

Note

Encapsulation of Ole e 1 in biodegradable microparticles induces Th1 response in mice: a potential vaccine for allergy

Eva Batanero*, Patricia Barral, Mayte Villalba, Rosalía Rodríguez

Departamento de Bioquímica y Biología Molecular; Facultad de Ciencias Químicas, Universidad Complutense, 28040 Madrid, Spain

Received 11 April 2003; accepted 8 July 2003

Abstract

Ole e 1 is the most prevalent allergen of olive (*Olea europaea*) pollen. The suitability of poly(D,L-lactide-co-glycolide) (PLG) microparticles as a vaccine delivery system for Ole e 1 in therapy against olive allergy has been evaluated. BALB/c mice were immunized intraperitoneally with Ole e 1 in PLG microparticles. Encapsulated Ole e 1 induced specific and long-lasting antibodies that were predominantly of the IgG2a isotype. Splenic cells from immunized mice secreted in vitro IFN- γ , but not IL-4, after stimulation with Ole e 1. These results suggest that Ole e 1-containing PLG microparticles elicit a specific Th1-type immune response and may become a promising concept for allergy vaccine.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Poly(D,L-lactide-co-glycolide) microparticles; Vaccine; Allergen; Olive pollen allergy

1. Introduction

At present, desensitization of allergic patients by systemic injection of increasing doses of the allergen is used as an immunotherapy for the treatment of allergy. Although it has shown to be effective, the risk of systemic reactions, however small, represents a major drawback for general application. Thus, it is of great importance to develop safer and more effective immunotherapies. Since IgE-allergic reactions are mediated by specific Th2 cells [1], it should be useful to design new approaches that promote a Th1 response and thereby downregulate the Th2 ones, for

interfering with allergic reaction [2–4]. Biodegradable poly(D,L-lactide-co-glycolide) (PLG) microparticle-based vaccines could represent a promising strategy for the treatment and prevention of allergy disorders, since immunizations with antigen encapsulated in PLG preferentially induce a Th1 immune response [5]. Moreover, PLG microparticles have been used for many years in humans as resorbable sutures, prothesis and drug carriers [5]. They exhibit capacity to release an entrapped antigen over extend periods, and have been shown to be effective adjuvants for a number of antigens [5].

Olive pollen represents one of the most important causes of pollen allergy in the Mediterranean countries [6]. Ole e 1, a polymorphic glycoprotein of 145 amino acids, is the most prevalent allergen from olive pollen, affecting more than 70% of patients suffering from olive pollinosis [7]. In a previous work,

* Corresponding author. Tel.: +34-91-3944155; fax: +34-91-3944159.

E-mail address: ameva@bbm1.ucm.es (E. Batanero).

it was shown that PLG microparticles are a suitable vehicle for Ole e 1 [8]. The aim of this study has been to evaluate the ability of PLG microparticles to stimulate a Th1 response to Ole e 1 in mice.

2. Material and methods

2.1. Preparation of PLG microparticles

Ole e 1 was purified from olive pollen (Allergon, Ängelholm, Sweden) as previously described [7]. PLG microparticles with encapsulated Ole e 1 were prepared by the solvent evaporation method and characterized [8].

2.2. Immunization protocol

BALB/c mice ($n=8$ per group, 6 weeks old) were obtained from Harlan Interfauna Ibérica (Barcelona, Spain) and maintained at the University Animal Care Services, according to the local guidelines. Mice were immunized by intraperitoneal (i.p.) route with either PLG-microparticulated (5 $\mu\text{g}/\text{dose}$) or $\text{Al}(\text{OH})_3$ -adsorbed (1 $\mu\text{g}/\text{dose}$) Ole e 1 in PBS at days 0, 7, 14 and 21. Control groups received alternatively equivalent amounts of free Ole e 1 (5 $\mu\text{g}/\text{dose}$), placebo microparticles or $\text{Al}(\text{OH})_3$ under the same conditions.

2.3. Determination of antibody levels

Blood samples were collected from the retro-orbital plexus at day 28 and sera were stored at -20°C until analysis. An ELISA was performed to detect IgG isotypes and total IgE levels [9]. Optical density (OD) was measured on an ELISA reader at 492 nm.

2.4. Cytokine assays

Spleens were removed on day 28 and cells were isolated and stimulated with or without concanavalin A (Con A, 2 $\mu\text{g}/\text{ml}$) or Ole e 1 (preliminary studies demonstrated optimal cytokine production at 10 $\mu\text{g}/\text{ml}$) to produce IL-4 and IFN- γ as previously described [9]. IL-4 and IFN- γ were determined by sandwich ELISA (PharMingen, San Diego, CA, USA). The sensitivity of the IL-4 assay was 5 pg/ml and that of IFN- γ was 25 pg/ml.

3. Results

I.p. immunization with Ole e 1 encapsulated in PLG elicited high levels of specific IgG2a antibodies, and low levels of both total IgE and specific IgG1 antibodies (Fig. 1) in mice. In contrast, immunization with Ole e 1 adsorbed in alum induces high levels of both specific IgG1 and total IgE. Serum-specific IgG

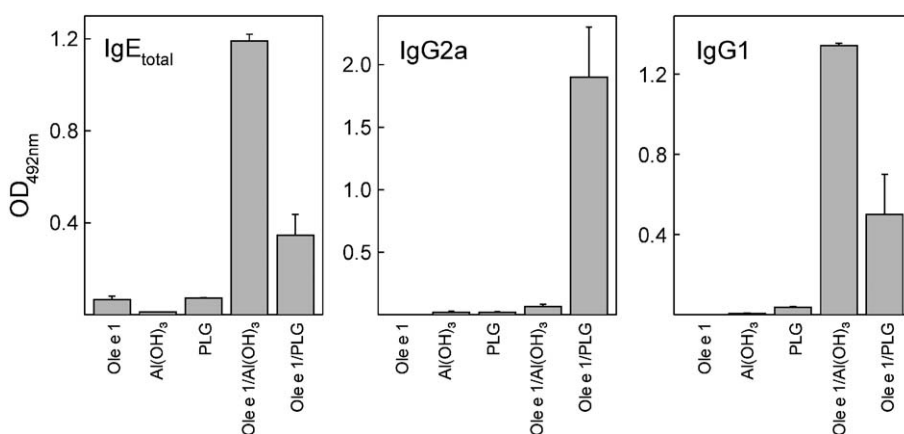


Fig. 1. Serum antibody response to Ole e 1 was measured by ELISA on pooled sera from each group of eight mice after immunization with the antigen encapsulated in PLG microparticles or adsorbed on alum on day 28 after the first immunization. Control mice were administered with Ole e 1, $\text{Al}(\text{OH})_3$ or placebo microparticles (PLG). Data are expressed as the mean optical density (OD) of duplicate assays. One representative experiment out of two is shown. Error bars show standard deviation.

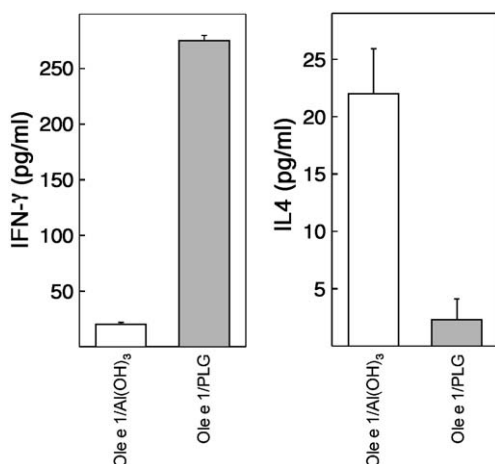


Fig. 2. Cytokine production by spleen cells from immunized mice after stimulation with Ole e 1 for 48 h. IL-4 and IFN- γ productions were determined by sandwich ELISA. Data are expressed as the mean of duplicate determination and expressed in pg/ml. Results are representative of two independent experiments. Error bars show standard deviation.

and total IgE levels in control mice were negligible. These results indicated a Th1 dominance after immunization of mice with PLG-encapsulated Ole e 1 versus Th2 dominance after immunization with Ole e 1 adsorbed in alum.

IFN- γ was detectable in supernatant cultures of splenocytes from mice immunized with Ole e 1 in PLG after *in vitro* stimulation with the allergen, whereas IL-4 production was found in splenocyte cultures from mice immunized with Ole e 1 in alum exclusively (Fig. 2). Neither IFN- γ nor IL-4 could be detected in cultures from control mice (data not shown). Cells from both immunized mice and control mice produced IFN- γ (710 ± 54 pg/ml) and IL-4 (48 ± 5 pg/ml) in response to Con A. According to their cytokine pattern, immunization with PLG-Ole e 1 stimulated the production of Th1-like cytokines, whereas the immune response induced by Ole e 1 in alum could be classified as Th2-like.

4. Discussion

In recent years, the potential use of PLG microparticles as a vaccine delivery system has been investigated for a number of diseases such as Alzheimer

[10], tetanus [11] or influenza [12], but there are scarce studies about the suitability of using such an antigen delivery system within the field of allergy [13,14].

Previous studies performed by our group have shown that Ole e 1 encapsulated in PLG microparticles is immunologically similar to the natural allergen [8]. Moreover, the features of the Ole e 1-microparticles (size $< 2 \mu\text{m}$, rapid antigen release and high allergen/polymer ratio) made them a good candidate as vehicle vaccine for therapy to olive pollen allergy. In this study, Ole e 1 encapsulated in PLG was tested for use as a parenteral delivery system in a mouse model. Data show that *i.p.* immunization with PLG-encapsulated Ole e 1 induced high levels of antigen-specific IgG2a. As BALB/c mice have an inherent tendency to developed a Th2 response [15], the dominance of IgG2a antibodies (9-fold increase compared to mice immunized with Ole e 1/alum) over IgG1/IgE levels (3-fold lower than in the Ole e 1/alum group) in the sera of mice immunized with Ole e 1/PLG indicates that PLG microparticles induce Th1-like responses to Ole e 1 *in vivo*. Moreover, *in vitro* stimulation of spleen cells from mice immunized with Ole e 1 in PLG produced at least five times more IFN- γ compared with mice immunized with the allergen in alum. In contrast, as it is known, *i.p.* immunization with Ole e 1 in alum promoted a Th2-like immune response that is characterized by high levels of IgE and IgG1 antibodies and production of IL-4 [9].

5. Conclusion

Ole e 1-PLG microparticles are able to induce a Th1-like immune response. This suggests that PLG-encapsulated allergen could be utilized to formulate antigen-specific vaccines against allergic disorders. Although in this study a single antigen was used as a model, the possibility to encapsulate multiple antigens will increase the potential of PLG as a vaccine delivery system for allergy.

Acknowledgements

This work has been supported by Grant 08.4/0022/98 from the Dirección General de Investigación of the Comunidad Autónoma de Madrid (Spain).

References

- [1] G.F. Del Prete, M. De Carli, M.M. D'Elis, P. Maestrelli, M. Ricci, L. Fabbri, S. Romagnani, Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders, *Eur. J. Immunol.* 23 (1993) 1445–1449.
- [2] S. Sehra, L. Chugh, S.V. Gangal, Polarized TH1 responses by liposome-entrapped allergen and its potential in immunotherapy of allergic disorders, *Clin. Exp. Allergy* 28 (1998) 1530–1537.
- [3] M. Kumar, A.K. Behera, H. Matsuse, R.F. Lockey, S.S. Mohapatra, A recombinant BCG vaccine generates a Th1-like response and inhibits IgE synthesis in BALB/c mice, *Immunology* 97 (1999) 515–521.
- [4] M. Toda, H. Sato, Y. Takene, Y. Taniguchi, S. Saito, S. Inouye, T. Takemori, M. Sakaguchi, Inhibition of immunoglobulin E response to Japanese cedar pollen (Cry j 1) in mice by DNA immunization: different outcomes dependent on the plasmid DNA inoculation method, *Immunology* 99 (2000) 179–186.
- [5] D.T. O'Hagan, M. Singh, R.K. Gupta, Poly (lactide-co-glycolide) microparticles for the development of single-dose controlled-release vaccines, *Adv. Drug Deliv. Rev.* 32 (1998) 225–246.
- [6] J. Bousquet, P. Cour, B. Guerin, F.B. Michel, Allergy in the Mediterranean area. I: pollen counts and pollinosis of Montpellier, *Clin. Allergy* 14 (1984) 249–258.
- [7] M. Villalba, E. Batanero, C. López-Otín, L.M. Sánchez, R.I. Monsalve, M.A. González de la Peña, C. Lahoz, R. Rodríguez, The amino acid sequence of *Ole e* I, the major allergen from olive tree (*Olea europaea*) pollen, *Eur. J. Biochem.* 216 (1993) 863–869.
- [8] E. Batanero, P. Barral, M. Villalba, R. Rodríguez, Biodegradable poly (DL-lactide glycolide) microparticles as a vehicle for allergen-specific vaccines: a study performed with *Ole e* I, the main allergen of olive pollen, *J. Immunol. Methods* 259 (2002) 87–94.
- [9] E. Batanero, P. Barral, M. Villalba, R. Rodríguez, Sensitization of mice with olive pollen allergen *Ole e* I induces a Th2 response, *Int. Arch. Allergy Immunol.* 127 (2002) 269–275.
- [10] D.J. Brayden, L. Templeton, S. McClean, R. Barbour, J. Huang, M. Nguyen, D. Ahern, R. Motter, K. Johnson-Wood, N. Vasquez, D. Schenk, P. Seubert, Encapsulation in biodegradable microparticles enhances serum antibody response to parenterally-delivered β -amyloid in mice, *Vaccine* 19 (2001) 4185–4193.
- [11] G.F. Kersten, D. Donders, A. Akkermans, E.C. Beuvery, Single shot with tetanus toxoid in biodegradable microspheres protects mice despite acid-induced denaturation of the antigen, *Vaccine* 14 (1996) 1627–1638.
- [12] S.C. Chattaraj, A. Rathinavelu, S.K. Das, Biodegradable microparticles of influenza viral vaccine: comparison of the effects of routes of administration on the in vivo immune response in mice, *J. Control. Release* 58 (1999) 223–232.
- [13] S. Sharif, A.W. Wheeler, D.T. O'Hagan, Biodegradable microparticles as a delivery system for the allergens of *Dermatophagoides pteronyssinus* (house dust mite): I. Preparation and characterization of microparticles, *Int. J. Pharm.* 119 (1995) 239–246.
- [14] S. Pecquet, E. Leo, R. Fritsché, A. Pfeifer, P. Couvreur, E. Fattal, Oral tolerance elicited in mice by β -lactoglobulin entrapped in biodegradable microspheres, *Vaccine* 18 (2000) 1196–1202.
- [15] C.S. Hsieh, S.E. Macatonia, A. O'Garra, K.M. Murphy, T cell genetic background determines default T helper phenotype development in vitro, *J. Exp. Med.* 181 (1995) 713–721.