

INTRADERMAL ROUTE FOR MALARIA SYNTHETIC PEPTIDES LOADED MICROPARTICLES VACCINATION.

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Abstract

The aim of this study is to promote and characterize the immune response induced in mice after immunization with 2 antimalarial synthetic peptides, 3D7 and FC27, entrapped into microparticles (MP) of poly-D,L-lactide-co-glycolide acid (PLGA) and administrated intradermally (id), against Montanide ISA-720™ (M-ISA720) adjuvant, accepted for clinic assays, and based on a (w/o) emulsion administered by the traditional immunization subcutaneous (sc) route.

FC27 peptide loaded into PLGA MP administered id induced greater antibody titres than emulsified in M-ISA720 by sc conventional route, whereas 3D7 peptide elicited minor differences between both formulations. A booster dose was able to improve immune response with regard to IgG2a production; moreover, FC27 loaded MP given id overcame M-ISA720 adjuvant in that enhance of IgG2a subclass. The id immunization with microencapsulated FC27, although it evoked higher levels of specific IgE, they were well-correlated with those of IgG1, whereas emulsified in M-ISA720 induced superior levels of IgE than IgG1.

Resumen

El objetivo del presente estudio consiste en promover y caracterizar la respuesta inmune inducida en ratones tras su inmunización con 2 péptidos sintéticos antimaláricos, 3D7 y FC27, encapsulados en micropartículas (MP) de poli-láctico-co-glicólico (PLGA) y administradas intradérmicamente (id), frente al adyuvante Montanide ISA-720™ (M-ISA720), ya aceptado en ensayos clínicos, y basado en una emulsión (w/o) administrada por la tradicional vía de inmunización subcutánea (sc).

El péptido FC27 formulado en microesferas de PLGA (PLGA MP) y administrado id indujo mayores títulos de anticuerpos que emulsificado en M-ISA720 por la vía convencional sc, mientras que el péptido 3D7 originó menores diferencias en cuanto a la producción de anticuerpos entre ambas formulaciones. Una dosis de recuerdo es capaz de mejorar la respuesta inmune en lo referente a la producción de IgG2a; es más, las MP cargadas con FC27 administradas por vía id superaron al adyuvante M-ISA720 en dicho incremento de la subclase IgG2a. La inmunización id con el péptido FC27 microencapsulado, a pesar de que originó mayores niveles de IgE específica, mantuvo una buena correlación entre estos y los de IgG1; mientras que emulsificado en M-ISA720 indujo niveles superiores de IgE que de IgG1.

Introduction

Today 300-500 million acute illnesses and at least one million deaths annually, are caused by malaria. Most of them occur in Sub-Saharan Africa, and principally affects young children. Numerous efforts have been carried out since 90's to control and prevent this infectious disease, but vaccination could be the best strategy in this war. One obstacle in this sense is the necessity of well-defined structures like synthetic peptides. They are in general, poor immunogenic by themselves and require several strategies to improve their immune response (1). The used of adjuvants to raise immunogenicity is a possible strategy, but often conventional adjuvants like aluminium salts, are unable to stimulate an appropriate immune response (2). Poly-D,L-lactide-co-glycolide acid microspheres (PLGA MP), have been presented as a promising alternative to those conventional adjuvants, improving immune response, increasing it,

reducing the number of inoculations and total amount of antigen (3), and eliciting a shift of the immune response from an exclusive

humoral immunity (Th2-type) towards cellular (Th1) (4). Intradermal (id) immunization with microparticles represents another alternative for traditional vaccination routes such as subcutaneous (sc) or intramuscular (im). Skin is a possible site to elicit potent immune responses and constitute an immune organ itself (5). Recent works reported from our laboratory have demonstrated that id route overcomes sc even with an antigen dose 10 times minor (6).

The aim of this study is to promote and characterize the immune response elicited by two malarial synthetic peptides formulated into biocompatible and biodegradable microparticles administrated by intradermal route and compare to Montanide ISA™ 720, an emulsion based adjuvant tested on clinical trials (7).

Materials and Methods

Malaria synthetic peptides, around 100-120 amino acids, correspond to the merozoite surface protein 2 (MSP-2) of *Plasmodium falciparum*. They were called as the two principal allelic families to whom they correspond due to the dimorphism presented at the central region, 3D7 and FC27. They were supplied by the Institute of Biochemistry, University of Lausanne, Switzerland.

PLGA MP were prepared by a modified (w/o/w) double emulsion solvent extraction technique using Resomer® RG 503 with a 50:50 lactic/glycolic copolymer ratio, supplied by Boehringer Ingelheim (Ingelheim K.G., Germany). An aqueous solution of peptide 10% (w/v) was emulsified with PLGA dissolved 5% (w/v) in dichloromethane by sonication for 30 s (Branson® T-sonifier 250) giving a theoretical peptide load of 5% (w/w). This emulsion (w/o) was poured to a polyvinyl alcohol aqueous solution 8% (w/v) (PVA, average *M_w* 30,000–70,000, Sigma Chemical Co., Madrid, Spain) to perform a second double emulsion (w/o/w) by turbine homogenization during 5 min at 9,500 rpm (Ultraturrax® T-25). Then a 2% isopropanol solution was added and stirred for 1 h to facilitate the organic solvent extraction to the external phase and evaporation.

Finally microspheres were collected and washed by centrifugation / resuspension on distilled water and freeze-dried.

Principal parameters were studied to characterize PLGA MP. Size distribution was determined by laser diffractometry (Coulter Counter® T-LS130) and zeta potential was measured using laser doppler velocimetry (Malvern Zetasizer 3000). Total loaded peptide and surface adsorbed peptide were estimated by micro-BCA assay, with a working linearity for peptides diluted in the range of 5-20 µg/ml.

Montanide ISA™ 720 (M-ISA720, SEPPIC, Paris, France), is an oil composition containing a natural metabolizable nonmineral oil and a highly refined emulsifier from the mannide mono-oleate family (8, 9). Just immediately before the administration, an aqueous peptide solution was mixed with M-ISA720, in a 7:3 (v/v) oil /aqueous phase ratio, for a final concentration of 200 µg/ml. The emulsion (w/o) was performed by making up and down through a crystal syringe with a needle diameter of 21G.

For immunization study, 32 female C57BL/6J mice, aged 7 weeks (Charles River Laboratories, Barcelona, Spain), were divided into four groups of eight and were each vaccinated with one of the peptides id or sc. Two groups received 3D7 peptide either loaded into PLGA MP and by id route, "3D7MPid", or emulsified with M-ISA720, "3D7Mont" by sc. Another two groups were given FC27 peptide, like for 3D7, formulated as "FC27MPid" or "FC27Mont". All groups received the same amount of peptide, an initial dose of 20 µg and a booster dose of 2 µg at 20 weeks. Groups vaccinated with M-ISA720, were sc injected with a volume of 100 µl at the nape of the neck. Groups immunized with MP formulations by id route, were given 50 µl of the particles suspension in PBS divided into 4-5 injections at the shaved back skin.

Blood samples were collected prior to immunization and periodically until 30 weeks bleeding animals by the retroorbital plexus under ether anaesthesia. These samples were centrifuged at 3,000 x *g* for serum collection and stored frozen at -80°C until being assayed by ELISA.

Results and Discussion

The principal parameters that characterize microparticles were evaluated. The mean particle size was around 1 μm with a narrow size distribution. Encapsulation efficiency was better for 3D7 peptide than FC27 (93% and 58%, respectively). The final peptide loading was 46 $\mu\text{g}/\text{mg}$ MP for 3D7 and 29 $\mu\text{g}/\text{mg}$ MP for FC27. Both peptides presented an important percentage of themselves adsorbed to the microspheres surface (around 50%).

For the immunization study, we compared the two formulations, MP given id and M-ISA720 by sc route, for both peptides. Mean IgG titres from 2 up to 30 weeks were represented (Figures 1, 2). 3D7 peptide elicited slight higher IgG production for PLGA MP given id than M-ISA720. "FC27MPid" group also overcame M-ISA720, and induced significantly greater antibody levels than it at all time points ($p < 0.01$).

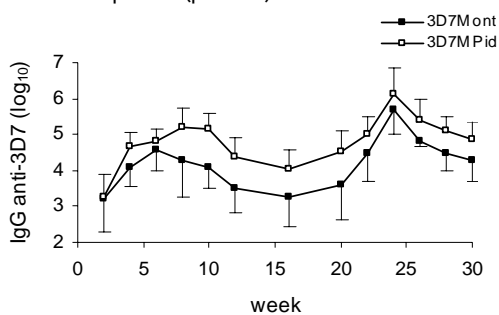


Figure 1. Specific IgG production in C57BL/6j mice vaccinated with 3D7 at 0, 20 weeks

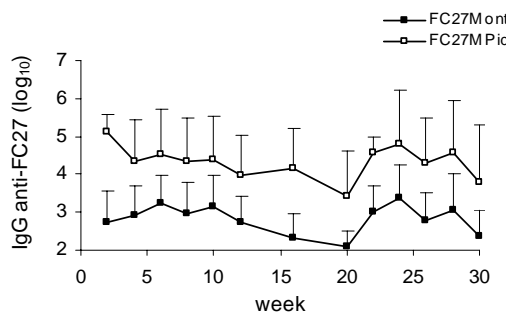


Figure 2. Specific IgG production in C57BL/6j mice vaccinated with FC27 at 0, 20 weeks.

To evaluate the Th subset (Th1 or Th2), IgG subclasses production and their ratios were measured at 8 and 26 weeks, in order to establish the possible effect of a booster dose on the type of response. Although a mixed

IgG1/IgG2a response was elicited, IgG2a levels were hardly detectable and IgG1 subtype predominated after first dose, with similar titres for 3D7 peptide formulations, but higher for "3D7MPid" after boosting ($p < 0.05$); whereas FC27 PLGA MP produced superior IgG1 titres than M-ISA720 before and postboosting ($p < 0.01$). "FC27Mont" group was the only one whose IgG1 levels were reduced after booster dose. All groups elicited similar IgG2a subclass levels, even after boosting; except "FC27Mont" group whose increase postboosting was minor than that of the microencapsulated group (Figures 3, 4).

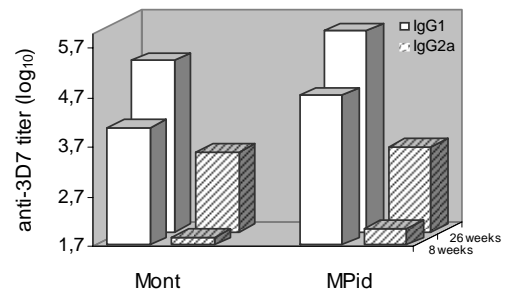


Figure 3. IgG isotypes at 8 weeks and 26 (6 weeks after boosting) for 3D7 immunization.

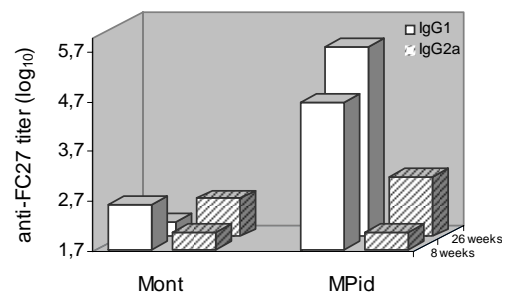


Figure 4. IgG isotypes at 8 weeks and 26 (6 weeks after boosting) for FC27 immunization.

The booster dose evoked an important increase of IgG2a subclass levels, and a consequent decrease in the IgG1/IgG2a ratio of all groups (Figure 5), suggesting an enhanced Th-1 type activation. The ratios were similar for 3D7 peptide; but not for FC27, where M-ISA720 emulsion gave a lower ratio than PLGA MP. This was due to the slight antibody response elicited by FC27 M-ISA720 immunized group, with IgG levels notably inferior than that of PLGA MP, resulting in a remarkably lower IgG1 secretion, but not for IgG2a subclass.

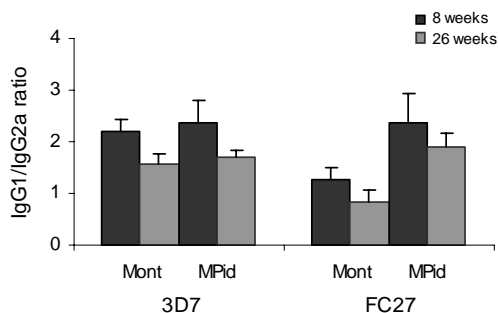


Figure 5. IgG1/IgG2a ratio for both formulations after a first dose of immunization (■ 8 weeks) and a booster dose (▒ 26 weeks).

Finally, IgE antibody was studied as a component of immune response positively controlled by Th2-type cytokines (8) (Figures 6, 7). The specific IgE secretion was similar for both MP and M-ISA720 in 3D7 peptide immunized groups, but FC27 mice vaccinated with MP showed superior levels than M-ISA720. However, IgE antibody was well-correlated with the IgG1 subclass, with similar IgE/IgG1 ratios for all cases except for "FC27Mont" group, whose ratio was superior after first dose, and it was reversed to higher IgE levels than IgG1 after boosting.

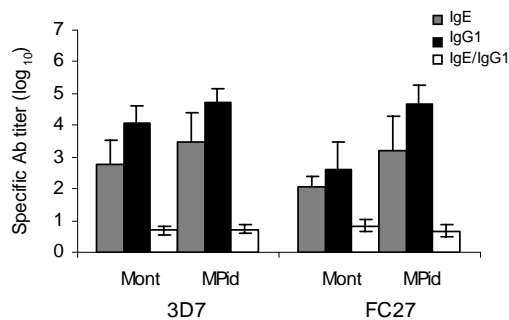


Figure 6. IgE secretion compared to IgG1 production at 8 weeks of immunization.

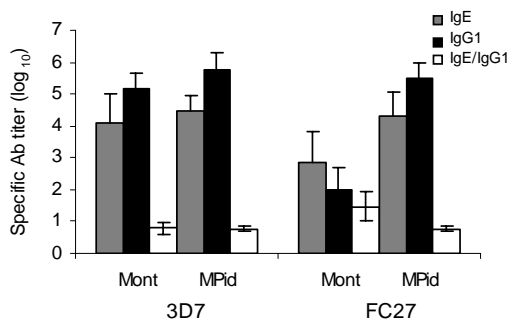


Figure 7. IgE secretion compared to IgG1 production at 6 weeks post-boosting (26 weeks).

The results obtained have demonstrated that PLGA MP id immunization is able to overcome the immune response elicited by M-ISA720 given sc. Overall for FC27 peptide, the MP not only enhanced the antibody levels, but also promoted a shift to a Th1 subset, and guaranteed a well-balanced IgE response, which was not reached with the M-ISA720 emulsion immunization.

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Acknowledgements

This project was supported by a Basque Government's research grant given to E. Mata, and by the Ministry of Education and Culture of Spain (SAF 2004-02742).

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