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Anti-inflammatory effects of Tat-Annexin protein on ovalbumin-induced airway inflammation in a mouse model of asthma

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ABSTRACT

Chronic airway inflammation is a key feature of bronchial asthma. Annexin-1 (ANX1) is an anti-inflammatory protein that is an important modulator and plays a key role in inflammation. Although the precise action of ANX1 remains unclear, it has emerged as a potential drug target for inflammatory diseases such as asthma. To examine the protective effects of ANX1 protein on ovalbumin (OVA)-induced asthma in animal models, we used a cell-permeable Tat-ANX1 protein. Mice sensitized and challenged with OVA antigen had an increased amount of cytokines and eosinophils in their bronchoalveolar lavage (BAL) fluid. However, administration of Tat-ANX1 protein before OVA challenge significantly decreased the levels of cytokines (interleukin (IL)-4, IL-5, and IL-13) and BAL fluid in lung tissues. Furthermore, OVA significantly increased the activation of mitogen-activated protein kinase (MAPK) in lung tissues, whereas Tat-ANX1 protein markedly reduced phosphorylation of MAPKs such as extracellular signal-regulated protein kinase, p38, and stress-activated protein kinase/c-Jun N-terminal kinase. These results suggest that transduced Tat-ANX1 protein may be a potential protein therapeutic agent for the treatment of lung disorders including asthma.

1. Introduction

Asthma is a chronic inflammatory disease of the lungs that is characterized by airway inflammation, airway hyperresponsiveness (AHR) and airflow obstruction [1,2]. The incidence of asthma has markedly increased worldwide to become one of the most common respiratory diseases [3]. New agents are needed for the treatment of asthma. In the majority of asthmatics, the response in the lungs involves excessive activation of Th2 cells, eosinophil infiltration, mucus over-production, and airway hyper-reactivity [1,3]. The cellular response in allergic airway inflammation is controlled by an abroad range of bioactive mediators, including immunoglobulin E (IgE), cytokines, and chemokines. In asthma, Th2 plays a central role and controls the allergic response through the production of cytokines such as interleukin (IL)-4, IL-5, and IL-13 [4–6]. IL-4 plays important roles in the infiltration of eosinophil into lung tissues, B cell maturation, and IgE synthesis. IL-5 is involved in eosinophil activation and synthesis. IL-13 plays a role in eosinophilic inflammation, mucus hypersecretion, and AHR [2,4,6–8]. Also, other pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and IL-6 are involved in asthma pathology in humans. The results suggest that the inhibition of these cytokines is clinically useful in the treatment of asthma [3,9,10].

The activation of mitogen-activated protein kinases (MAPKs) by inflammatory stimuli is important in immune cells. Three MAPKs – extracellular signal-regulated protein kinase (ERK), c-Jun terminal kinase (JNK), and p38 MAPK – have been studied in the transcriptional regulation of cyclooxygenase-2 expression [11–13]. MAPK activity is markedly increased in the lungs of asthmatic mice compared with normal mice, suggesting that the inhibition or regulation of the MAPK pathway could be a potential therapy for asthma [14,15].

Annexin-1 (ANX1) is a calcium-dependent phospholipid binding protein originally identified as a glucocorticoid inducible 37 kDa protein. ANX1 induced by glucocorticoids inhibits phospholipase activity and regulates diverse cellular functions such as

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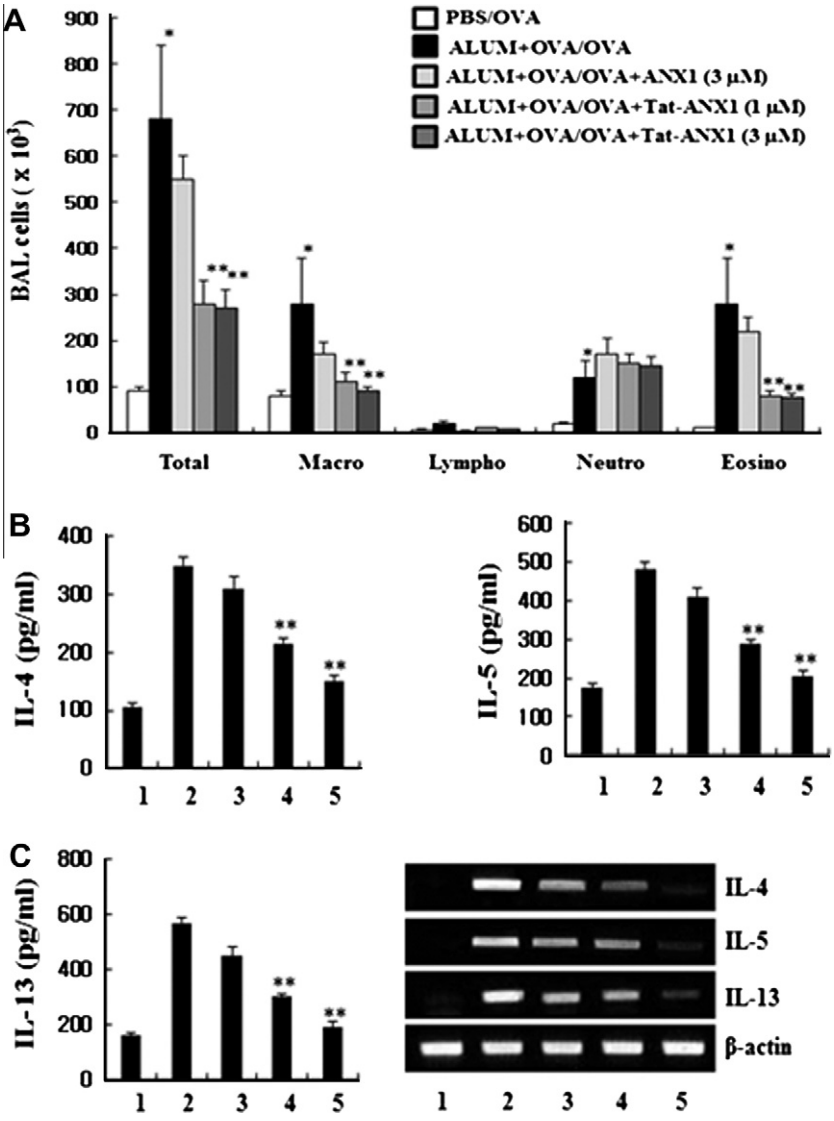

Fig. 1. Effect of Tat-ANX1 on OVA-induced inflammation. (A) Effect of Tat-ANX1 on recruitment of inflammatory cells in BAL fluid taken from mice 48 h after the last OVA challenge. Cells were isolated by centrifugation and stained with Diff-Quik stain reagent. Cell numbers were assessed by direct microscopic counting with a hemocytometer. $^{*}P < 0.05$ compared with control group, $^{**}P < 0.05$ compared with OVA-treated group. (B) Effect of Tat-ANX1 on the Th2 cytokine levels. BAL fluid samples were collected after the last OVA challenge and analyzed by ELISA. (C) Total RNA was extracted from the lung tissues. IL-4, IL-5, IL-13, and β-actin mRNA were analyzed by RT-PCR using specific primers. Lanes in panels A and B are as follows: lane 1, PBS treated control mice; lane 2, OVA-treated mice; lane 3, OVA + ANX1 (3 μM) treated mice; lane 4, OVA + Tat-ANX1 (1 μM) treated mice; lane 5, OVA + Tat-ANX1 (3 μM) treated mice. $^{**}P < 0.05$ compared with OVA-treated group.

cellular proliferation, anti-inflammatory effects, and cell differentiation [16–18]. Although ANX1 has been implicated as an anti-inflammatory therapeutic, the biological function and molecular mechanism of the ANX1 protein in inflammation are not clearly understood.

Protein transduction technology is the successful delivery of exogenous full-length fusion proteins into living cells *in vitro* and *in vivo* by protein transduction domains (PTDs) or cell penetrating peptides (CPPs) [19]. In previous studies, we demonstrated that various fusion proteins efficiently protect against cell death *in vitro* and *in vivo* using this technology [20–24]. In the present study, we show that the Tat-ANX1 protein significantly attenuates the pathogenesis in a mouse model of asthma as determined by a histological examination, cytokine enzyme-linked immunosorbent assay (ELISA), enumeration of cells in bronchoalveolar lavage (BAL), and serum IgE levels. The results support the suggestion that the Tat-ANX1 protein could be useful as a potential therapeutic agent for the treatment of inflammations including asthma.

2. Materials and methods

2.1. Purification of Tat-ANX1 fusion proteins

A Tat-ANX1 expression vector was constructed to express the Tat peptide as a fusion with human ANX1. Expression and purification of the ANX1 fusion proteins were carried out as described previously [24].

To produce the Tat-ANX1 proteins, a plasmid was transformed into *E. coli* BL21 cells and grown in 100 ml of LB medium at 37 °C to an optimal density at 600 nm value of 0.5–1.0 and induced with 0.5 mM isopropyl-β-D-thio-galactoside at 37 °C for 3–4 h. Harvested cells were lysed by sonication at 4 °C and clarified cell extracts were purified using a Ni^{2+}-nitrilotriacetic acid Sepharose affinity column (Qiagen, Valencia, CA, USA) and PD-10 column chromatography (Amersham, Piscataway, NJ, USA). The protein concentration was estimated using bovine serum albumin as a standard [32].