IMPROVEMENT OF THE IMMUNE RESPONSE TO VACCINES BY THE ORAL ROUTE USING POLYMERIC FORMULATIONS

A. Montero[a], R.M. Hernández[a], M. Igartua[a], J.E. Rosas[b], M.E. Patarroyo[b] and J.L. Pedraz[a]

[a] Pharmacy and Pharmaceutical Technology Laboratory, Pharmacy Faculty, University of the Basque Country (UPV-EHU), Paseo de la Universidad nº 7, 01006 Vitoria-Gasteiz, Spain

[b] Fundación Instituto de Inmunología de Colombia (FIDIC), Carrera 50, nº 2600, Santafé de Bogotá, Colombia

Introduction

SPf66 was the first synthetic vaccine able to induce a partial protective immune response to malaria when it was formulated with alum as adjuvant in several pre-clinical and clinical trials (1). Recent investigations by subcutaneous route in animal models have demonstrated the applic ability of the PLGA microencapsulation to this antigen in order to improve the immune responses and protection induced by alum, both in mice (2) and monkeys (3), without integrity changes in the molecule. The humoral immune response induced by the SPf66 antigen was clearly increased when encapsulated in PLGA microparticles, even with a single immunization, suggesting the possibility of simplifying immunization protocols. The search for an ideal vaccine prompted us to test the oral immunogenicity of these microparticle formulations in Balb/c mice. Systemic IgG responses to the administration of an oral formulation were assessed. IgG isotype (IgG1, IgG2a) was also characterized in order to indirectly evaluate the activation of a Th1-like response.

Materials and methods

PLGA microparticles (MP) of two different polymers were formulated by a modification of the solvent extraction technique using double emulsion. Administered microparticles were a 1:1 mixture of Resomer® RG 506 particles, with a copolymer ratio of 50:50 lactic/glycolic (%), and Resomer® RG 756 particles, with a copolymer ratio of 75:25 lactic/glycolic (%), both supplied by Boehringer Ingelheim K.G. (Ingelheim, Germany).

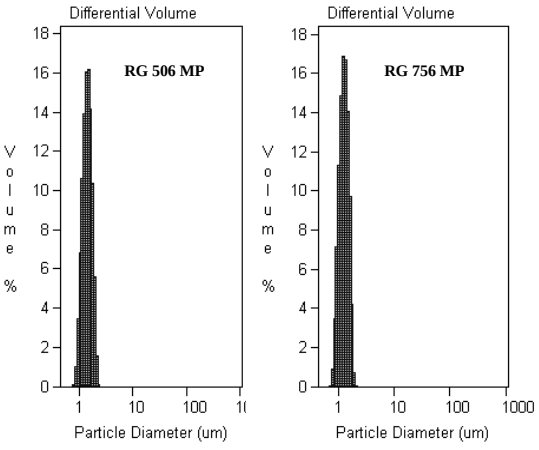

Figure 1. *Size distribution of the PLGA formulations*

For antibody induction against SPf66, 40 female Balb/c mice, aged 7 weeks (Harlan Interfauna Iberica S.L., Barcelona, Spain), were ramdomly divided into 4 groups of 10 and were immunized with the antigen.

Two groups of 10 mice were orally immunized at days 0, 1 and 2 with 500 µg of the antigen each day, with SPf66-loaded microparticles (MP), in 0.3% sodium bicarbonate by intragastric intubation. One of these groups was boosted with the same dose at days 21, 22 and 23. One group as control received orally 500 µg of free peptide at days 0, 1, 2, 21, 22 and 23. The fourth group of 10 mice was subcutaneously immunized in the

nape of the neck with SPf66 adsorbed onto aluminum hydroxide, Alum (100 µg SPf66 at days 0, 14 and 28, with 1,25 mg Al^{+3} per dose).
Blood samples were collected from the retroorbital plexus of the mice, under anaesthesia with ether, at weeks 3, 6, 9 and 12.
A conventional ELISA was used to determine anti-SPf66 antibodies. An affinity-purified goat anti mouse IgG (peroxidase conjugate, from Sigma) was used and total IgG titers were measured at all time points, while IgG1 and IgG2a isotypes were assessed with goat anti mouse IgG1 and goat anti mouse IgG2a (peroxidase conjugates, purchased from Southern Technology) at 6 weeks. The end-point titers were expressed as the maximum dilution which gave an OD405 above the mean OD405 of the preimmune sera plus 2 standard deviations.
The results were expressed as mean ± standard deviation (s.d.) of the log10 antibody titer of individual mice for each group. Normal distribution of samples was assessed by Shapiro-Wilk trial using the SPSS 10.1 program (SPSS®, Chicago, USA). Differences among groups of animals at significance levels of 95% were calculated by the non-parametric Mann-Whitney U-test.

Results and discussion

Freeze-dried, sphere-shaped microparticles were obtained. The mean particle size for RG 506 and RG 756 microparticles was 1.40 and 1.33 µm, respectively, with a narrow size distribution (Figure 1). Table 1 shows the results for peptide loadings and encapsulation efficiency for both formulations.

Table 1. *Microparticle characterization (n=3)*

Formulation	Size (µm)	Encapsulation efficiency (%)	Peptide loading (%)
RG 506	1.40 (95% 0,82-1,96)	84.0 ± 6.1	8.4 ± 0.6
RG 756	1.33 (95% 0,79-1,74)	77.3 ± 6.4	7.7 ± 0.6

The development of serum anti-SPf66 IgG antibody responses was investigated. As presented in figure 2, none of the animals immunized by the oral route with the peptide solution induced antibody levels above background values. The administration of three consecutive oral doses of 500 µg of SPf66 peptide encapsulated into microparticles elicited detectable antibody levels in only 10% (week 3) to 30% (weeks 9, 12) of the mice, and the difference was not statistically significant with the group immunized with the free peptide. Only when the animals were orally boosted at week 3 with microparticles the number of responders increases up to 80% at week 9, and the difference among boosted group and free antigen group was statistically significant from week 6. The subcutaneous administration of three doses of 100 µg of free antigen in alum at weeks 0, 3 and 6 elicited responses in 70% of mice at week 9, and no differences with the orally boosted group could be found.

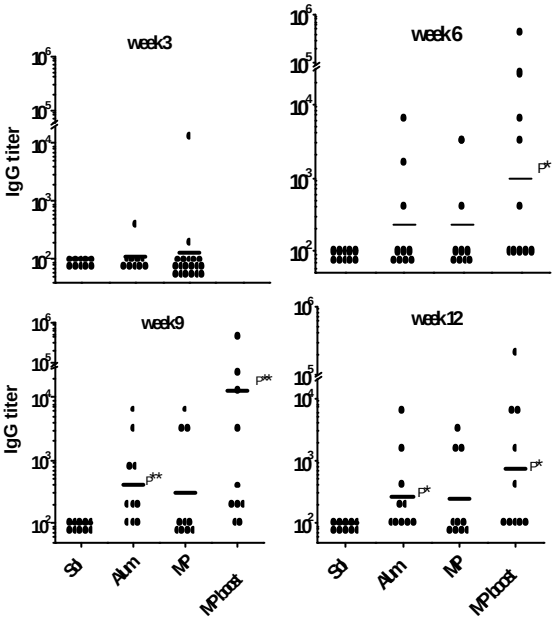

Figure 2. *Anti-SPf66 antibody responses (individual (•) or mean (—)) in sera of Balb/c mice immunized orally using free peptide (Sol), SPf66 loaded microparticles (MP), SPf66 loaded microparticles with boosting (MPboost) compared to subcutaneous SPf66 adsorbed on $Al(OH)_3$ gel (Alum).*
P: P<0,05; P**: P<0,01 (significantly different compared to Sol group)*

We next determined whether differences on the isotype profiles existed, measuring SPf66-

specific IgG1 and IgG2a levels. None of the mice immunized with free peptide elicited neither IgG1 nor IgG2a antibodies. Compared to the free peptide group, the alum group presented significantly higher (P<0,01) IgG1 levels, and similar IgG2a profile. Concerning to oral microparticles, only the boosted animals presented differences (P<0,01) with the free peptide group. Figure 3 shows that the IgG responses in mice vaccinated with alum formulation were biased towards IgG1 and, moreover, no IgG2a antibodies could be detected. On the other side, significantly higher IgG2a titers, with 60% of responders, were present in the sera of orally boosted mice, and also a 30% of the non-boosted animals were possitive for IgG2a isotype. IgG1 titer for the MP groups was significantly higher for the boosted group.

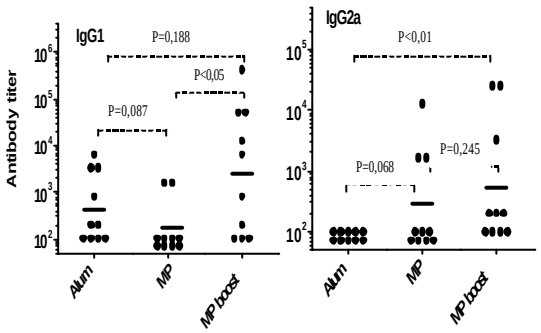

Figure 3. *Anti-SPf66 antibody IgG isotypes at 6 weeks in sera of Balb/c mice (groups of figure 2)*

By the oral route, PLGA particles protect the antigens from degradation into the gastrointestinal tract, and direct them to be captured by the gut associated lymphoid tissues (GALT), mainly represented by the Peyer's patches (4). Anyway, many attempts of eliciting immune responses using oral PLGA particles have failed and modifications on the vaccination protocol such as boosting doses are usually needed. In our case, only the boosted animals elicited a significant response.

The major difference between the immune response elicited by conventional alum formulation and PLGA particles is the induction of Th1-type responses to the PLGA particles. A marked shift was noted in the quality of the antibody responses in our study, characterized by significant levels of IgG2a antibodies by the oral PLGA formulations, compared to the absent levels of this isotype elicited by the alum adjuvant or by the free oral peptide. Since IgG2a is the isotype associated to the stimulation of B lymphocytes by the T helper type 1 (Th1) lymphocytes, capable of activating the cellular immune response, which is crucial for malaria, we hypothesize that immunological advantage could be obtained by this novel route of vaccination.

Conclusion

Significant immune responses are achieved after primary immunization and boosting with PLGA microparticles by the oral route. The response to conventional alum administration is qualitatively improved since IgG2a secretion is obtained by the oral polymeric formulation.

References

1. Patarroyo, M.E., Romero, P., Torres, M.L., Clavijo, P., Moreno, A., Martínez, A., et al., Nature, 328, 629, (1987).
2. Rosas, J.E., Hernández, R.M., Gascón, A.R., Igartua, M., Guzmán, F., Patarroyo, M.E., Pedraz, J.L.., Vaccine, 19, 4445, (2001).
3. Rosas, J.E., Pedraz, J.L., Hernández, R.M., Gascón, A.R., Igartua, M., Guzmán, F., Rodríguez, R., Cortés, J., Patarroyo, M.E., Vaccine, 20, 1707, (2002).
4. O'Hagan, D.T., In: O'Hagan, D.T. (Ed.), Novel delivery systems for oral vaccines, CRC Press, Boca Raton, Fl., 175, (1994).

Acknowledgements

This project was partly supported by grant AP99 16061993 from the Ministry of Education, Culture and Sports of Spain to A. Montero, and by the University of the Basque Country, UPV-EHU (UPV 00101.125-TB-8227/2000).

Corresponding author:

José Luis Pedraz Muñoz

E-mail: knppemuj@vc.ehu.es

Universidad del País Vasco

Paseo de la Universidad, 7, 01006, Vitoria-Gasteiz

Telephone: + 34 – 945013091 Fax: + 34 - 945013040