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Inhibition of high-mobility group box 1 in lung reduced airway inflammation and remodeling in a mouse model of chronic asthma

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ABSTRACT

The role of high-mobility group box 1 (HMGB1) in chronic allergic asthma is currently unclear. Both airway neutrophilia and eosinophilia and increase in HMGB1 expression in the lungs in our murine model of chronic asthma. Inhibition of HMGB1 expression in lung in ovalbumin (OVA)-immunized mice decreased induced airway inflammation, mucus formation, and collagen deposition in lung tissues. Analysis of the numbers of $CD4^+$ T helper (Th) cells in the mediastinal lymph nodes and lungs revealed that Th17 showed greater increases than Th2 cells and Th1 cells in OVA-immunized mice; further, the numbers of Th1, Th2, and Th17 cells decreased in anti-HMGB1 antibody (Ab)-treated mice. In OVA-immunized mice, TLR-2 and TLR-4 expression, but not RAGE expression, was activated in the lungs and attenuated after anti-HMGB1 Ab treatment. The results showed that increase in HMGB1 release and expression in the lungs could be an important pathological mechanism underlying chronic allergic asthma and HMGB1 might a potential therapeutic target for chronic allergic asthma.

1. Introduction

Allergic asthma is a chronic inflammatory disease; the clinical characteristics of asthma are chronic airway inflammation, airway hyperresponsiveness (AHR), airflow obstruction, and airway remodeling [1,2]. Allergen-triggered allergic asthma induces the activation of $CD4^+$ T helper 2 (Th2) cells and the release of Th2 cytokines, causing airway inflammation, IgE production and mucus production [3,4]. However, recent clinical and animal studies have shown that Th17 cells and neutrophils may be important for the pathological mechanism of chronic asthma, especially in severe asthma or steroid-resistant asthma. Wilson et al. found that airway sensitization with allergen primes modest Th2 cell responses and also primes strong Th17 cell responses that promote airway neutrophilia and acute AHR [5]. In asthma patients, IL-17A and IL-17F are upregulated, and the levels of IL-17 correlate with disease severity [6]. Th17 cells can directly secrete IL-8, which recruits and activates neutrophils, and IL-17, which activates airway epithelial cells, airway fibroblast cells, and endothelial cells to release IL-1, IL-6, IL-8, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF). These modulators indirectly induce grianulopoiesis, neutrophil chemotaxis, and antiapoptotic effects [7]. Activated neutrophils cause the upregulation and release of metalloproteinase, proinflammatory cytokines, elastase, lipid mediators, and reactive oxygen species (ROS) that might contribute to bronchoconstriction, bronchial hyperreactivity, mucus hypersecretion, and remodeling in asthma [8].

High mobility group box1 protein (HMGB1) was first found in 1973 as a nuclear protein that regulates transcription, replication, and DNA repair [9]. Recently, extracellular HMGB1 has been identified as a crucial cytokine involved in many inflammatory conditions such as endotoxemia and sepsis [10], intestinal inflammatory disorder [11], rheumatoid arthritis [12], stroke [13], and lung inflammatory diseases [14,15]. Receptors for HMGB1 include receptor for advanced glycation products (RAGE), Toll-like receptor (TLR) 4, TLR2, and TLR9 for the activation of inflammation, and CD24 and Siglec-G for the inhibition of inflammation [16,17]. The interaction of HMGB1 with RAGE, TLR2, and TLR4 transduces intracellular signals involving mitogen-activated protein kinases

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(MAPKs) and nuclear factor kappaB (NF-κB); thereby, it mediates cellular responses such as chemotactic cell movement [18] and the release of pro-inflammatory cytokines (*e.g.*, TNF and IL-1) *in vitro* and causes fever, epithelial barrier dysfunction, and acute inflammation *in vivo*.

A previous study found that HMGB-1 levels in induced sputum were significantly higher in asthmatic patients and were significantly correlated with the percentage of neutrophils in induced sputum, compared to healthy people [19]. Furthermore, HMGB1 has been associated with the pathogenesis of experimental acute asthma [20]. However, the effects and mechanisms of HMGB1 in chronic asthma are still unclear. Asthma is a chronic inflammatory airways disease which involved genetic factors [21] and environmental factors [22] for development. There are several differences in pathological mechanisms between acute and chronic airway inflammation [23]. The acute inflammatory response is limited to the proximal airways and is not associated with tissue remodeling [24]. The cytokine profile of the lungs also varies with the number of allergen challenges [25] and increase in IFN-γ release in BALF in chronic asthma animal model [26]. Treatment of chronic asthma remains highly complex and problematic, especially severe asthma. Previous studies have demonstrated that corticosteroids have little or no effect on airway remodeling [27,28]. Investigation of the clear mechanisms and development of novel therapeutic approaches targeting airway remodeling and chronic airway inflammation in asthma are necessary. Therefore, in this study, we examine the role of HMGB1 in airway inflammation and remodeling in a murine model of chronic asthma. We found that inhibition of HMGB1 showed potential therapeutic effects in chronic asthma.

2. Methods

2.1. Animals and experimental protocol

Female BALB/c mice aged 6 weeks were obtained from the National Laboratory Animal Center (Taiwan). The animal care and handling protocols were approved by the Animal Committee of China Medical University. The animal study was modified based on a previous study [29]. As shown in Fig. 1A, mice were intraperitoneally sensitized by a 50-μg injection of OVA from Sigma Chemical (St. Louis, MO, USA), dissolved in 100 μL phosphate-buffered saline (PBS) and emulsified in 100 μL PBS containing 2 mg aluminum hydroxide (AlumImuject; Pierce Chemical, Rockford, IL, USA) on days 0, 10, and 20. Removed endotoxin from OVA by endotoxin removal resin according to the manufacturer's instructions

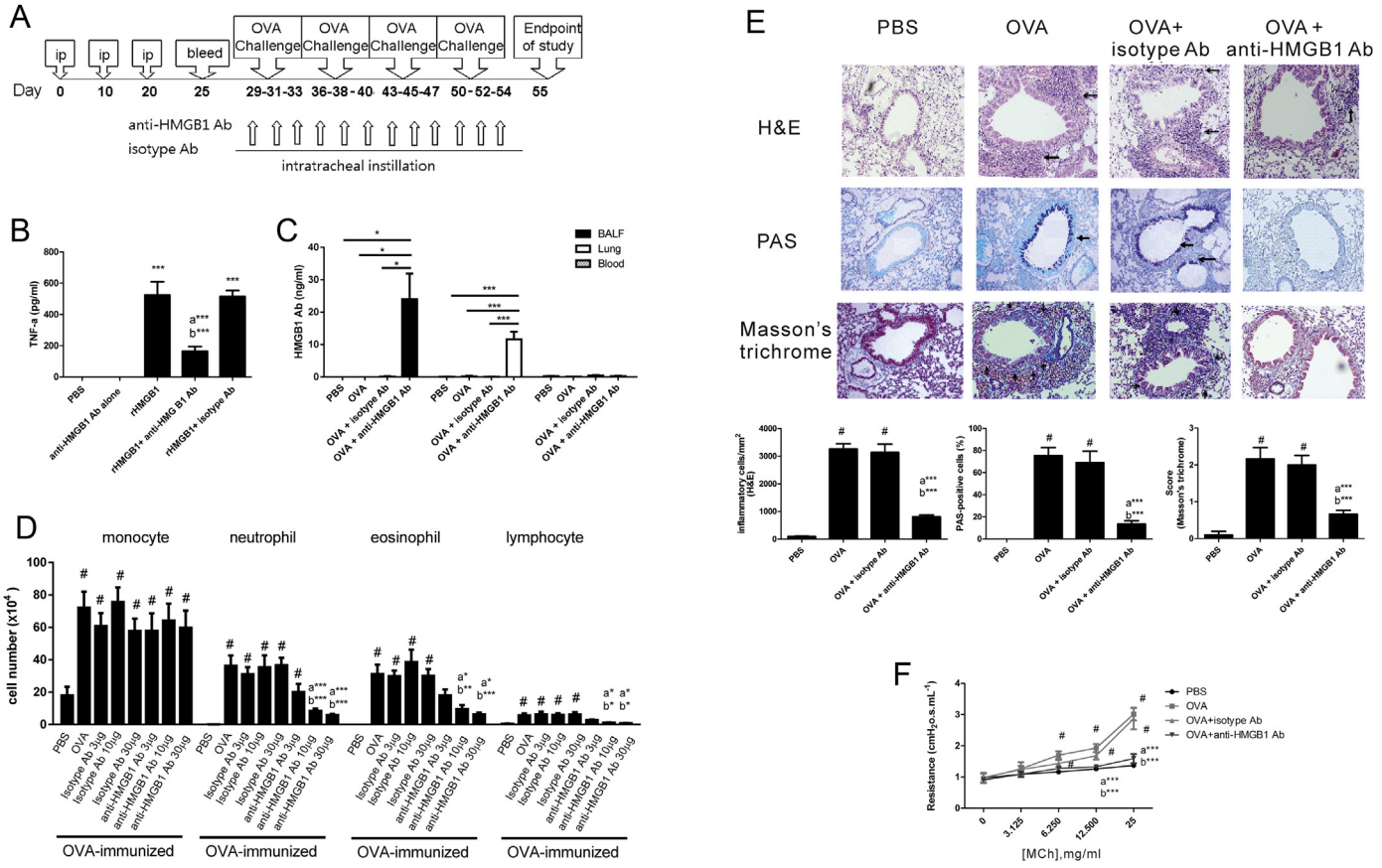

Fig. 1. Inhibition of symptoms of chronic allergic asthma after i.t. instillation of anti-HMGB1 Ab. (A) Brief scheme of animal sensitization and challenge with OVA. The PBS group represents mice injected intraperitoneally with and exposed to PBS only. i.p., intraperitoneal; i.t., intratracheal. (B) RAW cells were pretreated with 2 μg/mL anti-HMGB1 Ab for 1 h and then stimulated with 1 μg/mL recombinant human HMGB1 (rHMGB1) for 3 h. The cell culture medium was collected for detection of TNF-α by ELISA. Data are expressed as mean ± SEM values (n = 3). $^{***}p < 0.001$, compared with the PBS group. a$^{***}p < 0.001$, compared with the rHMGB1 group. b$^{***}p < 0.001$, compared with the rHMGB1 + isotype Ab group. (C) The expression of HMGB1 Ab in BALF, lung tissue, and serum were detected by ELISA. Data are expressed as mean ± SEM values (n = 10). $^{*}p < 0.05$ and $^{***}p < 0.001$, compared between groups. (D) Total cell counts were determined from 3 mL of BALF, and differential cell counts were assessed by Wright and Giemsa staining. Data are expressed as the mean ± SEM values (n = 10). (E) Lung sections were stained by H&E, PAS, and Masson trichrome staining. Quantification of the numbers of H&E-stained cells and PAS-stained cells, and the score of the Masson trichrome staining are presented in bar graphs. Data are expressed as mean ± SEM values (n = 6). Black arrows indicate inflammatory cells in H&E, mucus in PAS and collagen deposition in Masson-trichrome-stained lung tissue. (F) Airway resistance as measured by invasive body plethysmography. Data are expressed as the mean ± SEM values (n = 6). #$p < 0.05$, compared with the PBS control. #$p < 0.05$, compared with the PBS control. a$^{*}p < 0.05$, a$^{***}p < 0.001$, compared with the OVA group. b$^{*}p < 0.05$, b$^{**}p < 0.01$, b$^{***}p < 0.001$, compared with the OVA + isotype Ab group.