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## The use of phenylalanine- and polyethylene glycol-modified nano-chitosan in house dust mite allergen immunotherapy

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House dust mite (HDM) is recognized as the most common cause of household allergy, and leads to chronic allergic diseases that cannot be completely cured. Recently, considerable research has focused on allergen specific immunotherapy to overcome the limitations of conventional therapeutics. Nano-chitosan-based delivery systems are of great interest since they have immunoadjuvant properties and provide enhanced cellular uptake of protein-based allergens. Here we report the application of nano-chitosan modified with phenylalanine (Phe) and polyethylene glycol (PEG) as a new adjuvant for use in HDM allergen immunotherapy. In this study, the HaCaT human keratinocyte cell line was used as a model for topical allergen delivery to evaluate cellular toxicity and intracellular localization of allergen. Peripheral blood mononuclear cells (PBMCs) from HDM-allergic patients and normal volunteers were used to study the cell-mediated immune response. The allergen entrapped within a nano-chitosan based adjuvant was characterized by transmission electron microscope (TEM) and was found to be able to penetrate the test cells. Analysis of cellular viability, morphology, reactive oxygen species (ROS) generation, and cell cycle progression indicates that the cellular structure, quantity, and function of the cells were not significantly changed after allergen treatment. Interestingly, chitosan-HDM nanoparticles significantly reduced the rate of PBMC death caused by HDM allergen. This adjuvant system modulates the innate immune response by reducing interferon-(IFN)- $\gamma$  and interleukin-(IL)-10 secretions in PBMCs from allergic patients compared with normal controls. Furthermore, our results indicate that co-modification of CS with both Phe and PEG modulates the cytokine responses in allergic patients, suggesting that this delivery method may facilitate HDM allergy treatment.

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