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Intestinal dysbacteriosis potentiates ovalbumin-induced allergic airway inflammation by inhibiting microRNA-130a to upregulate tumor necrosis factor α

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ABSTRACT

Allergic airway diseases (AAD), including chronic disorders such as allergic rhinitis, are resulted from complicated immunological interactions. Intestinal dysbacteriosis (ID) has been implicated in immune response to respiratory infections. We aimed to investigate the effect of ID on a mouse model of AAD, and the potential molecular factors involved. Ovalbumin (OVA) was employed to sensitize and challenge mice to elicit allergic inflammation in the upper as well as the lower airways. OVA-induced AAD model mice and control mice were raised with or without antibiotics treatment to establish the combinational AAD + ID mouse model. Characteristic symptoms of AAD were evaluated in regard to allergic symptoms, serum OVA specific IgE level, as well as inflammation cells, cytokines and microRNA expression profile in nasal lavage fluid (NALF) and bronchoalveolar lavage fluid (BALF). In AAD mice, ID caused increased nasal rubbing, sneezing, serum OVA specific IgE level and pro-inflammatory cytokine tumor necrosis factor α (TNF-α) in NALF and BALF. ID also inhibited microRNA-130a of AAD mice. Further molecular experiments indicated that microRNA-130a could specifically target and repress TNF-α. ID increases the susceptibility to AAD and allergic inflammatory response, possibly by inhibiting microRNA-130a to upregulate TNF-α.

1. Introduction

Allergic airway diseases (AAD), such as asthma and allergic rhinitis, are reversible chronic atopic disorders as a result of complicated immunological interactions between environmental factors and genetic susceptibility [1]. Recently, a growing body of evidence demonstrates that the upper and lower airways share common pathological mechanisms underlying the proliferation of allergen specific T-helper (Th) 2 lymphocyte associated with excessive interleukin (IL)-4 and other Th2 cytokines [2,3]. Th2-like immune activities also include goblet cell metaplasia in sub-epithelial mucosa, eosinophil inflammation, and up-regulation of allergen specific immunoglobulin (Ig) E in the serum, all of which synthetically contributing to allergic airway inflammation [4]. During the past decades, there has been a dramatic increase of the prevalence of AAD around the globe, drawing worldwide attention concerning public health [1]. Meanwhile, substantial disparities exist between developed and developing countries, and between urban and rural areas in the same country, regarding the prevalence of asthma and allergic rhinitis [5].

Tumor necrosis factor α (TNF-α), a pro-inflammatory cytokine in systemic inflammatory responses, is one of the cytokines responsible for the acute phase reaction [6]. It is mainly produced by activated macrophages, but it can also be generated by several other types of cells such as NK cells, $CD4^+$ lymphocytes, neutrophils, neurons, mast cells, and eosinophils [7]. The primary function of TNF is to regulate immune cells. As an endogenous pyrogen, TNF can induce apoptotic cell death, fever, cachexia, and inflammation, inhibit viral replication and tumorigenesis, as well as respond to sepsis via interleukin (IL)-1 and IL-6 producing cells [6]. Dysregulation of TNF is implicated in various human diseases including AAD. For instance, it is required for Toll-like receptor (TLR) ligand-mediated allergic airway inflammation [8]. In murine AAD models, TNF-α was found to be critical for respiratory

Abbreviations: AAD, allergic airway diseases; ID, intestinal dysbacteriosis; OVA, ovalbumin; NALF, nasal lavage fluid; BALF, bronchoalveolar lavage fluid; TNF-α, tumor necrosis factor α

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syncytial virus-induced exacerbations [9], and blocking TNF-α could stabilize local airway hyper-responsiveness during TLR-induced exacerbations [10]. The TNF-related apoptosis-inducing ligand was also a key molecule in mediating resolution of allergic inflammation [11].

Dysbacteriosis refers to the microbial imbalance or maladaptation on or inside the body [12]. For instance, a part of the human microbiota, the gut flora, can become disorganized, where normally dominating species are underrepresented and normally outcompeted species increase to fill the void. Intestinal dysbacteriosis (ID), a condition in the gastrointestinal tract [12], is often reported during small intestinal bacterial or fungal overgrowth [13]. It is suggested to be associated with a variety of illnesses, including inflammatory bowel disease [14], cancer [15] and allergic diseases [16,17]. ID has been implicated in immune response to respiratory infections [18,19]. Recently, our group has demonstrated the beneficial immunomodulatory effects of *Escherichia coli* and intestinal microbiota against AAD in a mouse model [20,21]. However, to date little is known on the potential involvement of ID in AAD.

In the present study, we aimed to investigate the effect of ID on a mouse model of AAD, and the potential molecular factors involved. Ovalbumin (OVA) was used to sensitize and challenge mice to induce allergic inflammation in the upper as well as the lower airways. OVA-induced AAD model mice and control mice were then raised with or without antibiotics treatment to establish the combinational AAD + ID mouse model. Characteristic symptoms of AAD were evaluated in regard to allergic symptoms, serum OVA specific IgE level, as well as inflammation cells, cytokines and microRNA expression profile in nasal lavage fluid (NALF) and bronchoalveolar lavage fluid (BALF).

2. Materials and methods

2.1. Combinational AAD + ID mouse model

Specific pathogen-free (SPF) female Balb/c mice (11–13 g, 3 week old) were housed under standard SPF conditions supplied with sterile food and autoclaved water. Animal protocols were approved by the Animal Care Committee of Shandong Provincial Hospital Affiliated to Shandong University. Mice were randomly assigned into four experimental groups (n = 8 each group), as shown in Fig. 1. OVA (Sigma-Aldrich, A5503, USA) as the allergen, and aluminum hydroxide (Thermo Scientific Imject Alum, 77161, USA) as the immune adjuvant were used for sensitization and challenge to induce ADD in the mice, as described in a previously established protocol [21]. 1 g/l ampicillin, 0.5 g/l vancomycin, 1 g/l neomycin and 1 g/l metronidazole were freshly supplied in drinking water on a daily basis to induce ID in the mice.

2.2. Measurement of allergic symptoms

The frequency of nose rubbing and sneezing behavior in each mouse was counted immediately after the last OVA challenge for 10 min in a blinded manner as previously described [22].

2.3. Cell counts for nasal lavage fluid (NALF) and bronchoalveolar lavage fluid (BALF)

All mice were sacrificed 24 h following the final challenge, and the nasal sections and the lungs were perfused with 1.2 ml PBS containing 1% fetal bovine serum (FBS) to collect NALF and BALF. After centrifugation of lavage fluids at 2500 rpm for 7 min at 4 °C, lavage supernatant was collected and stored for further analysis. Lavage cells were resuspended in 150 μl PBS and counted with a hemocytometer. Smear preparations were made and stained with Wright-Giemsa for classification and counting. The inflammatory cells were classified into monocytes, eosinophils, lymphocytes, and neutrophils based on standard morphology [23], and counted at ×400 magnification under a light microscope (Leica, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

Blood samples were obtained 24 h after the final OVA challenge via heart puncture to extract serum for further assessment of OVA-specific IgE levels using ELISA kit, with a linear range of 0.4–25 ng/ml. Commercial ELISA kit from R&D Systems (Minneapolis, MN, USA) was used to examine levels of TNF-α in both NALF and BALF following instructions of the manufactures.

2.5. MicroRNA assays

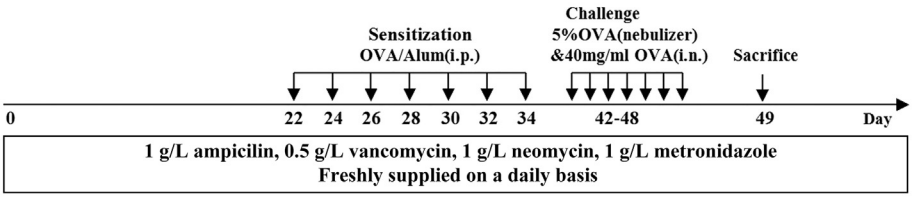
MystiCq microRNA assay kits (Sigma-Aldrich, St. Louis, MO, USA) were utilized to evaluate expressions of microRNAs as shown in Fig. 6, normalized to RNU6 control microRNA assay (MIRCP00001; Sigma-Aldrich, USA). The Mission miRNA lentiviral mimic for miR-130a-3p (HLMIR0187; Sigma-Aldrich, USA) and negative control (NCLMIR001; Sigma-Aldrich, USA) were packaged for transduction in stable cell lines.

2.6. Luciferase reporter assay

Wild-type (TNFα-UTR) targeting sequence of miR-130a-3p on 3′-UTR of TNF-α mRNA, and the mutated version (TNFα-mut), were respectively cloned into pGL-3 luciferase reporter plasmid (Promega, Madison, WI, USA) using double-digestion method. The indicated reporter plasmid was transfected into either scramble or miR-130a-3p expressing cell line, then cultured for another 48 h. Relative luciferase activity was determined using Bright-Glo luciferase assay system (Promega, USA) per manufacturer's instructions.

2.7. RT-PCR

The relative expression of mRNA was examined by qRT-PCR using the commercial PowerUp SYB Green Master Mix (ThermoFisher, Waltham, MA, USA) following the manufacturer's instruction. Briefly, the total RNA was extracted from cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). The quality and quantity of RNA samples were assessed by BioAnalyzer 2100 (Agilent, Santa Clara, CA, USA). 1 μg



Groups:	(Antibiotics treatment, sensitization & challenge)
Control group:	No antibiotics treatment, sensitized & challenged with PBS
Control+ID group:	Antibiotics treatment, sensitized & challenged with PBS
AAD group:	No antibiotics treatment, sensitized & challenged with OVA
AAD+ID group:	Antibiotics treatment, sensitized & challenged with OVA

Fig. 1. The experimental protocol and different groups.
Mice were sensitized and challenged with ovalbumin (OVA) or phosphate-buffered saline (PBS) solution, and were treated with or without antibiotics freshly supplied in drinking water on a daily basis, as described in the Methods section. The mice were divided into four different groups.