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Spleen tyrosine kinase inhibition ameliorates airway inflammation through modulation of NLRP3 inflammosome and Th17/Treg axis

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ABSTRACT

Repeated exposure to the fungal pathogen *Aspergillus fumigates* triggers spleen tyrosine kinase (SYK) signalling through dectin-1 activation, which is associated with deleterious airway inflammation. β-Glucan-induced dectin-1 signalling activates the NLRP3 inflammasome, which in turn rapidly produces IL-1β, a master regulator of inflammation. IL-1β expression results in Th17/Treg imbalance, pulmonary inflammation, and bystander tissue injury. This study reports that 3,4 methylenedioxy-β-nitrostyrene (MNS), a potent SYK inhibitor, markedly decreased the expression of pro-inflammatory cytokines and increased the expression of anti-inflammatory cytokines *in vitro*. Furthermore, SYK inhibition markedly decreased β-glucan-induced IL-1β expression, suggesting that SYK is indispensable for NLRP3 inflammasome activation. Decreased IL-1β expression correlated with reduced Th17 response and enhanced immunosuppressive Treg response. Notably, SYK inhibition ameliorated inflammation caused by repeated intranasal β-glucan challenge in BALB/C mice. SYK inhibition also restored the Th17/Treg balance *via* decreased Th17 and increased Treg responses, as evidenced by decreased IL-17 and *ror*-γ levels. Additionally, inhibition of SYK increased IL-10 secreting $CD4^+$ $FOXP3^+$ T cells that accompanied reduced T cell proliferation. Decreased IgA in the Bronchoalveolar lavage (BAL) fluid and serum also indicated the immunosuppressive potential of SYK inhibition. Histopathology data revealed that repeated β-glucan challenge caused substantial pulmonary damage, as indicated by septal thickening and interstitial lymphocytic, neutrophil and granulocyte recruitment. These processes were effectively prevented by SYK inhibition, resulting in lung protection. Collectively, our findings suggest that SYK inhibition ameliorates dectin-1- mediated detrimental pulmonary inflammation and subsequent tissue damage. Therefore, SYK can be a new target gene in the therapeutic approach against fungal induced airway inflammation.

1. Introduction

Opportunistic fungi are clinically harmful pathogens, especially in immunocompromised or nosocomial patients, where the fungi may cause life-threatening infections. In particular, fungi and fungal allergens cause allergic airway diseases such as allergic asthma [1]. Past studies have suggested that exposure to β-glucan, the main cell wall component of a variety of fungi, contributes to the development of asthma [2]. Dectin-1, constitutively expressed on the innate immune cells, recognizes β-glucan and leads to initiation of phagocytosis and the inflammatory response [3]. Spleen tyrosine kinase (SYK) is an immunoregulatory kinase that operates downstream of dectin-1. SYK is coupled to immune receptor tyrosine-based activation motif (ITAM), which turns on CARMA1-related adaptor protein (CARD9) to activate NF-κB and the NLRP3 inflammosome [4]. SYK plays an indispensible role in the activation of immune cells as well as in lymphocyte development. SYK is expressed in immune cells and in non-hematopoietic cells such as human fibroblasts, breast epithelium, and hepatocytes [5]. SYK is a crucial player in diverse biological functions [6]. Previous studies have shown that SYK overexpression induces inflammation, allergy and autoimmunity [6]. SYK activation triggers a sequelae of cytokines, including the release of IL-1β. Although IL-1β is required to combat the invading pathogen, its excessive and chronic secretion is responsible for a number of inflammatory disorders, including asthma.

Repeated inhalation of *Aspergillus fumigatus* (*A. fumigatus*) conidia can trigger the host immune response. Generally, healthy individuals can eradicate these conidia without developing deleterious inflammation. However, the conidia pose a problem in patients with immunodeficiencies, which leads to allergic asthma. Allergic asthma pathogenesis involves dysregulated inflammation and aberrant activation

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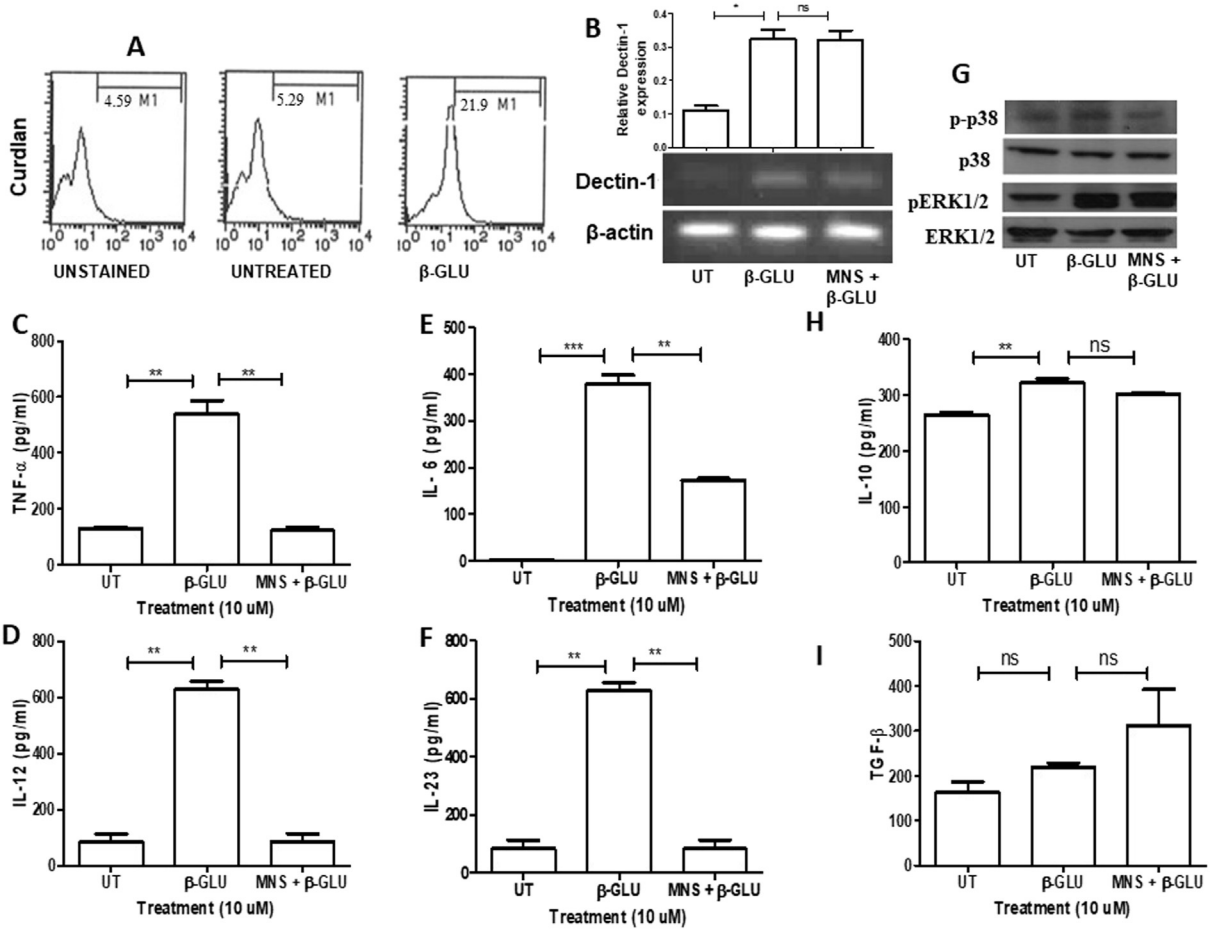

Fig. 1. SYK inhibition dampens β-glucan-induced inflammation in BMDCs. BMDCs were treated with or without β-glucan for 18 h. Surface dectin-1 expression was assessed by flow cytometry (A). BMDCs were treated with or without SYK inhibitor for 30 min, then stimulated with β-glucan for 6 h. Dectin-1 mRNA levels were assayed by semiquantitative RT-PCR, and β-actin was used as an endogenous control (B). (C–H) BMDCs were pre-treated with or without SYK inhibitor for 30 min, then stimulated with β-glucan for 24 h. Proinflammatory cytokines TNF-α and IL-12 (C and D) and Th17 polarizing cytokines IL-6 and IL-23 were analyzed using ELISA (E and F). Phosphorylation of ERK 1/2 and p38 levels were assayed using Western blot (G). Anti-inflammatory cytokines IL-10 and TGF-β were also assayed using ELISA (H and I). Data are representative of a minimum of three independent experiments (*$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; ns = non-significant).

of innate immune cells (such as dendritic cells, macrophages and neutrophils), which subsequently cause tissue damage. The release of IL-1β promotes differentiation of pathogenic IL-17-producing $CD4^+$ T cells (Th17), which contribute to inflammation and pulmonary damage [7]. Concurrently, regulatory T cells (Treg) inhibit the inflammatory Th17/Th1 response and are critically involved in the limitation of chronic inflammation and the development of tolerance [8,9]. The Th17 response is necessary for early clearance of the fungal pathogen [9], but repeated fungal challenge induces inflammation and persistent activation of Th17 cells, which results in neutrophil recruitment and collateral tissue damage [10]. Restoration of the Th17/Treg balance is critical for protection against inflammation-mediated self-perpetuating tissue damage [11]. Therefore, it is worth exploring the regulation of the SYK-inflammasome pathway as it relates to the modulation of the Th17/Treg axis and deleterious inflammation. We have investigated the role of SYK in the context of inflammasome activation and maintenance of the Th17/Treg axis. Using MNS (3,4-methylenedioxy-β-nitrostyrene), a cell-permeable and selective inhibitor of SYK kinase activity [12], our study aims to answer the following key questions: Does SYK inhibition (i) manipulate β-glucan-induced dectin-1 expression and inflammatory response? (ii) regulate β-glucan-induced NLRP3 inflammasome activation? (iii) modulate β-glucan-induced Th17/Treg balance? and (iv) protect against pulmonary inflammation and pathogenic T cell modulation caused by repeated inhalation of β-glucan?

2. Materials and methods

2.1. Materials

β-Glucan (Sigma-Aldrich, St. Louis, Missouri, USA), 3,4-Methylenedioxy-β-nitrostyrene (MNS, SYK inhibitor, PubChem CID: 672296, Cayman Chemical, Michigan, USA), anti-dectin-1 PerCP-eFluor710 antibody (eBioscience, San Diego, CA, USA), anti-NLRP3 antibody (IMGENEX, San Diego, CA, USA), anti-caspase-1 antibody (Santa Cruz Biotechnology, Dallas, Texas, USA), anti-GAPDH antibody (Life Technologies, Carlsbad, CA, USA) and enhanced chemiluminescence (ECL) kit (BioRad, Hercules, CA) were used in this study. All ELISA kits, the $CD4^+$ T cell enrichment kit, and the Treg staining kit were purchased from eBioscience. Recombinant (r) GM-CSF, IL-4 and TNF-α were obtained from BD Biosciences, San Diego, CA, USA. All the other reagents were obtained from Life Technologies (Carlsbad, CA, USA) unless noted otherwise.

2.2. Preparation of bone marrow-derived dendritic cells (BMDCs)

BALB/c mice were used for the study. Animal studies were approved by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) at B.V. Patel PERD Centre, Ahmedabad, India. Bone marrow-derived dendritic cells (BMDCs) were prepared as described previously