

# The Effect of Water-soluble Chitosan on Macrophage Activation and the attenuation of Mite Allergen-induced Airway Inflammation

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## I. Introduction

Chitin and chitosan are polymers of b-D-glucosamine and of N-acetylglucosamine, respectively, and is a key structural component of helminths, arthropods and fungi [1,2]. Water-soluble chitosan (WSC) derivatives has been reported to have anti-tumor, anti-fungal, and antimicrobial properties [4,5], to have potential as sustained release carriers for drugs [6], to accelerate wound healing [7], and to enhance the in vitro infectivity of adenovirus to mammalian cells [8]. The immune response to chitin that develops into T-helper type 1 (Th1) or Th2 response and its role in the allergic asthma remains controversial [10-14]. Oral administration of chitin, for example, has been shown to down modulate a murine model of allergic airway inflammation [11]. In this study, Shibata et al. [11] not only demonstrate the capacity of chitin to induce the secretion of Th1 cytokines, but also propose that chitin preparations could be an attractive therapy in allergic human disease. Another study by Strong et al. [12] demonstrates that direct application of chitin microparticles (sizes in the 1-20 mm range) to the respiratory tract can alleviate allergic symptoms in a mouse model of ragweed allergy and might be a useful treatment for respiratory allergy and allergic asthma. In contrast, Zhu et al. [13] have shown that during allergic airway inflammation, there is significantly increase of AMCcase production in Th2 immune response and IL-13 pathway activation. Further, Reese et al. [14] found direct administration of chitin induced eosinophil and basophil accumulation in helminth-infected murine model of Th2 inflammatory responses. Previously, we showed that house dust mites (*Dermatophagoides pteronyssinus*, Der p, and *Dermatophagoides farinae*, Der f) can directly activate innate immune cells such as alveolar macrophages and mast cells without previous in vitro or in vivo sensitization [17,18]. This activation led to eosinophils infiltration, goblet cell hyperplasia, and hyperplasia of peribronchial smooth muscles in mice repeatedly exposed to Der f. Moreover, we found that Der p can activate and induce nitric oxide (NO) production in the alveolar macrophage cell line (MHS) via CD14/toll-like 4 receptor (TLR4) signaling [19]. Therefore, it was of interest to evaluate the extent of WSC mediated activation of macrophages during allergen-stimulation. The results reported here demonstrate that water-soluble chitosan attenuates Der f-induced activation of macrophages in allergic asthma patients and reduces allergen-induced airway inflammation in Der f-sensitized mice.



## II. Experimental procedures and Results

### 2.1. The effect of WSC on Th2 cytokine production by MDMs

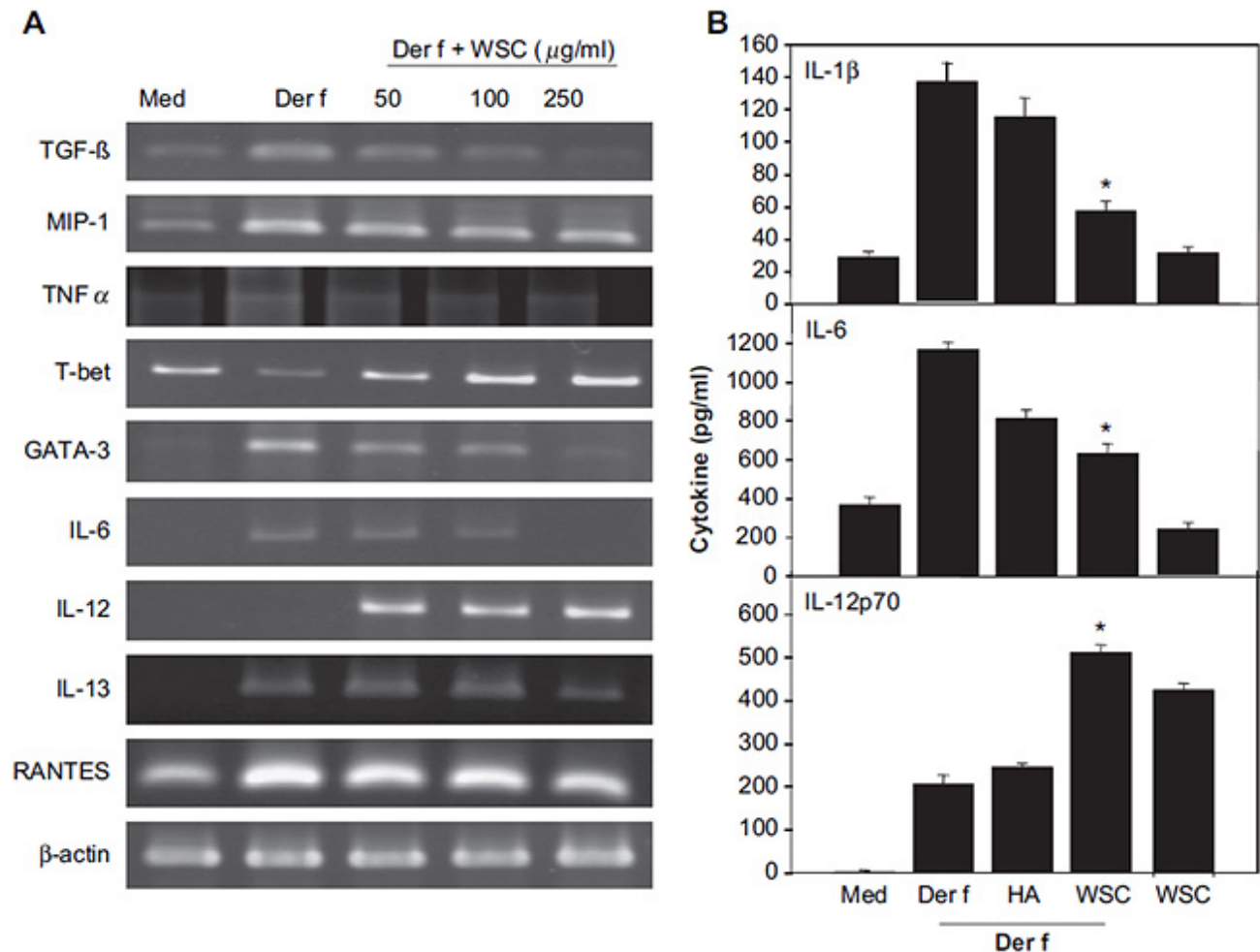


Figure 1. After pretreatment with various dosages of WSC for 6 h, there was a dose-dependent decrease in the mRNA expression of Th2 and inflammatory cytokines as well as GATA3, whereas the mRNA expression of the Th1 cytokine (IL-12) and its transcript factor (T-bet) were increased in WSC-pretreated, Der f-stimulated MDM. Although the mRNA expression of eosinophil chemotactic factor (RANTES) was increased in Der f-stimulated macrophages, pretreatment with WSC did not significantly affect the expression of this chemokine

### 2.2. The effect of WSC on Der f-induced change in MDM morphology

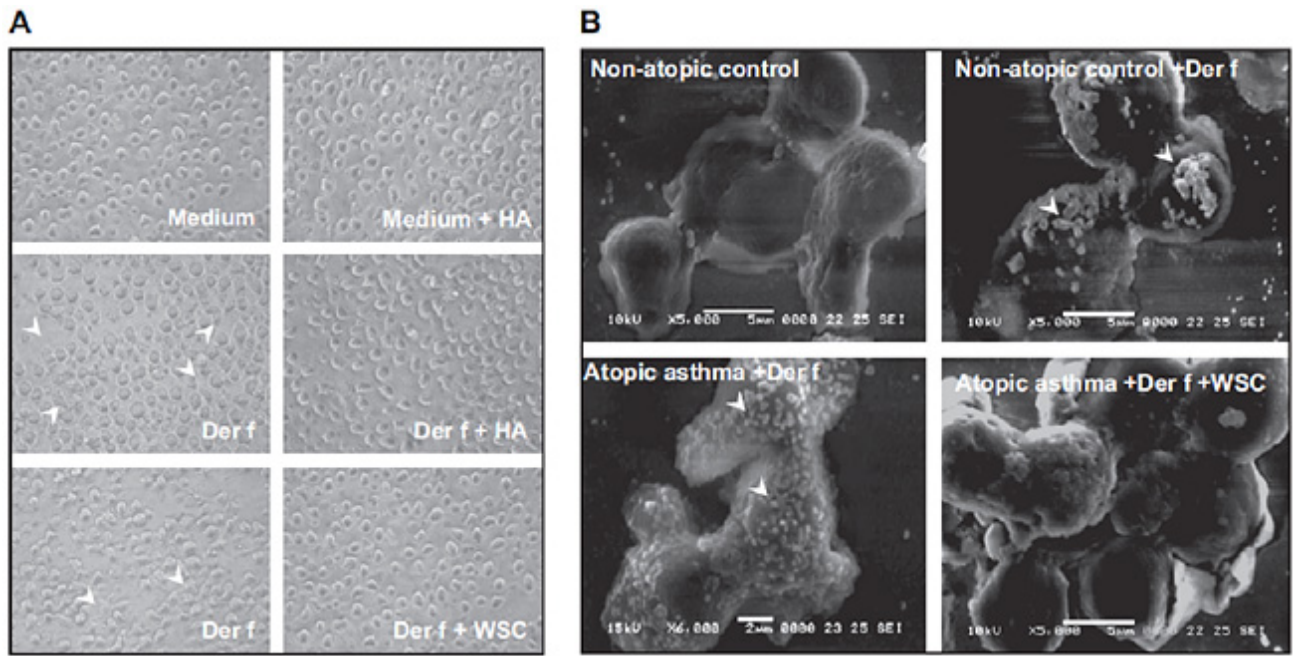


Figure 2, Changes in Der f-stimulated macrophage morphology in response to WSC or HA were observed under a phase contrast light microscope ( 100) (A). MDM isolated from non-atopic controls and Der f-sensitive asthmatic patients were pretreated with WSC (250 mg/ml for 6 h) and then stimulated with Der f (10 mg/ml for 24 h). SEM (X6000) revealed Der f-induced many changes in MDM morphology (white arrows) and that pretreatment with WSC prevented these changes. White bar is 2 μm in length.

2.3. The attenuation of Der f-induced airway inflammation by WSC in a murine model of asthma

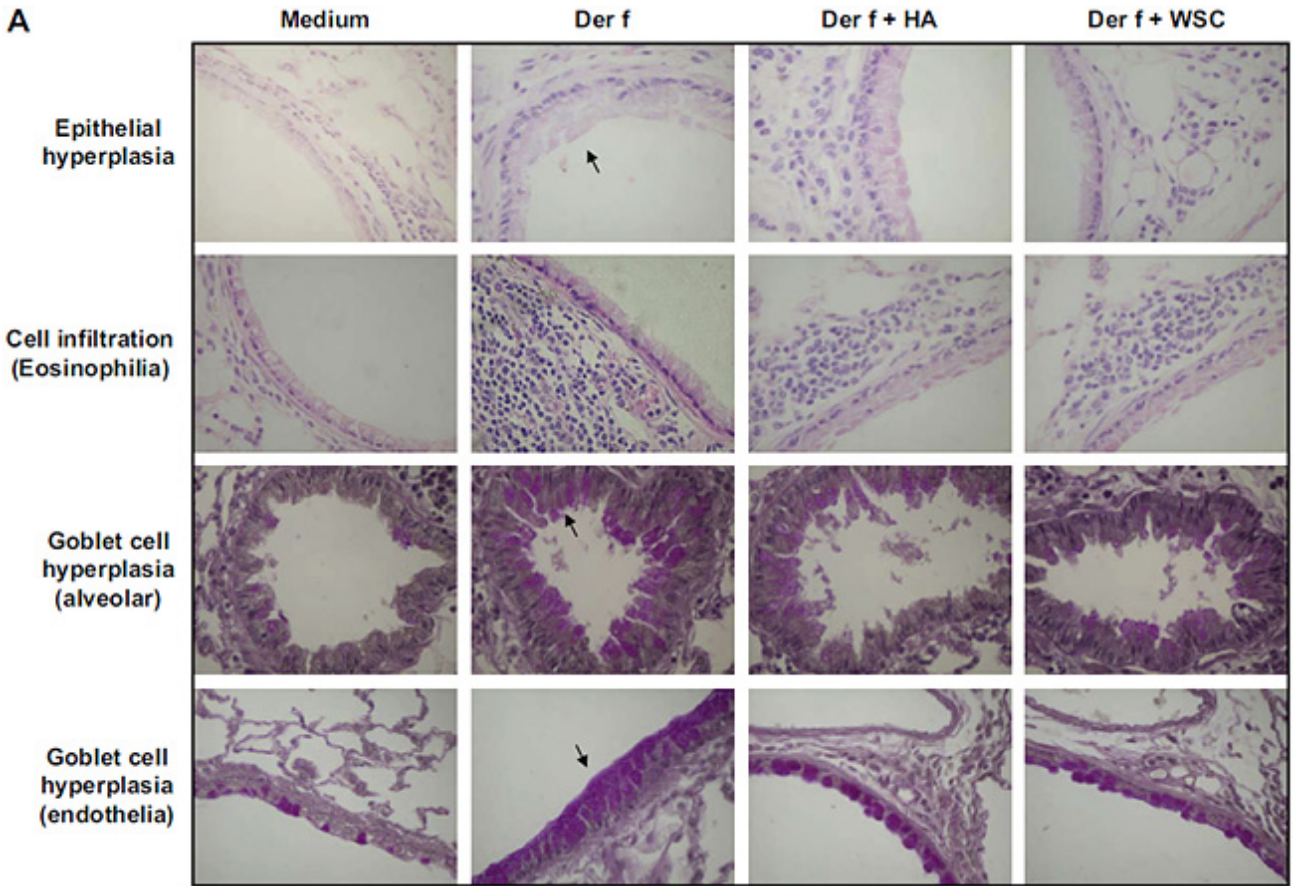


Figure 3 (A)

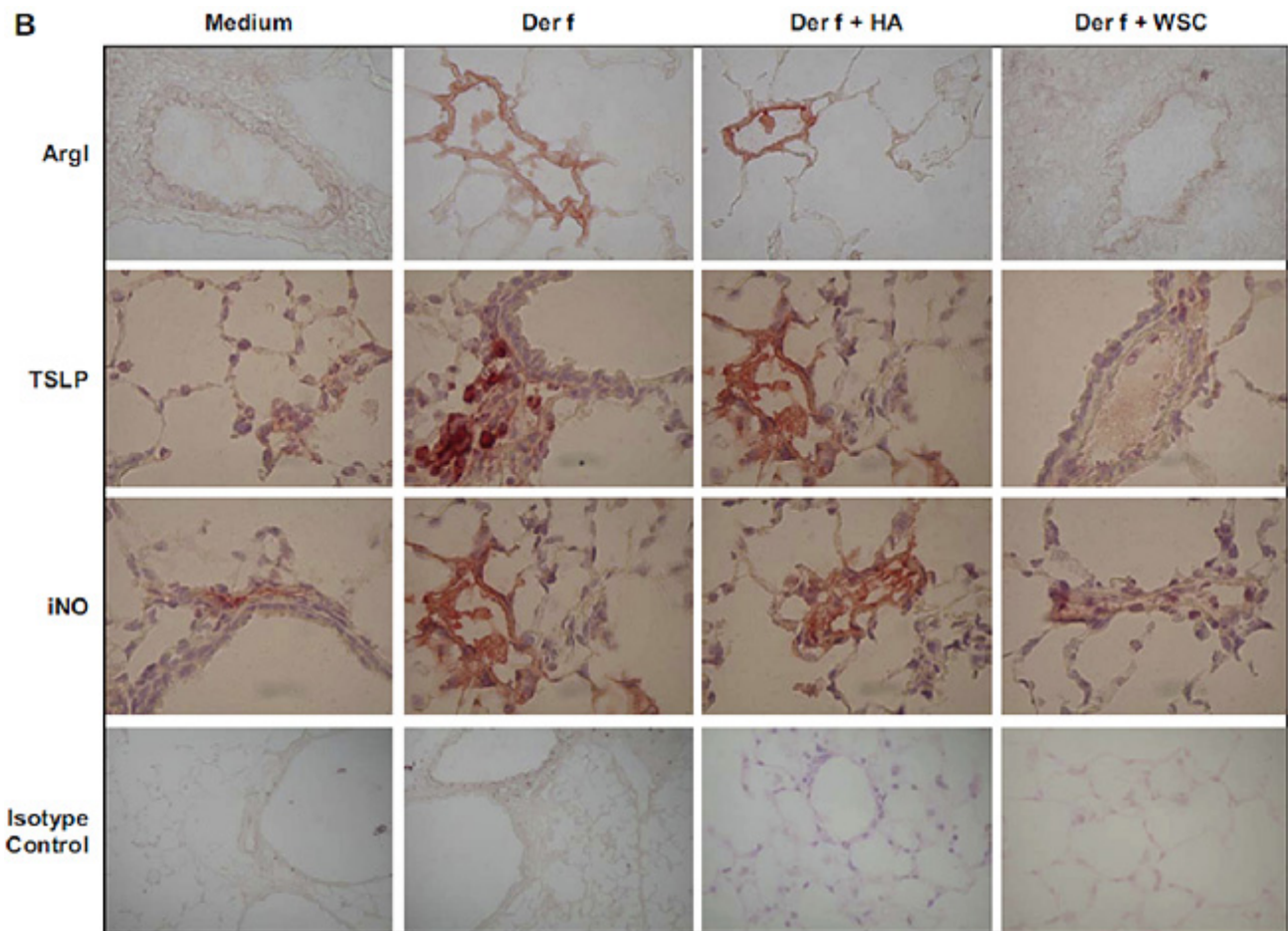


Figure 3 (B)

Finally, the *in vivo* effect of WSC on the Der f-induced airway inflammation was studied in Der f-sensitized mice. Tracheal instillation of Der f in Der f-sensitized mice induced marked changes in the airways, such as epithelial sloughing, goblet cell hyperplasia (Fig. 5A), and increased Arg I, TSLP, and inducible NOS (iNOS) expression in the subepithelial and peribronchiolar areas (Fig. 5B). These histopathological findings were similar to those in humans with asthma. Intranasal WSC, but not intranasal HA, significantly reduced the number of inflammatory cells such as eosinophils in the bronchoalveolar lavage (BAL) fluid, airway hypersensitivity (data not shown), epithelial damage, goblet cell hyperplasia (Fig. 3A), and Arg I, TSLP, and iNOs expression in the Der f-treated sensitized mice as compared to non-treated sensitized mice (Fig. 3B).

### III. Discussion

In this study, we demonstrated that water-soluble, low molecular weight chitosan had specific immunomodulatory effects on Der f-stimulated human MDM including the shifting of Th2 cytokine polarization, decreasing the production of the inflammatory cytokines IL-6 and TNF- $\alpha$ , down-regulating CD44, CD14, TLR14, and PAR2 receptor expressions, and inhibiting T cell proliferation in the presence of allergen stimulated MDM. These results further emphasize that the anti-inflammatory and anti-allergic effects of WSC are related to the macrophage activation. Furthermore, we found that Der f-stimulated MDM from allergic asthmatics (but not from non-atopic subjects) had multiple micropseudopodia (Fig. 2). This morphological difference in allergen-stimulated MDM between atopic and non-atopic subjects has not been reported before, and its significance in the pathogenesis of allergen-induced inflammation is not understood. We suspect that WSC prevents allergen-induced degranulation and perturbation in the MDM membrane events that trigger pathways leading to inflammatory activation.

In conclusion, our results showed that in addition to its Th1 adjuvanticity, water-soluble WSC is a strong immunomodulator of the alternative activation of macrophages to allergen-stimulation, i.e., it attenuates inflammatory processes frequently accompanying chronic asthma-induced airway remodeling. Such properties of WSC might be useful in combination, or as an alternative to treatment with current anti-asthma medications.

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