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Identification of α-tubulin, Der f 33, as a novel allergen from *Dermatophagoides farinae*

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ABSTRACT

Background: House dust mites are an important source of indoor allergens. More than 30 allergens of *Dermatophagoides farinae* (*D. farinae*) have been identified. Yet there may be many other allergens in mites remain to be characterized.
Methods: α-Tubulin (also named Der f 33) was cloned, expressed and purified. Reaction to specific-IgE, skin prick test and a mouse asthma model were employed to determine the allergenicity of Der f 33.
Results: The recombinant Der f 33 reacted to the serum of patients with mite allergy. The positive rate of skin prick test (SPT) was 23.5%. In an asthma mouse model, Der f 33 induced the airway allergy-like responses. Moreover, serum specific IgE and IgG1, interleukin-4 (IL-4) from bronchoalveolar lavage fluid (BALF) and spleen cell culture supernatant were markedly increased. In addition, Der f 33 upregulated the CD80 and TNF-α levels in dendritic cells (DCs).
Conclusions: Der f 33 is a novel allergen of *D. farinae*. It modulates the functions of DCs and induces airway allergy.

1. Introduction

Asthma is a common chronic respiratory disease (Fedorova et al., 2013). The prevalence of asthma is about 5%-16% worldwide (Platts-Mills et al., 2000). House dust mites (HDM) are one of the important allergens, and caused nearly 50% asthmatic disease (Calderon et al., 2015; Sun et al., 2013). The specific immunotherapy (SIT) with HDM extracts is recommended to be the only specific therapy for mite-related allergic asthma. But it is not easy to produce large amounts of pharmaceutical grade natural HDM extracts for SIT (Weghofer et al., 2013). In addition, the crude extracts of HDM comprise complex mixtures of proteins, and usually cause additional allergic response or adverse events (Valenta et al., 2011). Therefore, the use of recombinant allergens are suggested to be a breakthrough for SIT (Weghofer et al., 2013).

Dermatophagoides farinae is the predominant species in China and contains a large number of allergens. Its some allergens have been characterized, and the positive rates of Der f 1 and Der f 2 in patients with allergic disease (asthma) is the highest (van Der Veen et al., 2001). However, HDM allergic patients have variable profiles of IgE reactivity, and about 20% of patients have no IgE antibody to the Der f 1 and Der f 2 (Lin et al., 2015; Resch et al., 2011). Therefore, to identify and characterize new allergens is beneficial to diagnosis and treatment of allergic diseases.

DCs are antigen presenting cells (APC), and have the ability to dictate naive CD4+ T cells to differentiate into either Th1 or Th2 cells. A number of costimulatory molecules locate on the surface of DCs, such as CD80, CD40, which are associated with the activation of T cells (van Rijt et al., 2004). TNF-α can be produced by DCs, which plays a role in regulating CD4 + T cells and neutrophilia (Fei et al., 2011). Therefore, the function of DCs is important to T helper cell polarization. However, the factors modulating the function of DCs are not fully understood.

The *α-tubulin* genes which exist in most organisms have highly conserved amino acid sequences among different species. Some of

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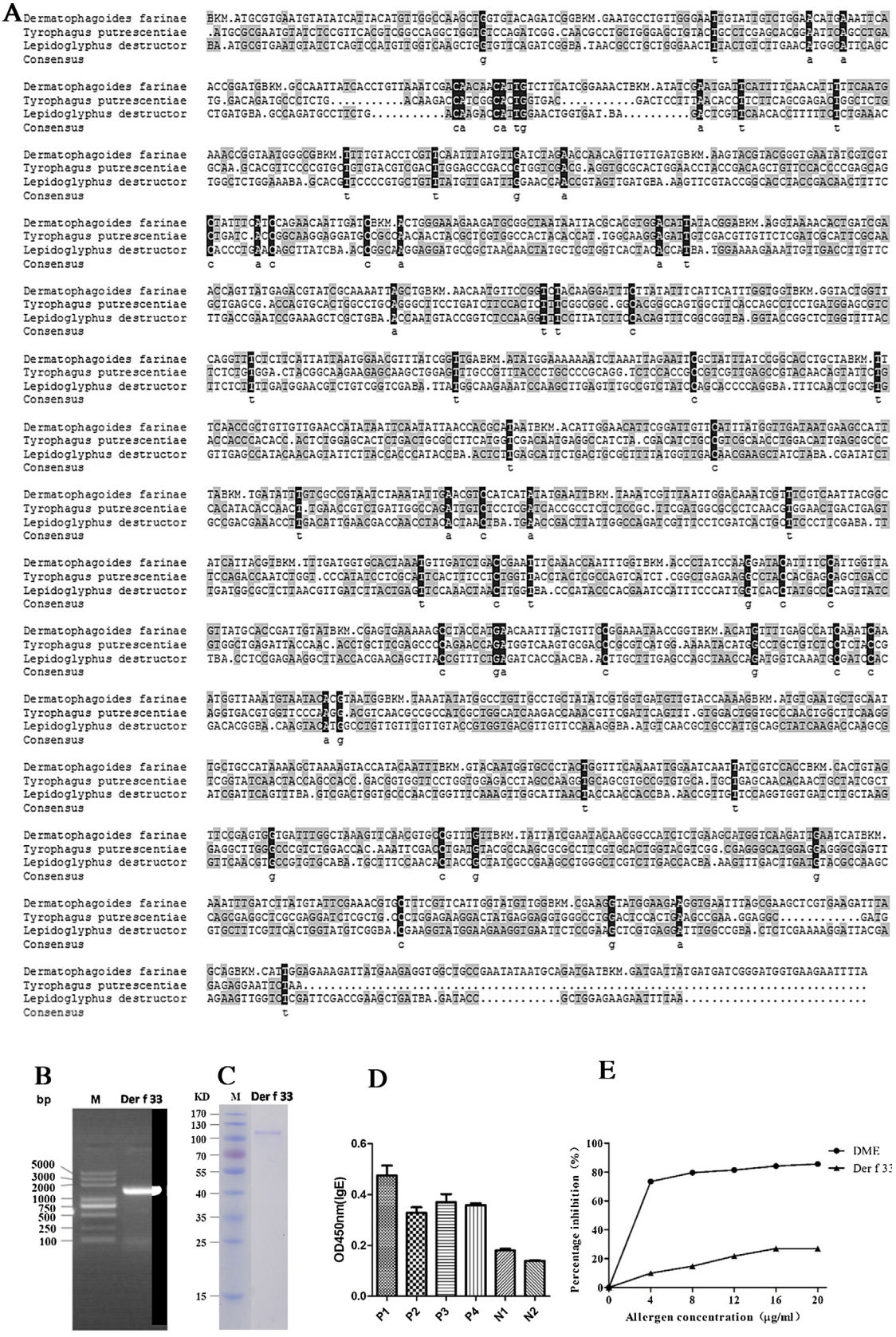

Fig. 1. Sequence alignment, expression and immunological characterization of *Dermatophagoides farinae* Der f 33. (A) The sequence alignment of α-tubulin in *D. farinae*, *Tyrophagus putrescentiae* and *Lepidoglyphus destructor*. (B) The PCR product of Der f 33. (C) SDS-PAGE analysis of the purified r-Der f 33. Lane M: Protein Marker. (D) The specific IgE reactivity to Der f 33 by ELISA. N1-N2, the serum from healthy subjects; P1-P4, the serum from DME positive patients. (E) ELISA inhibition assay.