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Oral administration of allergen extracts from mugwort pollen desensitizes specific allergen-induced allergy in mice

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ABSTRACT

Clinically, sublingual immunotherapy (SLIT) using allergen extracts effectively alleviates the symptoms of allergic rhinitis and asthma. We hypothesized that oral administration of a high-dose of allergen extracts imitates SLIT, which may prevent IgE-related responses in allergic diseases. In the present study, we investigated the effects of oral administration of allergen extracts from mugwort pollen (MP) on allergen-induced inflammation and airway hyperresponsiveness (AHR) in an allergic mouse model. After administration of MPdrop containing Art v 1 and Art v 4 extracts derived from MP specifically in MP-sensitized mice, the effects of MPdrop on AHR, inflammatory cell accumulation, cytokine production in the bronchoalveolar lavage fluid and lung tissue, and serum IgE and IgG levels were investigated. The results indicated that MPdrop not only prevented the AHR in response to methacholine in a dose-dependent manner but also significantly reduced the total serum and allergen-specific IgE levels. All of the maximal effects were achieved at a dose of 100 μg/(kg d) and were comparable to the effects of dexamethasone at a dose of 0.5 mg/(kg d). Furthermore, oral administration of MPdrop dose-dependently elevated allergen-specific serum IgG2a levels, reduced total and allergen-specific IgE levels and normalized the imbalance between the Th1 cytokine IL-12 and Th2 cytokines IL-4 and IL-5. Finally, oral administration of MPdrop significantly reduced goblet cell hyperplasia and eosinophilia in the MP-sensitized allergic mouse model. These data suggest that MPdrop effectively improves specific allergen-induced inflammation and AHR in MP-sensitized and -challenged mice and provides the rationale for clinical use of MPdrop in the specific allergen-induced asthma.

1. Introduction

Allergic diseases are the most common inflammatory diseases, and their prevalence and incidence have increased in many developed and developing countries [1]. Inhalation of allergens evokes deleterious immune and inflammatory responses, which further lead to allergic pathology and allergic symptoms [1]. A known risk factor for the development of atopic allergy is exposure and sensitization to the pollens. Pollens are important sources of outdoor allergens associated with asthma and other allergic disorders [2]. The overall prevalence of positive skin prick responses in Chinese individuals is 59.0% for Dermatophagoides farinae, 57.6% for Dermatophagoides pteronyssinus, 40.7% for Blomia tropicalis, 16.1% for American cockroach, 14.0% for dog, 11.5% for Blatella germanica, 11.3% for Artemisia vulgaris, 10.3% for cat, 6.5% for Ambrosia artemisiifolia, 6.3% for mixed mold I, 4.4% for mixed mold IV, 3.5% for mixed grass pollen and 2.2% for mixed tree pollen [3]. The most common and important outdoor aeroallergens are the pollens from Artemisia vulgaris (mugwort) and Ambrosia artemisiifolia (ragweed). To date, several clinically important allergens, such as Art v 1, Art v 2, Art v 3 and Art v 4, have been identified in crude mugwort extracts [4–6]. Recombinant Art v 1, Art v 2, Art v 3 and Art v 4 have been generated. They have been shown to exhibit allergic activity in humans and animals comparable with native products prepared from crude mugwort pollen (MP) extracts [6]. Because sensitivity to these allergens is present in 60–80% of patients with MP allergies, Art v 1 and Art v 3 could be valuable tools for the diagnosis and immunotherapy of allergic asthma and rhinitis caused by MP. Unlike symptomatic treatment, allergen-specific immunotherapy both improves symptoms and modifies the natural course of the disease in patients with allergic rhinoconjunctivitis, rhinitis and mild asthma [7–10]. Because of traditional therapies, such as subcutaneous immunotherapy (SCIT), carry the

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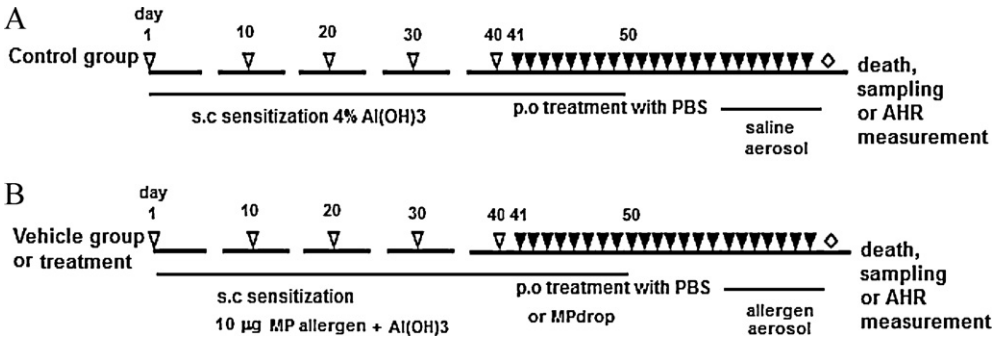

Fig. 1. Experimental procedure. Mice were subcutaneously (s.c.) sensitized five times with 10 μg of MP allergen emulsified in 0.5 ml of 4% aluminum hydroxide adjuvant. Negative control mice (Control group) were injected with 0.5 ml of 4% aluminum hydroxide adjuvant alone following the same protocol. Fifty-six days later, all the animals were placed in a plastic box and challenged via the airways with aerosolized MP allergen (1% in saline) or an equal volume of saline (Control group) in a jet nebulizer for 30 min daily for 7 consecutive days. Oral treatment was initiated at 24 h after the fourth immunization. In the 10 μg/(kg d) MPdrop group, each 20 g of mouse was fed 50 μl of 4 μg/ml MPdrop solution; in the 100 μg/(kg d) MPdrop group, each 20 g of mouse was fed 50 μl of 40 μg/ml MPdrop solution. In the control and vehicle groups (MP antigen-sensitized and vehicle-treated), each mouse was fed the same volume of PBS alone. These treatments were performed on 22 consecutive days. The treatment solutions were fed by mouth with a syringe until the solution was swallowed.

risk of systemic reactions (e.g., anaphylaxis) [11], more convenient alternatives to subcutaneous injections are being developed to improve safety and compliance. Sublingual immunotherapy (SLIT) was developed for these purposes [10]. Many clinical studies have demonstrated that SLIT is a safe and clinically effective treatment in patients sensitive to pollens with rhinoconjunctivitis, rhinitis and asthma [8–12]. However, the detailed effects of MP extracts on the modulation of immunoresponse in allergy are not fully understood. Specifically, questions remain regarding how MP extracts affect IgG and IgE levels in specific allergen-induced asthma and whether MP extracts work by modulating the Th1- or Th2-biased allergic immune response; these questions merit further study [13,14]. Established animal models were utilized to answer these questions [13,14]. In the present study, we used MPdrop solution containing Art v 1 and Art v 3 extracts derived from mugwort (Artemisia vulgaris) pollen (MP) as an example of allergen extracts. MPdrop solution was orally administrated to MP-sensitized and -challenged mice. The effects of MPdrop on airway hyperresponsiveness (AHR), cytokine production, inflammatory cell accumulation in the bronchoalveolar lavage fluid (BALF) and lung tissue, and total and allergen-specific IgE and IgG2a levels in serum were investigated. Our data suggested that oral administration of MPdrop effectively improved specific allergen-induced inflammation and AHR in MP-sensitized mice and provide the rationale for clinical use of allergen extracts for the treatment of allergic diseases.

2. Materials and methods

2.1. Animals

Female, 6–8-week-old BALB/c mice were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Certificate No. SCXK 2007-0005, Shanghai, China). All animals were housed in a room maintained at 23 ± 2 °C with 50 ± 10% humidity and a 12-h light, 12-h dark cycle (lights on from 8:00 A.M. to 8:00 P.M.). The animals were allowed free access to tap water and regular rodent chow. Rodent chow was withheld for 8 h prior to the experiments. All the animal care and handling procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang University. The animals were terminally euthanized by inhalation of CO_2.

2.2. Antigen

Endotoxin-free Artemisia vulgaris pollen (lot T20061020) was collected from a good agricultural practice (GAP) base of Zhejiang Wowu Biotech Co., Ltd. (Zhejiang Wowu Biotech Co., Ltd., Hangzhou City, China). The presence of endotoxin in the Artemisia vulgaris pollen was inspected using the limulus agent method. Allergens derived from Artemisia vulgaris pollen (MP) were purified by breaking the pollen wall using a mechanical method, defattening with acetone, extraction with 0.125 M ammonium bicarbonate, and further dialysis with distilled water. Additional purification was performed using DEAE ion exchange chromatography and gel filtration, and the preparation of MP extracts (MPdrop solution, lot T20061227) containing >50% of group 1 allergen (Art v 1) and >20% of group 3 allergen (Art v 3) was used for oral administration in mice (sublingual immunotherapy for human). However, the preparation used for antigen-sensitized mice (MP allergen, lot T20061124) contained >80% of Art v 1 and >55% of Art v 3.

2.3. Experimental procedure

The sensitization of the mice were used in the experiments was performed as previously described [14–16]. Briefly, 10 μg of MP allergen (approximately 5.8 μg Art v 1 and 3.1 μg Art v 3) emulsified in 0.5 ml of 4% aluminum hydroxide adjuvant was subcutaneously injected into the footpad, neck, back and groin of each animal, and the injections were performed on days 1, 10, 20, 30, 40 and 50. Negative control mice (control group) were subcutaneously injected with 0.5 ml of 4% aluminum hydroxide adjuvant only. Beginning on day 56, control and sensitized mice were placed in a plastic box and challenged for 30 min daily via the airways with aerosolized MP allergen (1% in saline) or equal volumes of saline (control group) by a jet nebulizer (BARI Co., Ltd., Germany) for 7 consecutive days (Fig. 1). The AHR to methacholine (Mch, Sigma, St. Louis, MO, USA) was measured 24 h after the last challenge in 5 groups of mice. In another 5 groups of mice, sera were harvested for assessment of immunoglobulin levels, and the BALF was prepared from the right lung to be used for inflammatory cell counting and cytokine measurement. A paraffin section from the left upper lobe was used for histological assessment, and the left lower lobe was homogenized for cytokine measurements.

2.4. Oral administration of MPdrop

Because SLIT is difficult to be performed in animals, we imitated clinical SLIT by feeding MPdrop solution into animal mouths using a syringe until the MPdrop solution was swallowed. We found that the solution was held in the mouth for a few minutes before swallowing; therefore, we considered most of the solution have been absorbed by the sublingual mucosa. Oral administration of MPdrop solution started at 24 h after the last immunization and