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Intranasal vaccination with poly(lactide-co-glycolide) microparticles containing a peptide T of Ole e 1 prevents mice against sensitization

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Clinical and Experimental Allergy

Summary

Background Biodegradable microparticles, in particular poly(lactide-co-glycolide) (PLGA), have been shown as potential delivery vehicles for intranasal (i.n.) vaccines in animal models. **Objectives** To evaluate whether i.n. administration of PLGA microparticles containing a peptide with the major T cell epitope of Ole e 1, the main allergen of olive pollen, prevented mice from allergic sensitization to the whole protein.

Methods Peptide-PLGA microparticles were prepared by a solvent evaporation double emulsion method. Microparticles in a size range of 0.8 µm were evaluated for peptide loading and *in vitro* antigen release. Stability and immunogenicity of the entrapped peptide were retained, as determined by dot blot and ELISA inhibition. BALB/c mice were intranasally treated with peptide-PLGA microparticles for 3 consecutive days, 1 week before sensitization/challenge to Ole e 1. Blood, lungs and spleen were collected and analysed for immune response. Biodistribution of microparticles was investigated using confocal microscopy.

Results I.n. pretreatment of BALB/c mice with peptide-PLGA microparticles before sensitization to Ole e 1 led to a significant inhibition of serum allergen-specific IgE and IgG1 antibody levels, but a marked increase of specific IgG2a antibodies as compared with sham-pretreated mice. Moreover, IL-5 and IL-10 levels in spleen cell cultures were suppressed in peptide-PLGA pretreated mice. The airway histopathologic parameters associated with inflammation were significantly suppressed by the pretreatment.

Conclusion: These results demonstrate that i.n. immunization with peptide T-PLGA microparticles is effective in preventing subsequent allergic sensitization to Ole e 1. Our data indicate that peptide-PLGA microparticles may be promising candidates for the design of nasal vaccines against allergic diseases in humans.

Keywords aeroallergens, immunoregulation, intranasal vaccine, mouse model, PLGA microparticles

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Introduction

Pollen allergy is a major public health problem that affects the quality of life of millions of children and adults. Olive pollen represents one of the most important causes of pollinosis in the Mediterranean area, America and Australia, where this tree is widely cultivated [1]. The most important olive pollen allergen is Ole e 1, which has been purified [2], cloned [3] and produced as recombinant protein in the yeast *Pichia pastoris* [4]. Moreover, Ole e 1 has been described as a diagnostic marker for sensitization to *Oleaceae* pollens [5]. Therefore, Ole e 1 has been

selected as a model allergen for the development of novel strategies for type I allergy.

The dramatic increase in the prevalence of pollinosis over the last decades together with the high risk of systemic reaction during allergen-specific immunotherapy highlight the urgent need for development of alternative preventive and therapeutic approaches. One of the principal approaches is the use of mucosal vaccines. Among the routes available for mucosal immunization, the intranasal (i.n.) one offers significant advantages [6]: (i) as the oral route, it is a practical site for easy self-administration of vaccines but (ii) requires much lower

doses of antigen, mainly because the antigen is not exposed to low pH and a broad range of secreted antigen-degrading enzymes; (iii) it induces more potent local and systemic response than other mucosal route; (iv) it shows the potential for induction of immune response in the genitourinary, respiratory and gastrointestinal tracts, as a consequence of the common mucosal immune system.

Vaccine delivery systems are generally particulates (e.g. liposomes and starch particles) to facilitate uptake by antigen-presenting cells (APCs) and the transport to secondary lymph organs [7]. Poly(lactide-co-glycolide) (PLGA) microparticles (MP) are the primary candidates for the development of microencapsulated vaccines because they are biodegradable and biocompatible, and have been used in humans for many years as reabsorbable suture and implants, as well as controlled release drug delivery systems [8]. PLGA MP have been applied as carrier vaccines for peptides, proteins or plasmid DNA [9, 10]. Microparticles act as potent immunological adjuvant, as they induce a Th1-type immune response as well as cytotoxic T cell activity [11, 12]. Therefore, the vaccination with PLGA MP could represent a promising strategy for allergy because it could modify the T helper type-2 (Th2)-based immune response of the allergic status.

Previous studies were performed to elucidate the T cell and B cell determinants of Ole e 1 by using overlapping synthetic peptides, and reported that the segment T109-K130 showed capability to stimulate T cells from allergic patients [13], but displays no IgE-binding capability [13, 14]. Moreover, this region was defined as the most important T cell epitope of Ole e 1 in humans [13] and a dominant T cell epitope in mouse (unpublished data). Based on these data, a peptide of 22 amino acids was designed, spanning the amino acids 109–130 of Ole e 1 (OE_{109–130}). This peptide would be of significant importance for novel approaches in immunotherapy because it would substantially decrease the risk of IgE-mediated anaphylactic side-effects. Therefore, the aim of this study was to evaluate the potential of PLGA MP as a vehicle for i.n. administration of OE_{109–130} peptide of Ole e 1 in a mouse model of sensitization to the whole allergen. We showed that OE_{109–130}-PLGA MP successfully prevented allergic sensitization to Ole e 1. The transport of antigen-PLGA MP towards lymphoid organs following i.n. administration in mice was also investigated.

Materials and methods

Mice

Female, 6–8-week-old BALB/c mice were obtained from Harlan Interfauna Ibérica SA (Barcelona, Spain). Animals were maintained at the Animal Care Services of the Faculty of Biology (Universidad Complutense, Madrid, Spain), according to the local guidelines for animal care.

The studies were approved by the Animal Experimentation Ethics Committee of the Complutense University.

Peptide and protein

OE_{109–130} (TVNGTTRTVNPLGFFKKEALPK) was synthesized by Thermo Electron GmbH (Ulm, Germany). Ole e 1 was purified from olive pollen as described [2].

Preparation of poly(lactide-co-glycolide) microparticles and characterization

Microparticles with encapsulated OE_{109–130} peptide were prepared by the solvent evaporation double emulsion method as reported [15]. Empty MP were prepared for control immunization. Particle size and surface morphology were evaluated by scanning electron microscopy (Jeol 6400, Jeol Instrument, Welwyn, Herts, Germany) using an instrument equipped with an image analyser. The lyophilized MP were dispersed onto metal stubs and coated with gold-palladium under an argon atmosphere. The loading efficiency of OE_{109–130} in PLGA MP was determined by extraction of lyophilized MP in SDS/DCM: acetone [15]. OE_{109–130} content was determined using the micro-bicinchoninic acid assay (Pierce Chemical, Rockford, IL, USA), with known dilutions of the peptide as standard. The percentage entrapped efficiency was calculated from the ratio between the experimental entrapped OE_{109–130} value and the theoretical value [15]. *In vitro* release kinetics study of encapsulated OE_{109–130} from MP was performed as described elsewhere [15]. Peptide release was calculated in terms of accumulative release (μg peptide/mg MP). The integrity of OE_{109–130} peptide following entrapment in PLGA MP was assessed using dot blot [14] and ELISA inhibition [15] assays with a polyclonal antibody (Ab) specific to Ole e 1.

Induction of experimental allergy (sensitization and challenge)

Mice were sensitized to Ole e 1 by two intraperitoneal injections with 1 μg Ole e 1 adsorbed on 2 mg Al(OH)₃ in 150 μL of phosphate-buffered saline (PBS) in 7 day-intervals. After 7 days mice were challenged by i.n. administration of 10 μg of Ole e 1 in 20 μL PBS on 3 consecutive days. Twenty-four hours after the last i.n. challenge, lung histology was examined.

Prophylactic protocol (intranasal vaccination)

Anaesthetized mice were i.n. pretreated three times on consecutive days with OE_{109–130}-PLGA MP in 20 μL PBS. Animals were given 1 mg of polymer equivalent to 3 μg of peptide because it was identified as the optimal dose in a pilot experiment. Control mice were sham-pretreated with

1 mg of empty PLGA MP under the same conditions. One week later, mice were sensitized and challenged as described above.

Determination of specific-allergen IgG1, IgG2a and total IgE in serum

Blood samples were collected from the retro-orbital plexus 7 days after the last i.n. challenge (terminal bleeding). Individual sera were prepared and stored at -20°C until used. Ole e 1-specific antibodies (Ab) were measured by ELISA as previously described [16]. Serum samples were diluted 1:25 000 for IgG1 and 1:400 for IgG2a. Antibody levels were expressed as optical density (OD) values at 492 nm. Total IgE (diluted 1:40) Ab levels in serum were measured by sandwich ELISA using the OptEIA mouse IgE set (PharMingen, San Diego, CA, USA) according to the manufacturer's instructions. In our mouse model it has been showed that specific IgE can be estimated by measuring total IgE levels in serum [16].

Determination of allergen specific-IgA in fecal extracts and serum

Fresh feces were collected 7 days after the last immunization. Feces (10% w/v) were homogenized in PBS containing 0.05% SDS and 1 mM phenylmethylsulphenyl fluoride, and incubated on rotary shaker for 15 min. Insoluble material was removed by centrifugation and the supernatants were stored at -20°C until analysed. Specific IgA Ab were assayed in sera and fecal samples by ELISA [16]. Sera and fecal samples were diluted 1:20 and 1:2, respectively. Specific-IgA Ab were detected using peroxidase-conjugated rabbit anti-mouse IgA (diluted 1:1000) Ab (Rockland, Gilbertsville, PA, USA). Antibody levels were expressed as OD values at 492 nm.

Proliferation and cytokine assays

Seven days after the last immunization, spleens were removed aseptically, minced, filtered through sterile filters, and a cell suspension was prepared as described [16] and resuspended in complete medium (RPMI containing 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 25 mM HEPES, 100 U/mL penicillin, 100 µg/mL streptomycin and 50 µM 2-mercaptoethanol).

For T cell proliferation assays, spleen cells (10^6 cells/mL, 0.2 mL/well) were stimulated with Ole e 1 (2 µg/mL), concanavalin A (Con A) (2 µg/mL, Sigma Chemical Co, St Louis, MO, USA) as positive control or media alone for 96 h in a 96-round bottom plates (Costar, Cambridge, MA, USA). The cultures were pulsed with 1 µCi/well [^3H]-thymidine (Amersham, Buckinghamshire, UK) for 16 h and cells were harvested on microbeta filter mats using a

96-well harvester and a proliferative response were measured by β plate scintillation counting. The stimulation index was calculated as the ratio of counts per minute of the unstimulated control and allergen-stimulated cells.

To measure the cytokine production, spleen cells were cultured in 48-well plates (Costar) at 10^7 cells/mL (0.5 mL/well) and stimulated with Ole e 1 (2 µg/mL), Con A (2 µg/mL) or media. Supernatants were taken after 48 or 72 h and stored at -20°C until analyses. Levels of IL-4, IL-5, IL-10 and IFN- γ were measured by mouse ELISA kits (PharMingen) according to the manufacturers' instructions. Values in pg/mL were calculated according to standard curves obtained with recombinant mouse cytokines. The sensitivity of the assays was 5 pg/mL for IL-4, 60 pg/mL for IL-5, 15 pg/mL for IL-10 and 25 pg/mL for IFN- γ .

Histopathology

Lungs were removed, fixed in 10% paraformaldehyde, cut into 5 µm sections and stained with haematoxylin-eosin. Lung sections were observed under light microscopy (Axiophot 2 model, Zeiss, Jena, Germany) and photographed with a digital camera (Spot Insight Color, Diagnostic Instruments, MI, USA). For each mouse, 10 airway sections were assessed for severity of the cellular inflammatory response by morphometric analysis, by individuals blinded to the protocol design. The intensity of the cellular infiltration around pulmonary blood vessels and airways was assessed on a semiquantitative score ranging from 0 to 5 $^{+}$. The number of eosinophils per unit airway area was determined by morphological criteria and enumerated per high-powered field.

In vivo trafficking of microparticle

Tetramethyl rhodamine isothiocyanate (TRITC, Molecular Probes, Leiden, the Netherlands) labelling of Ole e 1 was performed according to the manufacturer's instructions. PLGA MP containing TRITC-Ole e 1 was prepared as described for the peptide. BALB/c mice ($n=3$) received 2 mg of fluorescent Ole e 1-PLGA MP, suspended in 20 µL of sterile PBS, via i.n. route under light anaesthesia. Mice were killed 1, 24 and 168 h (7 days) after i.n. administration; one group of animals was treated with empty PLGA MP and used as negative control. Nasal associated lymphoid tissues (NALT), lungs, draining lymph nodes (LN) and spleen were removed, embedded in a cryostat medium and frozen in liquid nitrogen. Frozen samples were sectioned at thickness of 10 µm with a microtome and stained with TO-PRO-3 iodine for cell nuclei labelling (Molecular Probes). The semi-quantitative distribution of the number of fluorescent MP within a given tissue was determined by epifluorescence using a laser scanning confocal microscope (LSM510 Meta, Zeiss). Images were analysed using Imagine-Pro Plus software (Media

Cybernetics, Inc., Silver Spring, MD, USA). Fifteen cryo-sections were examined per time point. Results were expressed as the relative ratio of MP per cell, which was calculated from the number of red pixels (corresponding to MP) divided by the number of green pixels (corresponding to nucleus) for each section.

Statistical analysis

Data are expressed as means $\pm$ SEM from two independent experiments. Comparison of sensitized vs. pretreated group was performed by using Student's *t*-test.

Results

Characterization of OE_{109–130}-PLGA MP

Microparticles were prepared by double emulsion-solvent evaporation method under optimized formulation conditions. Scanning microscopy observation revealed that OE_{109–130}-PLGA MP were mostly spherical in shape with a smooth surface (Fig. 1a). The mean size of MP was $0.8 \mu\text{m}$. The loading of the peptide in MP was around $3 \pm 0.6 \mu\text{g/mg}$ of polymer. The *in vitro* release profile of the peptide from PLGA MP was characterized by a typical initial burst release of $74 \pm 10\%$ during the first hour (Fig. 1b). This was followed by a slow and sustained release of the peptide, reaching the $83 \pm 3\%$ over the next 28 days.

Structural integrity of encapsulated OE_{109–130}

Dot blot analysis revealed similar immunoreactivity for the encapsulated and original peptide, indicating that the structural integrity of OE_{109–130} was not altered during the entrapment procedure in PLGA MP (Fig. 2a). The integrity of the released peptide was also confirmed by ELISA inhibition assay because it showed similar inhibitory capacity compared with that of the original peptide (Fig. 2b).

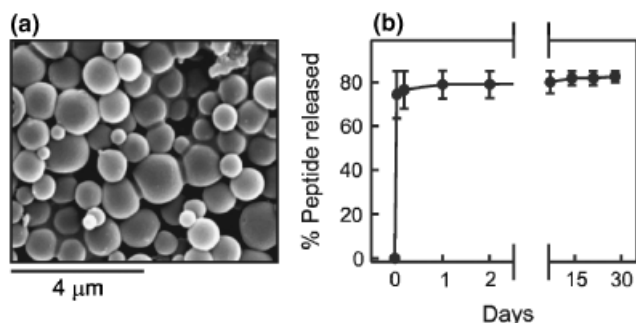


Fig. 1. Characterization of microparticles. (a) Scanning electron micrograph of OE_{109–130}-poly(lactide-co-glycolide) microparticles. (b) Analysis of the *in vitro* release of the peptide from PLGA MP in phosphate-buffered saline, calculated as a percentage of the total entrapped peptide, over a period of 28 days.

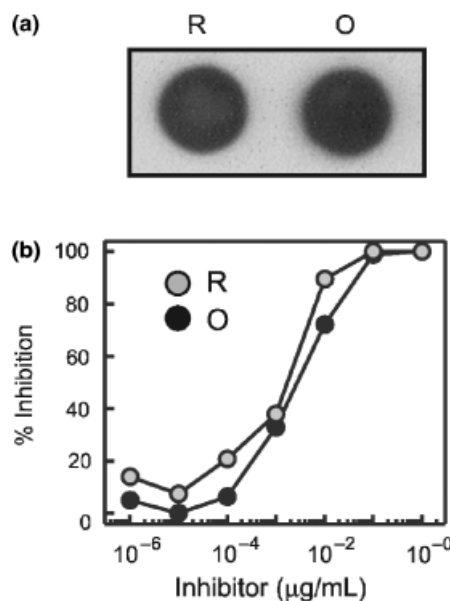


Fig. 2. Analysis of the integrity of OE_{109–130} released from poly(lactide-co-glycolide) microparticles. (a) Dot blot staining of released (R) and original (O) peptide with a polyclonal antibody (Ab) specific to Ole e 1. (b) ELISA inhibition assay of the binding of Ole e 1-specific polyclonal Ab to solid-phase OE_{109–130} using as inhibitors released (R) or original (O) peptide.

Protection of mice against Ole e 1 sensitization

A mouse model of sensitization to Ole e 1 with features similar to those seen in allergic patients was used to investigate whether i.n. delivery of OE_{109–130}-PLGA MP was able to prevent subsequent sensitization to the whole allergen. Mice were i.n. pretreated with peptide-PLGA MP or empty PLGA MP as sham-pretreated controls before sensitization with Ole e 1, and the allergen-specific response was analysed. Sham-pretreated animals developed an allergic state comparable to that observed in untreated mice sensitized and challenged with the allergen (data not shown).

Antibody response to Ole e 1. Allergen-specific Ab levels in serum were measured by ELISA 1 week after the last i.n. challenge (Fig. 3). Mice treated with $3 \mu\text{g}$ of the peptide in PLGA MP before sensitization displayed markedly reduced IgE Ab levels (85%) compared with sham-pretreated controls (Fig. 3). A decrease of specific IgG1 Ab levels (35%) were also observed within the group pretreated with encapsulated peptide. In terms of specific-IgG2a, pretreatment led to increase the Ab production compared with sham-pretreatment with empty PLGA MP (Fig. 3). Serum Ole e 1-specific IgA Ab levels were increased in mice pretreated with OE_{109–130}-PLGA MP (0.352 ± 0.003 OD) compared with mice pretreated with empty MP (0.175 ± 0.006 OD). Ole e 1-specific IgA Ab levels were not detected in feces of mice pretreated with OE_{109–130}-PLGA MP.

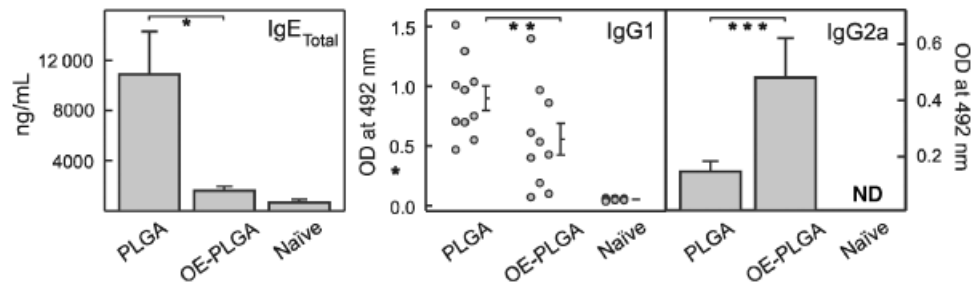


Fig. 3. Specific IgE, IgG1, and IgG2a Ab responses in sera from mice intranasal pretreated with OE₁₀₉₋₁₃₀-poly(lactide-co-glycolide) (PLGA) microparticles. Ab levels were determined by ELISA and expressed as the mean $\pm$ SEM of 2 separate experiments ($n = 10$ mice per group). For IgG1 Ab, data are presented as scatter plot. * $P < 0.002$; ** $P < 0.06$; *** $P < 0.02$ vs. sham-pretreated mice (PLGA). nd, not detected.

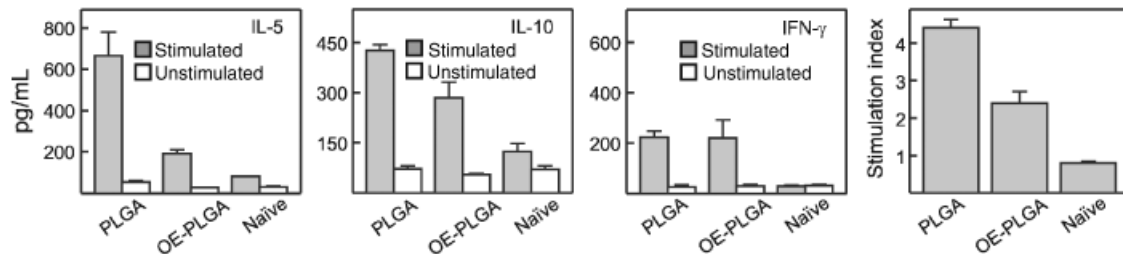


Fig. 4. T-cell proliferation and cytokine production in spleen cell cultures from mice pretreated with OE₁₀₉₋₁₃₀-poly(lactide-co-glycolide) (PLGA) microparticles. IL-5, IL-10 and IFN- γ levels (pg/mL) were measured in supernatants by ELISA and expressed as mean values from five mice per group. Results are shown as absolute cytokine levels compared with baseline levels of nonstimulated cultures. Proliferative responses were measured by [^{3} H]-thymidine-incorporation and indicated as mean stimulation indices of five mice per group. Two independent experiments were performed with similar results. PLGA, sham-pretreated mice.

Proliferative response and cytokine production of spleen cell cultures. I.n. pretreatment with 3 μ g of OE₁₀₉₋₁₃₀-PLGA MP resulted in a significant reduction of IL-5 (74%) and IL-10 (50%) production in comparison with the sham-pretreated controls (Fig. 4). No significant differences of IFN- γ levels were detected in spleen cells of these animals. In these experiments, IL-4 could not be detected *in vitro* under similar conditions probably because of insufficient secretion or too rapid use by these cells. The lymphoproliferative responses to Ole e 1 in spleen cell cultures of mice pretreated with OE₁₀₉₋₁₃₀-PLGA MP were reduced when compared with those of sham-pretreated group (Fig. 4).

Airway inflammation. The i.n. challenge with Ole e 1 induced severe infiltration of lymphocytes and eosinophils into the airways of mice pretreated with empty PLGA MP compared with naïve mice (Fig. 5a, A–D). Lungs of Ole e 1-sensitized/challenged mice that had previously been treated with OE₁₀₉₋₁₃₀-PLGA-MP showed a dramatic reduction in the degree of cellular infiltration compared with mice pretreated with empty PLGA MP (Fig. 5a, E). The cellular infiltrate was predominantly characterized by mononuclear cells (Fig. 5a, F). The number of eosinophils was reduced by 75% (Fig. 5c). These data indicate that i.n. administration of OE₁₀₉₋₁₃₀-PLGA MP can attenuate lung inflammation which was induced by the allergen challenge.

In vivo trafficking of microparticles

The fluorescent MP showed the same properties described for Ole e 1-PLGA MP [15]. Double colour confocal laser scanning microscopy was performed to determine the tissular distribution of Ole e 1-PLGA MP following i.n. administration (Fig. 6). The biodistribution of MP changed over the 7-day period. Many MP were observed within the NALT from 1 h of administration, and decreased significantly after 24 h. A significant number of MP was detected in lung up to 7 days, but in a significantly lower number as compared with earlier time-points. Significant particle accumulation within the LN was detected by 24 h following i.n. delivery of MP. This effect became more pronounced with time. Importantly, in terms of mucosal immunization, the first sign of MP in the spleen was observed at 24 h after i.n. delivery, but significant MP transference to spleen occurred in these animals by the seventh day after i.n. delivery of MP.

Discussion

PLGA MP have been used as delivery systems for a wide range of agents, including proteins and DNA [9, 10]. The aim of this study was to evaluate whether the PLGA MP could be used as a vehicle for i.n. administration of OE₁₀₉₋₁₃₀ peptide derived from Ole e 1 and, therefore, used to prevent sensitization to the whole allergen in a

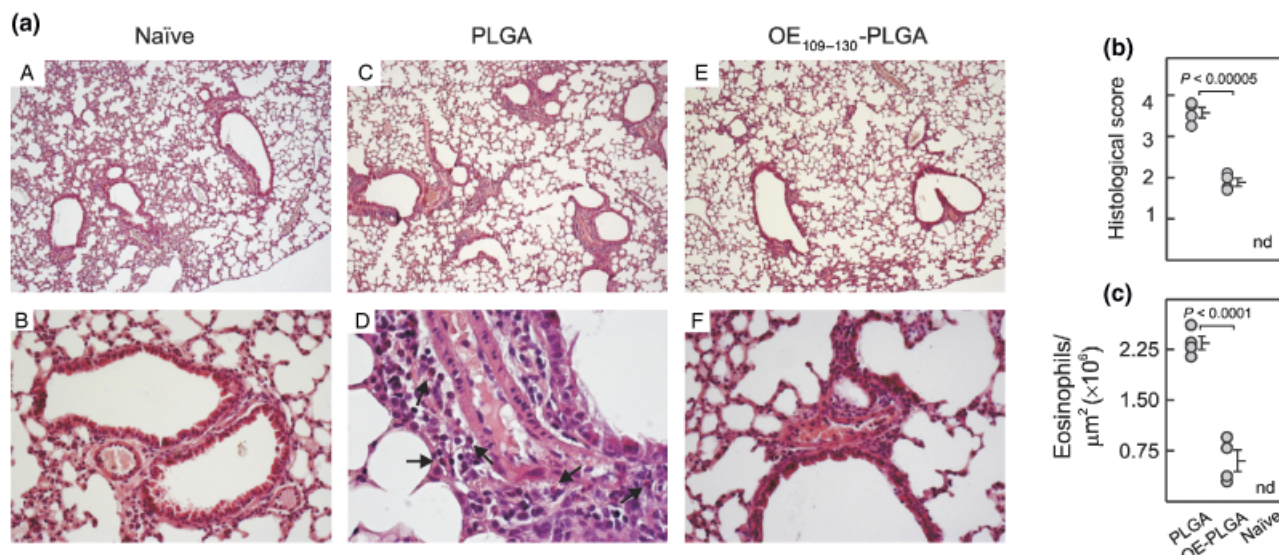


Fig. 5. (a) Representative photomicrograph of lung histology from mice pretreated with OE₁₀₉₋₁₃₀-poly(lactide-co-glycolide) (PLGA) microparticles (MP) compared with naïve and sham-pretreated mice (PLGA). (A) Normal airway mucosa and surrounding parenchyma. (B) Airway mucosa showing low cuboidal cells and absence of peribronchiolar inflammatory infiltrates. (C) Severe perivascular and peribronchial infiltration of inflammatory cells including eosinophils (D, arrows) are observed in lung tissue from sham-pretreated mice. (E) Minimal cellular infiltration around the blood vessels and airways are observed in mice pretreated with OE₁₀₉₋₁₃₀-PLGA MP, whereas eosinophils are not observed (F). Magnification: A, C and E, $\times 5$; B and D, $\times 20$; F, $\times 40$. (b) Lung inflammation and (c) number of eosinophils per unit of area. Data are the mean $\pm$ SEM from $n = 4$ per group from 2 independent experiments.

mouse model. OE₁₀₉₋₁₃₀ peptide was encapsulated in PLGA MP of 0.8 μ m mean range. Several studies have shown that MP smaller than 10 μ m generally show a higher uptake by M cells of NALT than larger ones [17–19]. Therefore, the size of the PLGA MP used in this study was suitable for M cells uptake and translocation to lymphoid organs to initiate antigen-specific immune responses. The loading of the peptide in MP was around 3 ± 0.6 μ g/mg of polymer. This value could be considered high enough for *in vivo* administration of the desired quantity of the peptide without the requirement of administering excessively high amounts of MP and number of doses. One of the major drawbacks to vaccine development by antigen microencapsulation using the solvent evaporation method is the potential degradation of antigen during the preparation process due to the use of organic solvent [9]. The antigenicity/integrity of the loaded OE₁₀₉₋₁₃₀ peptide was not significantly affected by the entrapping procedure as it has also been reported for other proteins and peptides [9, 15]. The peptide was released from PLGA MP in an initial burst of $74 \pm 10\%$, followed by a slow and sustained release over several weeks. It has been reported that an initial burst release of antigen is an advantage for mucosal vaccines to stimulate the immune system because of the limited period of exposure of the MP to the mucosal surface [20]. A slow but continuous release is also required to provide a long-lasting immune response [20]. In this study, a correlation

between the antigen release profile and immune response was observed.

In the current prophylactic study, three consecutive doses of 3 μ g of the peptide in PLGA MP were enough to prevent subsequent sensitization to the whole allergen Ole e 1. OE₁₀₉₋₁₃₀-PLGA pretreatment reduced the development of allergen-specific IgE and IgG1 Ab, and increased IgG2a Ab levels, but failed to elicit measurable IgA Ab levels in fecal extract. However, low levels of specific IgA were detected in the sera of mice pretreated with OE₁₀₉₋₁₃₀-PLGA MP, suggesting that IgA in serum could serve as a marker for mucosal response [21]. The low mucosal response after *i.n.* administration of the OE₁₀₉₋₁₃₀-PLGA MP could be due to the restricted absorption of the MP because of their fast clearance rate in the nasal cavity [22] after *i.n.* administration, in conjunction with scattered antigen-sampling M cells through the NALT epithelium [23], as observed under the confocal microscopy in this work. It is generally accepted that induction of mucosal Ab take place in the upper airway mucosal, while the antigen presentation in the lung is of importance for systemic but not for mucosal Ab responses [24]. Because NALT are the source of IgA precursor cells, it is tempting to speculate that a longer persistence of antigen in NALT might lead to increased stimulation of mucosal Ab production [25]. Further optimization of OE₁₀₉₋₁₃₀-PLGA MP may help to improve the efficiency antigen delivery and magnitude of mucosal responses.

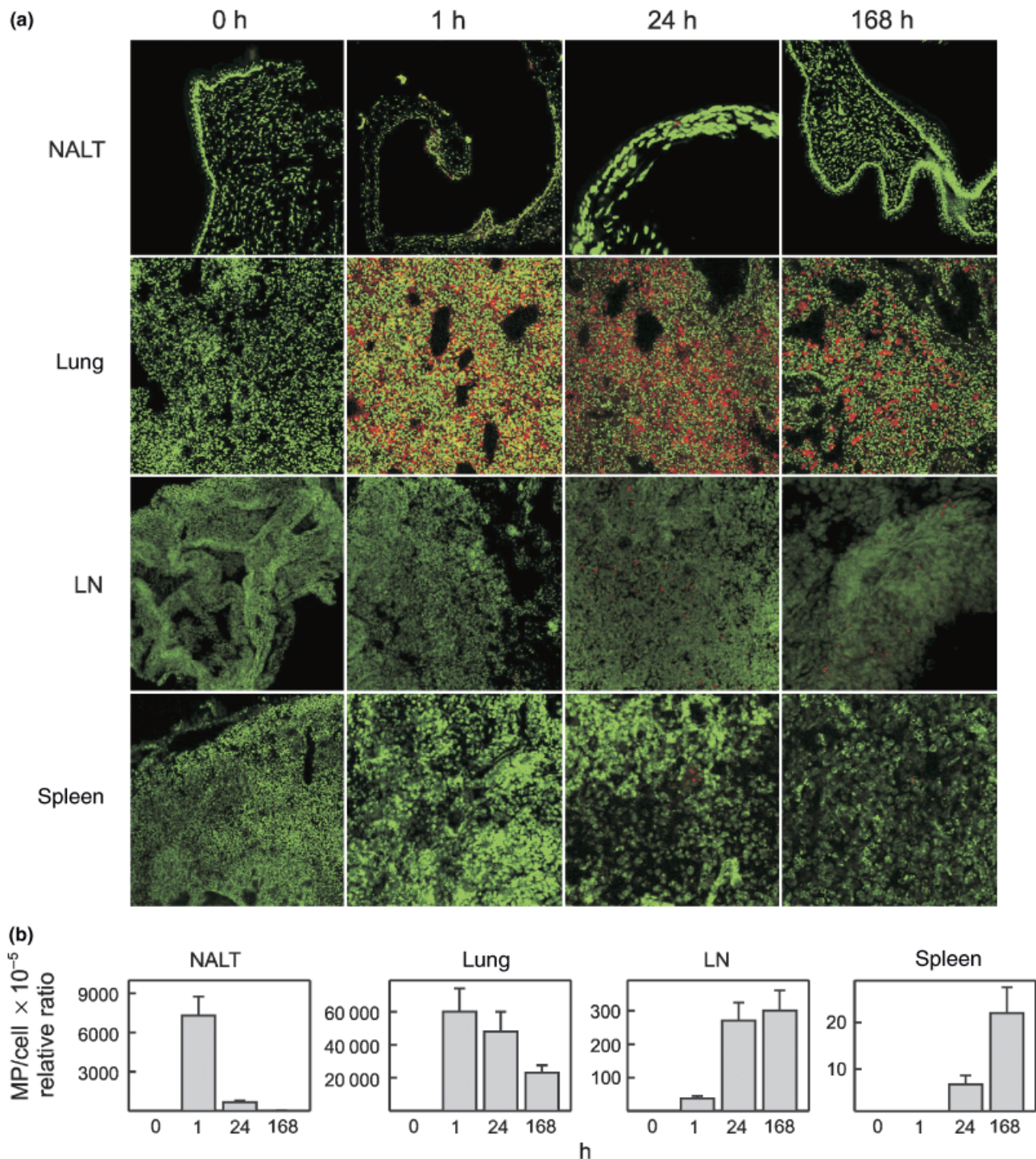


Fig. 6. (a) Confocal micrographs showing biodistribution of microparticles containing tetramethyl rhodamine isothiocyanate (TRITC)-Ole e 1 (red) into Nasal associated lymphoid tissues (NALT), lungs, draining lymph nodes and spleen following intranasal administration to mice. Time after i.n. delivery was 1, 24 and 168 h. Panels show overlaid images: TRITC-Ole e 1 loaded MP in red; the nucleus is stained green. (b) Semi-quantification of Ole e 1-poly(lactide-co-glycolide) microparticles in tissues at different timepoints. Data are presented as mean of five sections per mice ($n = 3$ per group) $\pm$ SD.

At the cellular level, i.n. pretreatment with OE₁₀₉₋₁₃₀-PLGA MP resulted in a notable decrease of IL-5 and IL-10 levels, but IFN- γ production was not significantly changed. Prophylactic treatment with

OE₁₀₉₋₁₃₀-PLGA MP also prevented lung inflammation which is one of the clinical features of allergy in humans. Because BALB/c strain of mice shows an inherent tendency to mount a Th2 response, both the increase of IgG2a

Ab levels (with a concomitant reduction in IgE and IgG1 Ab responses) and the presence of high levels of IFN- γ are evidences for the induction of a strong Th1 type response by OE₁₀₉₋₁₃₀-PLGA MP *in vivo*. Recent clinical and experimental investigations suggest that vaccination for immunomodulation to allergen-specific type 1 response may have prophylactic and/or therapeutic role in allergy [7, 26–28]. Thus, the use of PLGA MP containing appropriate antigen may be a useful approach for establishing a specific Th1-type response in humans.

Intranasal delivery of Ole e 1-PLGA MP to mice, in such way that MP are deposited through the respiratory tree, resulted in a time dependent translocation of MP to the spleen. These results are in agreement with the observations of other authors [19, 29, 30]. It is well established that M cells are highly efficient in the uptake of particulate antigens and delivery to underlying APCs in the local lymphoid structure [31]. After being route through the bronchial-associated lymphoid tissue and draining LN, MP probably enter the bloodstream, and thence reach the spleen via the thoracic duct. The delivery of different vehicles volumes causes significant differences in the immune responses observed in experimental animal model [19]. In this work, the administration of the OE₁₀₉₋₁₃₀-PLGA MP suspension in a 20 μ L volume of buffer was sufficient for significant particle transference to the spleen which results in the induction of systemic immunological response. These results suggest good immunomodulatory properties of the OE₁₀₉₋₁₃₀-PLGA MP upon combined nasal-pulmonary immunization (called total respiratory tract immunization) [24], as both systemic and mucosal immune responses were observed. These findings are important for the design of nasal vaccines. Already, several examples of safe and effective immunization of humans with vaccines administered via inhalation exist, such as measles and influenza vaccines [32, 33].

Although several important allergens have been successfully encapsulated in PLGA MP [15, 34–37], recently, there have been no data on the effectiveness of PLGA MP as a possible i.n. prophylactic treatment to prevent the development of allergy in mice. To our knowledge, there is just one report demonstrating that Bet v 1-PLGA MP modulate an ongoing Th2 response in a mouse model. Schöll *et al.* [37] demonstrated that a single subcutaneous immunization with Bet v 1-PLGA MP is effective in down-regulating serum IgG1 and enhancing IFN- γ and IL-10 production when given as treatment for Bet v 1 sensitization. But no changes in the specific IgE response were seen. One of the most significant findings of our study is that i.n. administration of micrograms of a peptide T in PLGA MP has a protective effect against sensitization to the whole allergen in mice. Although these results do not mean that protection will be obtained in humans, this model addresses the immunological effects on the most

important clinical features of allergy (IgE levels and airway inflammation), and it is, therefore, important for designing prophylactic allergy vaccines, whose possible use has been proposed for early prevention in atopic individuals or children at risk [38–40]. The feasibility of using PLGA MP for allergy vaccine in human was recently shown [41]. Oral immunization of allergic patients with timothy grass pollen-loaded MP proved to reduce effectively medication and symptom scores. Although further experiments are essential before this approach can be applied in humans, this study emphasizes the usefulness of i.n. vaccination with peptide T-PLGA MP for the treatment of allergy.

In summary, we demonstrated that i.n. administration of peptide T-PLGA MP prevented subsequent systemic allergic immune response and airway inflammation to the whole allergen. Furthermore, our results indicated a Th1 response for i.n. PLGA MP. These findings evidence the high potential of PLGA MP as a carrier for nasal peptide-based vaccines, indicating a promising alternative approach for conventional immunotherapy against allergy.

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