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Inhibition of BET bromodomains restores corticosteroid responsiveness in a mixed granulocytic mouse model of asthma

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ABSTRACT

Asthma is a heterogeneous disease characterized by different endotypes/phenotypes. Th2/Th17 driven mixed granulocytic asthma is one of them and shows resistance to corticosteroid therapy. Bromodomain and extra-terminal (BET) proteins are required for differentiation of Th17 cells which play a pivotal role in neutrophilic inflammation. Therefore, we sought to characterize the differential effects of BET inhibitor versus corticosteroids, and their potential synergism in cockroach allergen extract (CE)-induced mixed granulocytic (eosinophilic and neutrophilic) mouse model of asthma having Th2/Th17 endotype. Effects of BET inhibitor, (+)JQ-1 alone and in combination with dexamethasone (Dexa) were assessed on airway inflammation as well as Th2/Th17 related airway immune responses in CE-induced mixed granulocytic asthma. Markers of steroid resistance [histone deacetylase 2 (HDAC2), and oxidative stress] were also assessed in the lungs of mice and primary human bronchial epithelial cells (HBECs). BET inhibitor, (+)JQ-1 abolished Th17 driven neutrophilic inflammation in CE-induced mixed granulocytic asthma. Dexa had limited effect on overall airway inflammation despite having significant reductions in Th2 driven immune responses. However, combination of (+)JQ-1 with Dexa completely blocked both Th2 and /Th17 driven immune responses in the lung which led to significant reductions in eosinophils, neutrophils, and mucin secretion. (+)JQ-1 also reversed CE- and IL-17A-induced decrease in HDAC2 expression in murine and human airway epithelial cells respectively.

1. Introduction

Asthma is a chronic lung disease that occurs due to inflammation of the airways and is associated with airway remodeling, mucous cell metaplasia, and increased airway reactivity [1,2]. Indoor allergens originating from cockroach and house dust mite contribute significantly towards the pathogenesis of asthma through mucosal sensitization [3–5]. The prevalence of asthma has increased significantly over the past few decades globally and numbers suggest that it afflicts more than 300 million people worldwide which could go up to 400 million by 2025 due to increase in urbanization [6,7].

Asthma manifests itself with different patterns of airway inflammation that cannot be explained by the Th2 immune response alone. Recent studies in asthmatic patients and animal models have shown that in response to an allergen, there is development of not only Th2 immune response but also Th17 immune response in the lung, both of which contribute significantly towards the development of airway inflammation [8–11]. Th2-driven airway inflammation is mostly associated with eosinophils, whereas Th17 driven airway inflammation is usually associated with neutrophils [9,12,13]. Based on this, asthma has been classified into different endotypes/phenotypes depending upon the infiltration of leukocytes into the lung, e.g. Th2/eosinophilic, Th17/neutrophilic, and Th2/Th17 with mixed neutrophils and eosinophils [11,14–16]. The lowest lung function is found in asthmatic patients who have increased mixed granulocytes (both eosinophils and neutrophils) in their airways. They also have worse control of asthma and increased exacerbations [1,15–18]. Cockroach allergen extract (CE)-induced asthma has been shown to have mixed granulocytic airway inflammation which is c cytokines such as IL-4 and IL-17A respectively [8,10,19].

Post-translationally acetylated histones can affect the inflammatory gene expression by altering the chromatin structure through

Abbreviations: BAL, bronchoalveolar lavage; BET, bromodomain and extra-terminal; CE, cockroach extract; i.n., intranasal; STAT3, signal transducer and activator of transcription 3; RORC, retinoic acid receptor-related orphan receptor C; HDAC, histone deacetylase

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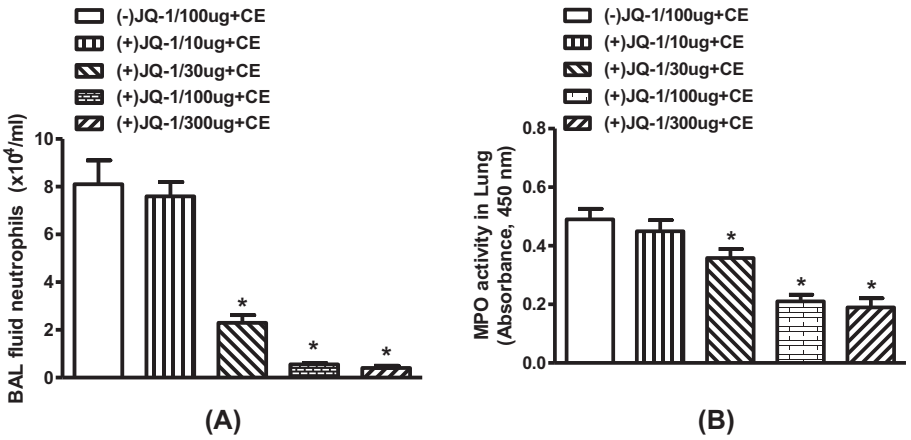

Fig. 1. Effect of BET inhibitor, (+)JQ-1 on CE-induced (A) neutrophilic influx in BAL, (B) lung MPO activity. Values are expressed as mean ± SEM, $n = 10$/group from two independent experiments. *$P < 0.05$, *vs.* (−)JQ-1/100 + CE group.

employment of histone modifiers known as readers of histone code/language [20–21]. One such family of readers for the acetylated histones is grouped under bromo- and extra-terminal (BET) bromodomain family. This family consists of Brd2, Brd3, Brd4, and BRDT members. BET family controls the assembly of chromatin complexes which are dependent on histone acetylation for inflammatory gene transcription. BET inhibitors such as (+)JQ-1 /IBET-762 initially showed efficacy in cancer and sepsis models [22,23]. However, recent studies have shown that inhibition of BET proteins in autoimmune/inflammatory disease models may also be an effective treatment option [24–27].

The discovery of Th17 cells whose differentiation is dependent on BET proteins, has added an additional layer of complexity to the regulation of airway inflammation [27]. In asthmatic patients, IL-17 expression is increased in the lungs, sputum, bronchoalveolar lavage (BAL) fluids, or sera, and the severity of airway hypersensitivity in patients correlates with IL-17 expression level [28,29]. Th17-derived cytokines, such as IL-17A not only regulate neutrophilic inflammation, but also enhances Th2 airway responses [30,31]. Usually Th17 driven airway inflammation is resistant to steroid therapy, however recent studies have shown efficacy of BET inhibitors in steroid resistant asthma [32,33]. Other studies have shown *in vitro* that BET inhibition could be an effective strategy in combating airway remodeling of asthmatic patients through down-regulation of inflammatory gene expression [34] However, the effect of BET inhibition on CE-induced mixed granulocytic airway inflammation has not investigated until now.

In the current study, we hypothesized that BET inhibition by blocking Th17 driven airway inflammation would be able to restore corticosteroid responsiveness in mixed granulocytic asthma. To test this hypothesis, we utilized CE-induced mixed granulocytic mouse model of asthma which has some features of difficult-to-control human asthma, i.e. mixed Th2/Th17 endotype and mixed neutrophilic/eosinophilic phenotype [8,11,15,35]. Using this model, we show for the first time that BET inhibitor, (+)JQ-1 abolishes CE-induced Th17 immune responses in the lung with concomitant reductions in airway inflammation, and mucus secretion. Further, BET inhibition restores corticosteroid sensitivity through blockade of Th17-driven airway inflammation. Our study suggests that a combined therapy of BET inhibitor with corticosteroids might be beneficial in difficult-to-treat mixed granulocytic airway inflammation.

2. Materials and methods

2.1. Animals

The current study utilized male Balb/c mice (8–10 weeks of age; 20–25 g), free of specific pathogens. These mice were housed under standard laboratory conditions such as 12-h light-dark cycle and surround temperature of 24–26 °C. The mice utilized in this study were provided by The Experimental Animals Center, College of Pharmacy, King Saud University. The research protocol for usage of mice in this study was approved by The Animal Care and Research Committee of College of Pharmacy, King Saud University.

2.2. Cockroach allergen extract (CE)-induced mixed granulocytic asthma model

Sensitization was performed according to the protocol described earlier by us with some modifications [3,10,19]. Mice were sensitized on days 1, 2, 3, 4, and 5 with intranasal (i.n.) administrations of 50 µg whole-body German cockroach (Blattella germanica) extract (CE) from Greer Laboratories (Lenoir, NC) under light anesthesia. Ten days after 1st sensitization, the mice were challenged i.n. under light anesthesia with 50 µg CE in 20 µl saline twice daily (morning and evening) on days 11, 12, 13, 14, 15, 16, and 17. Non-sensitized control animals received only saline with the same volumes.

To assess the role of BET bromodomains on CE-induced airway immune responses, a BET bromodomain inhibitor, (+)JQ-1 (Tocris, UK) was administered i.n. once every day before morning CE challenge at a concentration of 100 µg/mouse (dissolved in 10 µl dimethylsulfoxide) for 7 days. (−)JQ-1 (Tocris, UK) was used as an optically inactive enantiomer as a background control at a concentration of 100 µg/mouse (Fig. 2A). To assess the efficacy of corticosteroid therapy in this model, dexamethasone (Dexa) was also administered i.n. once every day alone or in combination with (+)JQ-1 before morning CE challenge at a concentration of 25 µg/mouse for 7 days (Fig. 4A).

Effect of IL-17A and neutrophils on histone deacetylase 2 expression in CE mixed granulocytic asthma was assessed by their respective depletion using antibodies against them. Mice were injected i.p. with rat anti-mouse Ly6G mAb, clone 1A8 (250 µg/200 µl/mouse, Biolegend, USA) or anti–IL-17A antibody (150 µg/200 µl/mouse, Biolegend, USA) or isotype control antibody (Biolegend, USA), 1 hr before administration of intranasal CE or saline on alternate days, i.e. on day 11, 13, 15 and 17.

2.3. Bronchoalveolar lavage (BAL)

The mice were sacrificed by isoflurane anesthesia and the trachea was cannulated to perform BAL 12 h after the final allergen challenge. A differential count of at least 300 cells was carried out as described previously [3,10,19].