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Research Paper

Dexmedetomidine mitigate acute lung injury by inhibiting IL-17-induced inflammatory reaction

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ABSTRACT

Interleukin-17 (IL-17) is considered to play an important role in the pathogenesis of a number of inflammatory conditions. Previous studies demonstrated that intranasal injections of IL-17 resulted in pulmonary inflammation and lung damage, we therefore hypothesize that dexmedetomidine, a potent α2 adrenergic receptor agonist that shows anti-inflammation effects in several animal models of inflammation, would attenuate IL-17 induced lung injury. We examined the lung damage using a histological approach, and assessed the number of lung-infiltrating neutrophils in the bronchoalveolar lavage fluid. We then compared the production of selected cytokines by measuring their serum concentration after various treatments. Finally, we evaluated the expression of selected inflammatory genes and activation of NF-κB in the lung epithelial cells, using real-time PCR and western blot assay, respectively. In every aspect of pulmonary inflammation investigated, dexmedetomidine significantly and dose-dependently attenuated the inflammatory effects of IL-17. Our results not only give a comprehensive description of the protective action of dexmedetomidine on IL-17 induced acute lung injury, but also provide insights to the underlying cellular and molecular mechanisms.

1. Introduction

Cytokines are key elements in many aspects of the immune responses, both under physiological and pathological conditions. Interleukin-17 (IL-17) is a family of cytokines with highly conserved cysteine residues consisting of six members identified so far: IL-17A to F (Gu et al., 2013). The founding member of the family, IL-17A (formerly known as “cytotoxic T lymphocyte-associated Ag-8”, CTLA-8) was first characterized nearly two decades ago. It is the signature cytokine mainly produced by T helper 17 (Th17) cells, a subset of $CD4^+$ T cells that is distinct from the classic Th1 and Th2 families of T cells (Ouyang et al., 2008; Park et al., 2005).

Amongst the six members, IL-17A (hereon referred to as IL-17) has been most-widely investigated. IL-17 signals through the ubiquitously-distributed receptor IL-17R (Moseley et al., 2003; Toy et al., 2006). Upon activation of the heteromeric receptor complex, an essential adaptor molecule Act1, with a U-box E3 ubiquitin ligase activity (Liu et al., 2009), is recruited to the receptor through a highly conserved ‘SEFIR’ (similar expression to fibroblast growth factor genes, IL-17 receptors and Toll–IL-1R) domain (Novatchkova et al., 2003), followed by the binding of tumor necrosis factor receptor–associated factor (TRAF) 6, which subsequently activates downstream NF-kB and MAPK pathways, leading to expression of a variety of genes (Cua and Tato, 2010; Liu et al., 2009; Moseley et al., 2003; Qian et al., 2007; Schwandner et al., 2000). Growing evidence demonstrates that IL-17 enhances the transcription of pro-inflammatory and neutrophil-mobilizing cytokines or chemokines such as granulocyte-macrophage colony-stimulating factor, tumor necrosis factor (TNF), IL-1, IL-6, chemokine C-X-C motif ligand (CXCL) 1 (or KC), chemokine (C-C motif) ligand (CCL) 2 (or MCP-1), CXCL2 (or MIP-2), and CCL20 (or MIP-3A) (Iwakura et al., 2011; Jovanovic et al., 1998). Hence, IL-17A is thought to mainly coordinate local immune responses in both auto-immunity and host defense against exogenous pathogens. It is hardly a surprise that dysregulation or aberrant expression of IL-17 has been shown to contribute to severe inflammation and tissue damage, and linked to a number of autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, allergic skin immune responses, asthma, and allograft rejection (Chang and Dong, 2011; Gu et al., 2013; Park et al., 2005). Consistent with the clinical observations, it is well established in prior reports that constitutive expression or intranasal administration of IL-17 may give rise to acute pulmonary inflammation and lung damage, as evidenced by histological changes, increased lung infiltration, and elevated level of cytokine production and expression (Bulek et al., 2011; Park et al., 2005; Zhong et al., 2013).

Abbreviations: IL-17, interleukin-17; TNF, tumor necrosis factor; Dex, dexmedetomidine; TRAF, tumor necrosis factor receptor–associated factor

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Dexmedetomidine (Dex) is a potent α2 adrenergic receptor agonist, with a strong sedative, anti-sympathetic, and analgesic effect (Hsu et al., 2004). It is widely used by intensive care units for sedation of patients under ventilation. Besides, prior studies also suggest that Dex possesses an anti-inflammatory property (Kurt et al., 2013; Xu et al., 2015). The mechanisms involved are still poorly understood, but it has been implicated that Dex controls systemic cytokine level via the cholinergic anti-inflammation pathway (Xiang et al., 2014). We therefore hypothesize that treatment with Dex may also exert protective effects against acute lung injury, in the model of IL-17 induced pulmonary inflammation. We explored this possibility through the combined use of histological comparison and quantitative analysis of various inflammatory molecules at both the mRNA and protein level in the present study.

2. Methods and materials

2.1. Animals

For all experiments, age and gender matched mice (3–4 months old, purchased from Binzhou Medical University Hospital Animal Facility) were used. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals and obtained ethics approval from Binzhou Medical University Hospital.

2.2. Drug administration

For intranasal injection of IL-17, mice were anesthetized with isoflurane. Mouse IL-17 (1 μg per mouse) (R & D System, Shanghai, China) resuspended in 50 μl sterile saline (0.9%) was injected into the nasal opening. Immediately after IL-17 injection, mice in the Dex-treatment group received an intraperitoneal injection of Dex (100 μl) at indicated doses.

2.3. Collection of the bronchoalveolar lavage fluid (BALF)

For the evaluation of pulmonary inflammation, PBS (0.8 ml) was used to collect BALF through the trachea 24 h after the treatment. The harvested samples was centrifuged at 4 °C for 10 min, then the precipitates, which contain the residual lung infiltrating cells, were collected with 1 ml PBS wash. Total cells were quantified, then stained with anti-Gr-1 and anti-CD11b in preparation for flow cytometry.

2.4. Histology

For the histological analysis, lung tissues were fixed in 4% paraformaldehyde immediately after the collection of BALF for subsequent embedment in paraffin. The samples were then cut into 400-μm-thick sections, and stained with hematoxylin and eosin (H & E).

2.5. Culture of mice lung epithelia cells

Primary cell culture was performed as previously described (Wang et al., 2005). In brief, two milliliters of dispase solution (3.6 unit/ml, Gibco, Shanghai, China) was instilled into the lungs through a tracheal catheter. The lungs were removed from mice and incubated in dispase solution at room temperature for 1 h. The lung tissues were microdissected in the dispase solution and the cell suspension was filtered through nylon monofilament and the recovered cells were resuspended in DMEM containing 10% FBS. The cells were incubated with rat anti-CD32/CD16 and anti-CD45 for 30 min at 4 °C, followed by incubation with anti-rat IgG micro beads and negative selection by Automacs. Cells were resuspended in DMEM containing 10% fetal bovine serum (FBS), 1% streptomycin-penicillin and 10 μM β-mercaptoethanol and seeded into 48-well plate at 1 × 105/well for overnight culture followed by various treatments.

Table 1
The gene-specific primers used for real-time RT-PCR.

Gene	Forward primer	Reverse primer
IL-6	CACAGAGGATACCACTCCCAACA	TCCACGATTTCCCAGAGAACA
TNF	CATCTTCTCAAAATTCGAGTGACA	CCAGCTGCTCCTCCACTTG
CXCL1	CTTGACCCTGAAGCTCCCTT	AGGTGCCATCAGAGCAGTCT
Actin	CGTGAAAAGATGACCCAGATCA	CACAGCCTGGATGGCTACGT

2.6. Quantitative real-time polymerase chain reaction (PCR)

Cells after different treatments were collect in TRIzol (Thermo, Shanghai, China) and first-strand cDNA was synthesized with a reverse-transcription kit (Thermo, China). Relative level of expression was assessed using an SYBR Green Real-Time PCR kit (Thermo, China). Data was normalized to the expression level of the reference gene, then standardized to the level of control group. Gene-specific primers are listed in Table 1.

2.7. Measurement of cytokine levels in the serum

Mice receiving various treatments were sacrificed 24 h after the IL-17 injection. Serum samples were immediately collected by centrifugation, and the concentrations of TNFα, IL-6, and CXCL1 in the supernatants were determined using ELISA kits (R & D Systems, China) according to the manufacturer's instructions.

2.8. Western blot

Lung tissues were harvested and snap-frozen by liquid nitrogen before homogenization. Protein concentration was assayed using the BCA protein assay kit. Samples (50 μg) were loaded and run for 2 h at 80 V, then transferred onto polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were blocked at room temperature for 2 h and washed with TBST. Primary antibodies specific to each target protein were incubated overnight with the membranes at 4 °C, followed by wash and then incubation with secondary antibodies at room temperature for 1 h. The amount of immunoactive proteins were determined by a Western blot detection kit.

2.9. Statistics

All results are presented as mean ± SD unless otherwise stated. Statistical significance between treatments was determined using one-way ANOVA followed by a Tukey's post hoc test. P values less than 0.05 were considered significant.

3. Results

In order to investigate the effect of Dex on IL-17 induced pulmonary inflammation, we first established the IL-17 mediated acute lung injury model in a similar manner as previously reported (Zhong et al., 2013). Histological analysis using H & E staining revealed marked pulmonary inflammation and tissue damage in the lung 24 h following intranasal administration of IL-17 (Fig. 1A and B). However, treatment with Dex immediately after IL-17 administration dose-dependently alleviated the acute lung injury, as evidenced by less infiltration of inflammatory cells and tissue morphology more similar to that of intact animals (Fig. 1C and D).

Since it has been well documented that stimulation with IL-17 could enhance recruitment of neutrophils at the site of inflammation, we next assessed the proportion of $Gr1^+$ $CD11b^+$ neutrophils in the lung infiltrating cells using flow cytometry analysis. The results indicated a