



Modulation of the specific allergic response by mite allergens encapsulated into liposomes

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Abstract

Liposomes are non toxic and biodegradable lipid vesicles, which are safe and effective adjuvants to induce Th1-skewed immune response. Therefore, the encapsulation of allergens into liposomes could be an attractive alternative for specific allergy immunotherapy. Previously, we obtained DPPC iposomes encapsulating purified allergens from *Dermatophagoides siboney*, with suitable stability and extremely reduced allergenicity. In this study, Balb/c mice were immunized with allergens encapsulated into liposomes (LP) and the induced immune response was evaluated in comparison with allergens dissolved in PBS (PBSA) or adsorbed in Alum (AL). The use of Alum or Liposomes induced a strong allergen specific IgG response. However, total IgE serum levels in the AL group were very high, while levels found in LP group were not significantly different from the control group receiving only PBS. The IgG2a/IgG1 subclass ratio was raised in the LP group. Allergen specific IgE, as measured by PCA assay, was similar for LP and PBSA groups, and approximately the half of the reaction size found in AL group. After allergen challenge by inhalation route, peripheral blood and airway eosinophil counts increased significantly in AL, but not in LP group. Additionally, histopathological analysis of lung tissue sections obtained from challenged mice indicated a reduced cellular infiltration in mice immunized with liposomes. These results support the potential use of liposomal formulations for allergen vaccines.

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1. Introduction

Dermatophagoides siboney has been commonly found in house dust of asthmatics in the Caribbean area [1]. In IgE-mediated allergy, allergen-specific immunotherapy has been one of the most frequently used and efficacious treatments [2]. Nevertheless, the development of more safe and efficient allergen formulations is needed to avoid anaphylactic reactions or to reduce the length of the injection regimens. Alum is the most common adjuvant used for allergy vaccination, but it stimulates T helper (Th) 2 lymphocytes [2]. Liposomes

(LP) are non-toxic and biodegradable lipid vesicles, which are safe and effective adjuvants to induce Th1-skewed immune responses. We determined the characteristics of the specific immune response against the major allergens from *D. siboney* encapsulated into LP. For comparison we used the same allergens adsorbed to Alum.

2. Material and methods

A fraction of the allergen extract, containing predominantly Der s 1 and Der s 2 proteins, was used as antigen. Multilamellar LP of dipalmitoyl phosphatidylcholine (DPPC) encapsulating the allergens were prepared according to the dehydration-rehydration method [3]. BALB/c

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mice were immunized intraperitoneally with 5 µg allergens encapsulated into LP, dissolved in PBS (PBSA) or adsorbed in Alum on weeks 0 and 2. Ten days after the last injection, mice were exposed to aerosolised *D. siboney* extracts during 30 min for 5 consecutive days. Afterward mice were bled and the lungs were collected for histopathological studies. Total IgE and specific IgG levels were determined by ELISA. Specific IgE was evaluated by passive cutaneous anaphylaxis assay (PCA).

3. Results and discussion

It was previously reported that allergens from *Aspergillus scoparia*, *D. pteronisinus* and *A. fumigatus* when encapsulated into LP induce a specific immune response with a Th1 signature [4,5]. We obtained DPPC-LP encapsulating the major allergens from *D. siboney* with high encapsulation efficiency and extremely reduced allergenicity. Here we report that immunization of Balb/c mice with the allergens administered with Alum or LP induced a strong allergen-specific IgG response. However, the total IgE serum levels in the Alum group were very high, while the IgE levels in the LP group did not differ from those present in the control group that received only PBS. Allergen-specific IgE was similar for the LP and PBSA groups, and approximately half that

found in the Alum group. After the allergen challenge by inhalation, peripheral blood eosinophils increased significantly only in the Alum group. Additionally, histopathological analysis of lung tissue sections indicated a reduced cellular infiltration in mice immunized with LP. These results support the potential use of liposomal formulations for allergen vaccines. At this moment we are carrying out experiments using DPPC and cholesterol containing LP with encouraging results.

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