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IL-21 alleviates allergic asthma in DOCK8-knockout mice

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ABSTRACT

Patients with DOCK8 deficiency are at increased susceptibility to develop allergic diseases such as food allergy and asthma. Here, we aimed to analyze the pathogenesis of asthma in DOCK8-deficient patients. In our mouse model, DOCK8-knockout (KO) mice sensitized with low-dose OVA were challenged with 1.5% OVA to induce allergic asthma. As compared to that in WT mice, remarkable airway hyperresponsiveness was observed in KO mice. Increased inflammatory cells and eosinophils infiltrated in airway lumen in KO mice especially around bronchi. KO mice showed higher levels of serum IgE and OVA-specific IgE and significantly elevated IgE-producing B cells in blood and in spleen. Surprisingly, nasal administration with rmIL-21 significantly reduced the airway hyperresponsiveness, inflammatory infiltration, as well as the serum IgE and IgE-producing B cells. DOCK8-knockout mice are susceptible to low-dose OVA induced allergic airway inflammation and airway hyperresponsiveness. Supplementary nasal administration of rmIL-21 alleviates allergic asthma in this mouse model.

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1. Introduction

DOCK8 immunodeficiency syndrome (DIDS) is caused by autosomal recessive loss-of-function mutation in the DOCK8 gene, which is characterized by recurrent infections, severe atopy, and predisposition to cancers [1–5]. As with hyper-IgE syndrome (HIES), other combined immunodeficiencies, such as the Wiskott-Aldrich syndrome, can be associated with eczematous rash, infection, and increased IgE level. However, as far as we know, only DOCK8 deficiency is typically associated with asthma and severe allergies including anaphylaxis to food allergens. Epidemiological investigation shows that, in the United States, the prevalence of asthma was 7.8% in 2015 [6]. However, more than 30% of DIDS patients develop reactive airway disease characterized as asthma indicating these patients are more susceptible to exert allergic asthma [1–3].

Moreover, despite the elevation of IgE associated with HIES, there were no cases of anaphylaxis observed among the patients with Job syndrome, one of the hyper immunoglobulin E syndrome (HIES) conditions caused by autosomal dominant STAT3 mutation [7]. DOCK8-deficient AR-HIES but not TYK2-deficient AR-HIES shows potential to develop anaphylaxis and asthma, indicates that DOCK8 gene plays a critical role in the development of

Abbreviations: rmIL-21, recombinant mouse interleukin-21; **DOCK8**, dedicator of cytokinesis 8; **STAT3**, signal transducer and activator of transcription 3; **TYK2**, tyrosine kinase 2; **DIDS**, DOCK8 immunodeficiency syndrome; **GEFs**, guanine nucleotide exchange factors; **Cdc2**, cell division cycle 42; **Rac1**, ras-related C3 botulinum toxin substrate 1; **HIES**, hyper-IgE syndrome; **AR-HIES**, autosomal recessive hyper-IgE syndrome; **CID**, combined immunodeficiency disease; **OVA**, ovalbumin; **BALF**, bronchoalveolar lavage fluid; **PBMCs**, peripheral blood mononuclear cells.

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anaphylaxis and allergic asthma [8]. However, the mechanism responsible for pathogenesis of asthma in DIDS has not yet been well established.

Additionally, there are lines of evidence that environmental allergens, such as soybean dust [9], *Alernaria* [10] and rye grass pollen [11], are an important cause of persistent asthma and asthma exacerbations [12,13]. Recent study provided evidence of a linear dose response relationship between allergen concentrations and asthma morbidity among allergen-sensitized children and adolescents [14]. Low-dose allergen challenge could not induce asthma exacerbation compared to high-dose challenge in asthmatic patients [15]. Thus, the dose of sensitization and challenge is an important factor to determine whether an asthma subject could present acute attack or exacerbation. Due to the increased serum IgE related to asthma [16,17], we hypothesized that DOCK8-knockout mice might tent to develop allergic asthma with low-dose sensitization and challenge.

Interleukin-21 (IL-21), produced by various kinds of cells, including activated $CD4^+$ T cells, Th2 cells, Th17 cells, follicular helper T cells and natural killer T cells [18-22], is a type I cytokine essential for immune cell differentiation and function [23]. In B cells, IL-21 promotes plasma cell differentiation [24,25], and in combination with IL-4, drives IgG1 and IgG3 class switch [25,26]. Tangye et al. found defects of IL-21 expression in DOCK8-deficient memory $CD4^+$ T cells ex vivo [27]. IL-21 produced by memory $CD4^+$ T cells is one of the main drivers of human B-cell activation, proliferation, and differentiation [28]. These reduced IL-21-producing T cells might affect the development of allergic asthma.

Thus, the goals of our present study were to determine whether low-dose ovalbumin could induce allergic asthma in DOCK8-knockout mice and whether nasal administration of rmIL-21 attenuates the allergic asthma.

2. Material and method

2.1. Mice

DOCK8-knockout (KO) mice were backcrossed to C57BL/6 background were purchased from Shanghai Biomodel Organism Science & Technology Development Co.Ltd. All mice were housed in individual filtered cages and used between 6 and 8 weeks of age. Cages, bedding, feeds without chicken ovalbumin, and water were sterilized before use. The room was maintained the temperature at 23 °C, and a 12-h on, 12-h off light cycle. The animal experiments were approved by the Experimental Animal Committee at Chongqing Medical University.

2.2. OVA-induced allergic asthma model and nasal administration of recombinant IL-21

OVA-induced allergic asthma was elicited by i. p. Sensitization with 5 μg chicken ovalbumin (OVA; Sigma-Aldrich, St Louis, MO, US) emulsified in 200 μl Imject Alum (Thermo Fisher Scientific, Waltham, MA, USA) on day 0, followed by two oropharyngeal aspiration challenges with 1.5% OVA dissolved in 50 μl PBS on day 14 and 21. Control mice received only PBS. Mice were harvested 72h after the second challenge. This exposure method referring to previous study was modified [29]. Some groups of mice were administered 20 ng of rmIL-21 (R&D Systems) into the nostrils daily on days 15–17, whereas other groups of mice were not treated with the cytokine (WT-OVA or KO-OVA). This three day protocol was decided by experiments in time gradient (Fig. S1). Dose of rmIL-21 was decided referring to previous study [30]. As a negative control, groups of mice received neither sensitization nor challenge treatment, except for the last challenge on day 21 (WT-con or KO-con).

2.3. Pulmonary function assessment

On day 24 or 39 (3d or 18d after the second OVA challenge), mice were anesthetized with 2% sodium pentobarbitaland their tracheas were cannulated via tracheostomy. Increasing doses (0, 3.125, 12.5, 25, 100 mg/ml)of aerosol acetyl-β-methylcholine chloride challenges were administered by nebulization with a nebulizer (EMKA Technologies, Paris, France). Data were plotted as lung resistance and compliance atbaseline and in response to a 10-s challenge of acetylcholine by plethysmography.(EMKA Technologies, Paris, France).

2.4. Cell counts in BALF

Mice were anesthetized with 2% pentobarbital (40μl/10 g body weight intraperitoneally) on day 24, and the trachea was surgically exposed, cannulated, and the lung was lavaged six times with 0.5 ml ice-cold PBS to obtain bronchoalveolar lavage fluid (BALF). The total number of cells in BALF was counted. The BALF was centrifuged, and the cell pellet was used to prepare slides for differential cell counting. Cytospin slides were fixed and stained with DiffQuik (Baxter Healthcare Corp, Deerfield, Miami, FL) for leucocyte counts, and the numbers of monocytes, lymphocytes, neutrophils, and eosinophils on each slide in a total of 200 cells were counted.

2.5. Lung histopathology

The lung tissue was fixed in 10% (v/v) neutral buffered formalin for 24 h, embedded in paraffin, cut into sections of 4 μm thickness and stained with H&E solution (hematoxylin, Sigma MHS-16; and eosin, Sigma HT110-1-32). The tissues were subsequently mounted and covered with coverslips using Dako-mounting medium (Dakocytomation, Denmark, CA). The degree of infiltration of airway inflammtory cells was scored in a double-blind screening by two independent investigators. Peri-bronchiole and peri-vascular inflammation were evaluated using a scoring system of 0–4, where 0 represented no cells; 1, a few cells; 2, a ring of inflammatory cells 1 cell layer deep; 3, a ring of inflammatory cells 2-4 cells deep; and 4, a ring of inflammatory cells >4 cells deep.

2.6. Measurement of total IgE, OVA-specific IgE and IL-21 in serum by ELISA

Serum IgE and OVA-specific IgE levels were determined using commercial ELISA kits purchased from Ray Biotech and Biolegend respectively according to the manufacturer's instructions.

2.7. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Axis-Shield PoC AS, Oslo, Norway) density gradient centrifugation and washed twice with phosphate-buffered saline (PBS). Spleen samples were mechanically disrupted in a 70-μm nylon strainer to obtain single-cell suspensions. Suspended 2×10^6 PBMCs and spleen cells in each tube were incubated with anti-B220 and anti-IgE for 30 min in darkness. 20000 events were acquired in terms of total B cells. Acquired data was analyzed by CellQuest software (Becton Dickinson).

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