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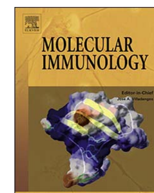

Vasoactive intestinal peptide overexpression mediated by lentivirus attenuates lipopolysaccharide-induced acute lung injury in mice by inhibiting inflammation

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ABSTRACT

Vasoactive intestinal peptide (VIP) is one of the most abundant neuropeptides in the lungs with various biological characters. We have reported that VIP inhibited the expressions of TREM-1 and IL-17A, which are involved in the initiation and amplification of inflammation in acute lung injury (ALI). However, the overall effect of VIP on ALI remains unknown. The aim of this study is to investigate the therapeutic effect of VIP mediated by lentivirus (Lenti-*VIP*) on lipopolysaccharide (LPS)-induced murine ALI. We found that the expression of intrapulmonary VIP peaked at day7 after the intratracheal injection of Lenti-*VIP*. Lenti-*VIP* increased the respiratory rate, lung compliance, and tidal volume, while decreased airway resistance in ALI mice, detected by Buxco system. Lenti-*VIP* significantly reduced inflammatory cell infiltration and maintained the integrity of the alveolar septa. Lenti-*VIP* also remarkably decreased the total protein level, the number of neutrophil and lactate dehydrogenase activity in the bronchoalveolar lavage fluid of LPS-induced ALI mice. In addition, Lenti-*VIP* down-regulated pro-inflammatory tumor necrosis factor (TNF)-α mRNA and protein expression, while up-regulated anti-inflammatory interleukin-10 mRNA and protein expression in lungs of ALI mice. Furthermore, we observed that VIP reduced the TNF-α expression in murine macrophages under LPS stimulation through protein kinase C and protein kinase A pathways. Together, our findings show that *in vivo* administration of lentivirus expressing VIP exerts a potent therapeutic effect on LPS-induced ALI in mice *via* inhibiting inflammation.

1. Introduction

Acute lung injury (ALI), triggered by direct (*e.g.* pneumonia) or indirect (*e.g.* sepsis) pathological events, is a severe clinical syndrome of pulmonary inflammation. The aggregation of neutrophil and over-activation of inflammatory response induced by alveolar macrophages increase the vascular permeability, pulmonary edema, and tiny atelectasis in ALI (Butt et al., 2016; Fanelli and Ranieri, 2015). Despite advances in therapeutic principles, ALI remains a leading cause of high morbidity and mortality in critically ill patients (Parvathaneni et al., 2017). Therefore, the innovative therapeutic targets for ALI are urgently needed.

Vasoactive intestinal peptide (VIP) is one of the most important sensory neuropeptides in lungs. VIP has various biological effects. For example, VIP can dilate pulmonary vascular (Anaid et al., 2007), increase the expressions of interleukin (IL)-4 and IL-10 (Xu et al., 2014), reduce apoptosis (Higyno et al., 2015), and decrease granulocyte recruitment (Onoue et al., 2013). Serum VIP level is significantly reduced in patients with asthma (Olopade et al., 2006), pulmonary hypertension (Leuchte et al., 2015), chronic bronchitis and pulmonary fibrosis. It's suggested that the occurrence of these diseases may be associated with the decrease of VIP. VIP analogue or agonists has shown protective effect against asthma, pulmonary hypertension, chronic bronchitis, and other pulmonary diseases (Laube, 2015).

Previously we have demonstrated that VIP inhibits IL-17A expression in murine macrophages induced by lipopolysaccharide (LPS) (Ran et al., 2015), and reduces the expression of triggering receptor expressed on myeloid cells 1 (TREM-1) in LPS stressed-pulmonary cells (Sun et al., 2011). Furthermore, IL-17A and TREM-1 are involved in the occurrence and development of ALI. IL-17A plays a pivotal role in lung injury by initiating inflammation and neutrophil recruitment after ischemia-reperfusion (Kim et al., 2015; You et al., 2014); monoclonal antibodies against IL-17 could be useful as a potential therapeutic remedy in S-OIV H1N1-induced ALI (Li et al., 2012). TREM-1 amplifies

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inflammatory response by inducing the expression of miR-155 in macrophages and aggravates ALI (Yuan et al., 2016). We have reported that downregulating or blocking TREM-1 can alleviate LPS-induced ALI (Dong et al., 2017; Liu et al., 2016b). These results suggest that VIP has its therapeutic potential for ALI. However, the exact effect of VIP on the ALI remains unclear.

Macrophages play an important role in the initiation of inflammation during the early stage of ALI (Duan et al., 2017). Macrophages stimulated by LPS can synthesize and release cytokines such as tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β_1 and IL-1β (Liu et al., 2016b), resulting in cascade amplification of inflammatory response during ALI. Illumination of the macrophages' inflammatory activation contributes to the therapy of ALI. So, in this study, we will investigate the effect of VIP on the activation of macrophages *in vitro*.

The half-life of VIP is short (Burian et al., 2012; Li et al., 2015). In order to explore the effect of VIP on ALI mice induced by LPS *in vivo*, we used lentiviral vectors to achieve steady expression of intrapulmonary VIP in this study. In recent years, researchers found that the lentivirus could be well integrated into the genome of mouse lung cells (Farrow et al., 2018). It suggests that the use of lentiviral vectors is an effective method of gene delivery. In this study, we will observe the effect of VIP overexpression mediated by lentivirus on the LPS-induced ALI in mice. Furthermore, we will investigate the effect of VIP on the inflammatory amplification of macrophages induced by LPS and the possible intercellular transduction mechanism in murine macrophages.

2. Materials and methods

2.1. Animal

Adult Swiss mice (male, 20 ± 2 g) were purchased from laboratory animal department of Central South University (Changsha, China). Mice were kept in clean cages with free access to water and food. All animal studies were approved by The Ethics Committee of Institute of Clinical Pharmacology at Central South University in accordance with the guidelines of National Institutes of Health.

2.2. Lentiviral transduction in lung of mice and VIP gene detection

Lentivirus carrying either GFP (Lenti-*Empty*) or both GFP and VIP (Lenti-*VIP*) was offered by Genechem (Shanghai, China). Swiss mice were anesthetized and intratracheally injected with Lenti-*Empty* (5×10^6 copies of vector/mouse) or Lenti-*VIP* (5×10^6 copies of vector/mouse). The method of exposure of the mice to lentivirus has been previously described. Mice were sacrificed at day4, 7, and 10 after intratracheally injection. The expression of VIP mRNA and protein in lung tissue were detected by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively.

2.3. Lentiviral VIP studies in vivo

Before the experiments, mice housed in microisolator cages were fasted for one night and were only fed water. In total, mice were randomly and equally divided into four groups: Lenti-*Empty* group, Lenti-*Empty* + ALI group, Lenti-*VIP* group, and Lenti-*VIP* + *ALI* group. Mice were anesthetized and intratracheally injected with Lenti-*Empty* (5×10^6 copies of vector/mouse) or Lenti-*VIP* (5×10^6 copies of vector/mouse). Seven days later, LPS (10 mg/kg, *E. coli* O111:B4; Sigma–Aldrich, USA) was intraperitoneally injected to induce ALI according to the previous report (Yu et al., 2017). Six hours later, the mice were undergone a series of detection as following.

2.4. Respiratory function testing by Buxco

Continuous respiratory data were collected throughout exposure periods using Buxco system (Sharon, Connecticut, CT, USA). After a brief acclimation to the chamber, the mice received an initial baseline challenge of saline. We focused on four measures: airway resistance, dynamic lung compliance, respiratory rate, and tidal volume (Zhou et al., 2017a).

2.5. Pulmonary histopathology analysis

To assess the pathological changes, samples of the lungs were taken after the mice were sacrificed. The samples were fixed in neutrally buffered 10% formaldehyde and were embedded in paraffin, and cut into 5 μm thick sections. Sections were stained with hematoxylin and eosin (HE), and observed using an optical microscope. Lung pathology was evaluated blindly by two pathologists using the light microscope, according to five independent variables: alveolar congestion, haemorrhage, infiltration or aggregation of neutrophils in air spaces or vessel walls, thickness of alveolar wall, and hyaline membrane formation. Each criterion was graded according to a 0- to 4-point scale, as previously reported (Zhou et al., 2017b).

2.6. Total and differential cell counts in bronchoalveolar lavage fluid (BALF)

Mice were sacrificed 6 h after the LPS injection. After the trachea was exposed, a catheter was inserted into the trachea for lavage. Cold phosphate buffered solution (PBS) (0.8 mL) was instilled into the mouse lungs followed by gentle aspiration for three times. The lavage fluid was centrifuged and the supernatants were used for cytokines and chemokines measurements. The cell pellets were resuspended in 0.5 mL of PBS, and the total cell counts were performed using a Neubauer chamber. For differential cells counts, Wright-Giemsa stained cytospin slides were used to obtain differential macrophage and leukocyte counts; 300 cells were counted per slide (Zhou et al., 2017b).

2.7. Protein leakage and lactate dehydrogenase (LDH) in BALF

Total proteins in BALF were quantified using the bicinchoninic acid (BCA) kit (Beyotime Biotech, Jiangsu, China) to evaluate lung vascular permeability. Briefly, supernatants from cell-free BALF were measured by colorimetry and read against a BCA protein standard curve. The LDH activity in the supernatant was measured using the corresponding kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.8. Real-time PCR

Total RNA was isolated from lung tissues or macrophages (10^6 cells in each sample) using TRIzol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized employing Superscript III (Invitrogen, Carlsbad, CA, USA) and gene expression was assayed with SYBR® Premix Ex Taq™ (Code No. RR420A, TaKaRa, Japan). PCR reaction and analysis were run on a deep-well Real-Time PCR Detection System (CFX96 Touch™, Bio-Rad, USA). All experiments were performed according to the manufacturer's instructions. Primer sequences are shown in Table 1. β-actin was used as an internal control. PCR reaction conditions were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 15 s, 60 °C for 30 s. Relative gene expression was measured by the $2^{-\Delta\Delta Ct}$ method (Huang et al., 2017a).

Table 1
Sequences of specific primers used in this study.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
VIP	TGTGCTGTTCTCTCAGTCGC	AAACGGCATCCTGTCATCCA
TNF-α	CCACCCGCTCTTCTGTCTA	TGGTTTGTGAGTGAGGGT
IL-10	GGTGAGAAGCTGAAGACCCT	ACACCTTGGTCTTGGAGCTT
β-actin	CACCATGTACCCAGGCATTG	CCTGCTTGCTGATCCACATC

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