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Encapsulation of hydrophobic allergens into nanoparticles improves the *in vitro* immunological diagnosis of allergic contact dermatitis

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Abstract

The diagnosis of allergic contact dermatitis (ACD) relies on *in vivo* patch testing. *In vitro* immunological assays based on the characterization of circulating allergen-specific memory T cells represent a promising alternative to patch testing. However, their development is hampered by the technical challenge of assessing hydrophobic allergens in serum-based assays. In this study, we show that the encapsulation of fragrance mix 1 (FMI, a mixture of 8 hydrophobic allergens) into poly-2-caprolactone nanoparticle (NP) vectors: (1) dramatically increases the solubilization of allergens in conventional cell culture media and (2) allows for a robust *in vitro* reactivation of allergen-specific T cells in large numbers of fragrance allergic patients. Therefore, the encapsulation of hydrophobic allergens into NP vectors opens new avenues to improve the *in vitro* immunobiological diagnosis of ACD.

Key words: Allergic contact dermatitis; Diagnosis; Immunobiological assays; Fragrances; Poly-ε-caprolactone nanoparticles

Introduction

Allergic contact dermatitis (ACD) is the most prevalent skin inflammatory disease in many European countries.[1,2] ACD is induced by the repeated contact of individuals with ubiquitous chemical allergens, called haptens. ACD is a delayed-type hypersensitivity reaction and is mediated by the recruitment and activation into the skin of allergen-specific effector T cells (Teff).[3]

Currently the diagnosis of ACD relies on clinical investigations by patch testing with suspected haptens. The patch test (PT) method aims at reproducing the ACD lesions in sensitized patients by applying occlusive patches containing the suspected allergens to the patient's healthy skin.[4]

Immunobiological assays based on the detection of allergen-specific Teff cells (and/or Teff cell products) circulating into the blood of allergic patients proved a valuable alternative to PT. Previously, promising results were obtained with hydrophilic compounds, such as metals or dyes.[5–7] Nevertheless, so far the development of such *in vitro* approaches was hampered by the lack of solubility into conventional cell culture media of the majority of allergens that are responsible for ACD; which is key to restimulate blood leucocytes collected from suspected patients.

In this study, we capitalized on the unique properties of poly-ε-caprolactone nanoparticles (NPs) to improve the solubilization of fragrance mix 1, a mixture of 8 hydrophobic fragrance

Abbreviations: ACD, Allergic contact dermatitis; EBS, European Baseline Series; FMI, Fragrance mix 1; GC, Gas chromatography; mAb, Monoclonal antibody; NP, Nanoparticle; PBMC, Peripheral blood mononuclear cells; PT, Patch tests; RPMIc, Complete RPMI medium; SI, Stimulation indices; Teff, Effector T cells.

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t1.1 Table 1
t1.2 Clinical characteristics of patients and their response to FMI patch testing.

t1.3 Patient number	Sex/age	Clinical symptoms	FMI PT result
t1.4 1	F/59	Generalized eczema	+++
t1.5 2	M/61	Generalized eczema	+++
t1.6 3	F/58	Face and generalized eczema	++
t1.7 4	F/34	Face and neck eczema	++
t1.8 5	F/50	Face and neck eczema	++
t1.9 6	F/51	Hand eczema	++
t1.10 7	F/55	Glossodynia	++
t1.11 8	F/51	Face and eyelid eczema	++
t1.12 9	F/51	Eczema	++
t1.13 10	F/60	Hand and forearm eczema	++
t1.14 11	M/61	Hand eczema	++
t1.15 12	M/64	Generalized eczema	++
t1.16 13	F/42	Generalized eczema	++
t1.17 14	M/79	Hand eczema	++
t1.18 15	M/74	Eyelid eczema	+
t1.19 16	F/49	Hand eczema	+
t1.20 17	F/52	Face eczema	+
t1.21 18	F/37	Forehead eczema	+
t1.22 19	F/57	Eyelid eczema	+
t1.23 20	F/70	Eczema	+
t1.24 21	F/61	Angular stomatitis/cheilitis, diffuse eczema	−
t1.25 22	F/40	Facial erythema, scalp seborrheic dermatitis, friction hives	−
t1.26 23	F/70	Leg and forearm eczema	−
t1.27 24	F/32	Eczema	−
t1.28 25	F/42	Eczema	−
t1.29 26	F/72	Erysipelas	−
t1.30 27	M/39	Atopic dermatitis	−
t1.31 28	F/38	Hand eczema	−
t1.32 29	M/72	Hand eczema	−
t1.33 30	F/24	Atopic dermatitis	−
t1.34 31	F/73	Eczema	−

F, female; M, male; PT, patch test. PT lesions were evaluated according to the ICDRG scoring: negative reaction (−); weak (non-vesicular) positive allergic reaction: erythema, infiltration and possibly papules (+); strong (vesicular) positive allergic reaction: erythema, infiltration, papules and vesicles (++); t1.35 extreme positive allergic reaction; bullous reaction (+++).

allergens, into cell culture media, and to demonstrate the efficacy of immunobiological assays for the diagnosis of ACD to fragrances.

Methods

See Supplemental Methods for complete information.

Preparation of fragrance mix I-loaded nanoparticles

FMI-loaded NPs were prepared using a nanoprecipitation process.[8,9] Free, i.e. unloaded NPs were also prepared. NP mean size and size distribution were determined by light scattering measurements. The concentration of each fragrance ingredient loaded into NPs was assessed using gas chromatography (GC).

Solubilization assay into cell culture media

FMI preparations (as allergen-loaded NP suspensions or free allergen solutions) were mixed into complete RPMI medium (RPMIc). Fractions, collected 15 min after introduction and mixing, were dissolved in acetone and next analyzed by GC. The percentage of FMI or individual FMI ingredients solubilized into RPMIc was determined as: fragrance concentrations titrated by GC into fractions/fragrance concentrations into FMI preparations (also validated by GC).

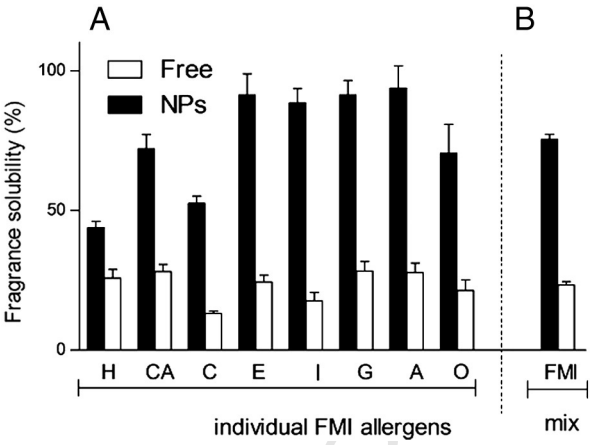

Figure 1. Increased solubilization of allergens and FMI into RPMI when encapsulated into poly-ε-caprolactone nanoparticles (NPs). **(A)** Percentage of the 8 individual fragrance allergens encapsulated in NPs versus free allergen, solubilized into RMPIc at 37 °C, measured by GC analysis and **(B)** determination of the solubility of the mix of the 8 allergens (FMI). Results are the mean of 6 measurements of 3 samples. H: hydroxycitronellal, CA: cinnalyl alcohol, C: cinnamal, E: eugenol, I: isoeugenol, G: geraniol, A: (α)-amylcinnamaldehyde, O: oak-moss, FMI: fragrance mix I. See details of solubility calculations in Supplementary Table S3.

Clinical study

A prospective clinical study was conducted to obtain PBMC from FMI-sensitized or non-sensitized subjects. Therein, 31 patients with clinical history of eczema were patch-tested with the European Baseline Series (EBS) (comprising FMI). Blood was collected before PT and PBMC were purified using standard Ficoll density gradient. The characteristics of each individual and PT results are reported in Table 1.

Immunobiological assays for the in vitro diagnosis of ACD

PBMC were cultured with graded amounts of FMI-loaded or free NPs for 5 days. Final concentrations of FMI introduced in each culture varied from 0.01 to 250 μg/mL.

Secondary T cell proliferation and cytokine secretion into culture supernatant were evaluated by radioisotope incorporation and a multiplex ELISA assay respectively.

Results

NP encapsulation of FMI allergens increases their solubility in culture medium

FMI, composed of 8 hydrophobic allergens (cinnamyl alcohol, cinnamal, hydroxycitronellal, amyl cinnamal, geraniol, eugenol, isoeugenol and oak-moss absolute), was encapsulated into poly-ε-caprolactone NP vectors according to a solvent displacement method. This nanoprecipitation process was