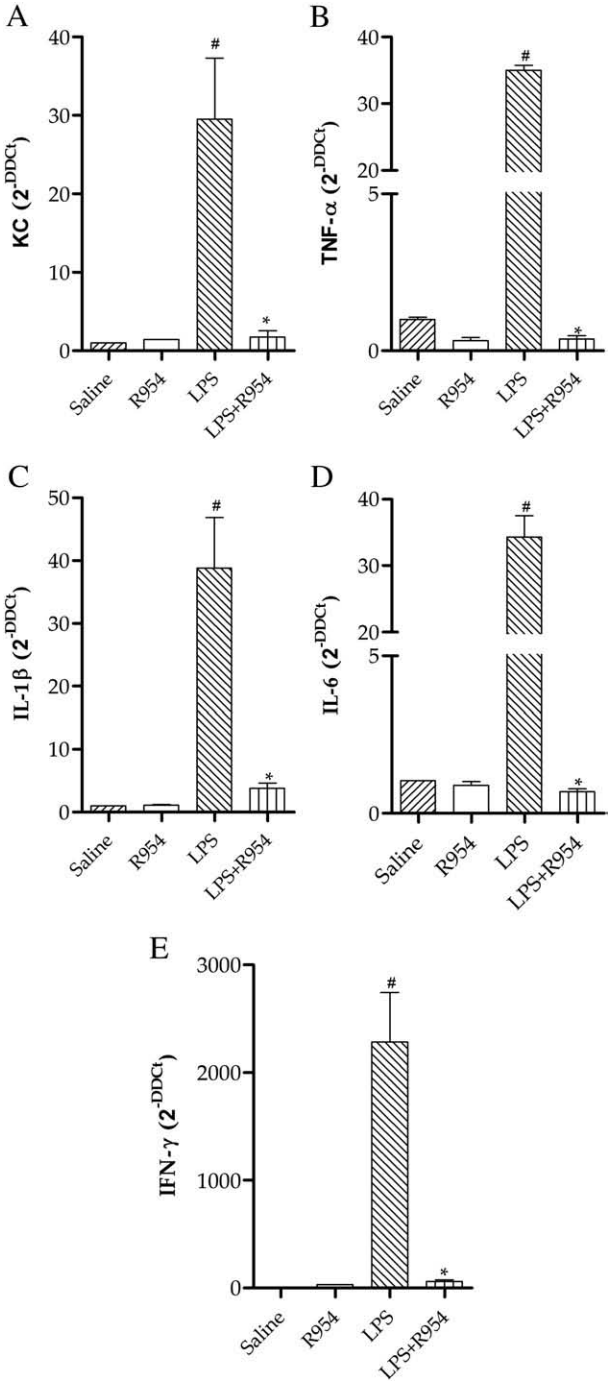

Fig. 5. Expression of pro-inflammatory mediators in the lung after R-954 treatment. All molecules were measured by real time PCR in the lung homogenates 24 h after lipopolysaccharide administration. Cytokine expression was higher in the lipopolysaccharide-treated group when compared to the control, and their expression was abrogated after treatment with R-954 (panels A–D). The same was observed for chemokine KC (panel E). Statistical analyses were performed by ANOVA followed by Tukey's post test. #LPS *versus* saline; *LPS + R954 *versus* LPS, $P<0.05$; $n=5$.

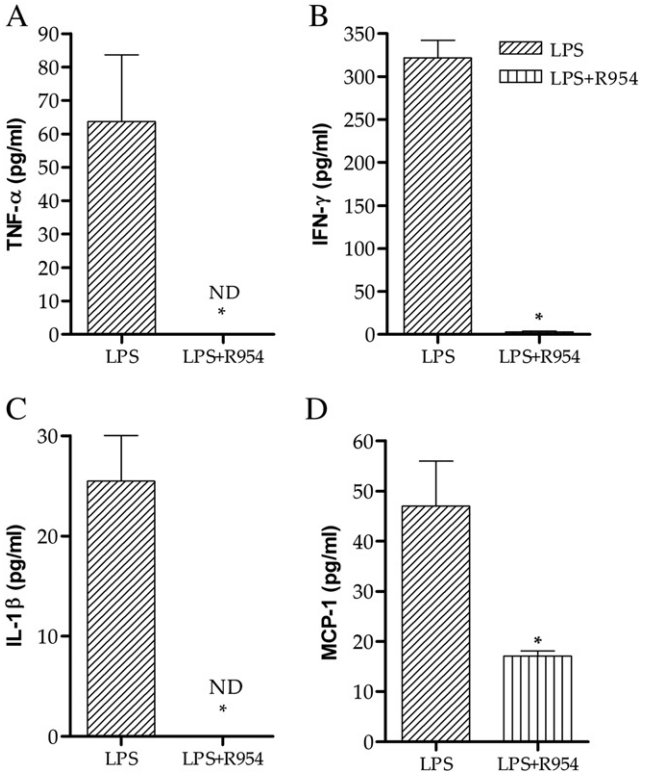

Fig. 6. Pro-inflammatory mediators in the bronchoalveolar lavage fluid after R-954 treatment. All molecules were quantified by Bioplex. Interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and monocyte chemotactic protein-1 (MCP-1) (panels A–D) levels were not detected in the control group. After lipopolysaccharide administration, these cytokines were increased in mice, whereas treatment with R-954 prevented this increase. Statistical analyses were performed by *t*-test. ND, not detected; *lipopolysaccharide + R954 *versus* iipopolysaccharide, $P<0.05$.

Many studies have aimed to elucidate possible mechanisms for lung injury caused by lipopolysaccharide. Schnyder-Candrian et al. (2005) have described that tumor necrosis factor-alpha is important for bronchoconstriction in mice that have inhaled lipopolysaccharide. However, bronchoconstriction and airway hyperreactivity to methacholine do not seem to be dependent on neutrophils (Lefort et al., 2001). Indeed, when tumor necrosis factor-alpha knockout mice were used, the bronchoconstriction was prevented after lipopolysaccharide administration, whereas the neutrophil infiltration was not reduced (Lefort et al., 2001; Schnyder-Candrian et al., 2005). Other studies have demonstrated that bronchoconstriction and airway hyperreactivity to methacholine after lipopolysaccharide inhalation are dependent on Toll-like receptor-4 pathway signaling (Togbe et al., 2006).

Here, we demonstrate that bradykinin is one contributor to acute lung injury caused by lipopolysaccharide. It is known that bradykinin promotes inflammatory effects by acting on the inducible bradykinin B_1 receptor, such as the activation of nuclear factor kappa B, and the release of inflammatory cytokines (interleukin-1 beta, tumor necrosis factor-alpha), chemokines, prostaglandin, and cellular migration (Tiffany and Burch, 1989; Dray and Perkins, 1993; Perretti et al., 1993). While bradykinin B_2 receptor is constitutive and widely expressed in different tissues, the bradykinin B_1 receptor is induced by different stimuli. The bradykinin B_1 receptor gene is regulated by a promoter region with binding sites for transcription factors such as activator protein-1 and nuclear factor kappa B, which are both up-regulated during inflammation (Marceau and Bachvarov, 1998). Interleukin-1 beta, tumor necrosis factor-alpha and activation of mitogen-activated protein kinase are involved in the up-regulation of the bradykinin B_1 receptor (Marceau et al., 1998). According to previous studies (Campos et al., 1996; deBlois and Horlick, 2001; Calixto et al., 2004), lipopolysaccharide up-regulates the bradykinin B_1 receptor expression in different types of cells and tissues, although not visualized in the lungs. Therefore, we analyzed that the expression of the bradykinin B_1 receptor and bradykinin B_2 receptor in the lung tissue homogenates 6 and 24 h after lipopolysaccharide inhalation. Our results showed that 6 h after lipopolysaccharide administration, the expression of both receptors was not significantly different from that in controls. However, 24 h after lipopolysaccharide, the bradykinin B_1 receptor was greatly up-regulated in comparison to

control mice. This result shows that the modulation of the bradykinin B_1 receptor expression by lipopolysaccharide was occurring in the lung 24 h after inhalation.

To study the role of the bradykinin B_1 receptor in acute lung injury, we neutralized the bradykinin B_1 receptor by treating animals with its antagonist (R-954) 30 min after lipopolysaccharide administration. Our results showed that treatment with R-954 was able to diminish cellular infiltration in the bronchoalveolar lavage fluid, as well as in the lung parenchyma. We showed that protein leakage in the bronchoalveolar lavage fluid and airway hyperreactivity to methacholine was also reduced with R-954 treatment. A previous work has demonstrated the involvement of the bradykinin B_1 receptor on allergic lung inflammation. Using an asthma model in mice, they demonstrated that neutralization of the bradykinin B_1 receptor was able to prevent mucus secretion, airway hyperreactivity, and eosinophils infiltration in the bronchoalveolar lavage fluid and lung parenchyma (Gama Landgraf et al., 2003). Here, we show that besides the bradykinin B_1 receptor's importance on allergic lung inflammation described previously (Gama Landgraf et al., 2003), the bradykinin B_1 receptor is also important to lung inflammation triggered by lipopolysaccharide, which involves mainly a T helper 1 response with neutrophil infiltrations, and release of nuclear factor kappa B-dependent cytokines such as tumor necrosis factor-alpha and interleukin-1 beta.

In the present study, the pro-inflammatory cytokines (tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6 and interferon-gamma) and the chemokines (monocytes chemotactic protein-1 and KC) in the bronchoalveolar lavage fluid and lung homogenates all increased after lipopolysaccharide inhalation. However, when the bradykinin B_1 receptor was blocked, the expressions of these cytokines and chemokines were similar to the control group. Moreover, the increase of interleukin-1 beta and tumor necrosis factor-alpha levels in the bronchoalveolar lavage fluid of mice treated with lipopolysaccharide was completely abrogated when the bradykinin B_1 receptor was blocked. These data are in accord with our previous study where the bradykinin B_1 receptor blockage protected the kidney from ischemia and reperfusion injury by decreasing interleukin-1 beta and monocyte chemotactic protein-1 expression in the kidney (Wang et al., 2008). Since bradykinin acting on B_1 receptor promotes the activation of nuclear factor kappa B, which is essential to tumor necrosis factor-alpha and interleukin-1 beta release, the bradykinin B_1 receptor neutralization resulted in less nuclear factor kappa B-dependent cytokines production. Our results show that by blocking the bradykinin B_1 receptor, the release of pro-inflammatory mediators, which are important to the development of acute lung injury, was abrogated.

In summary, the instillation of lipopolysaccharide into the airways had local consequences such as increased protein leakage and cellular infiltration in the bronchoalveolar lavage fluid as well as airways hyperreactivity, which are partially dependent on the bradykinin inducible receptor expression and activation. Thus, antagonists of the bradykinin B_1 receptor could be considered as a possible therapeutic tool in acute lung injury.

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