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Immunogenicity of synthetic peptides derived from *Plasmodium falciparum* proteins

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Abstract

To obtain antibodies suitable to be used in an antigen-capture assay, we have identified, synthesized, and evaluated a series of peptides from different *Plasmodium falciparum* excretory–secretory proteins: glutamate-rich protein (GLURP); histidine-rich protein 2; histidine-rich protein 3; Falciparum interspersed repeat antigen and, serine-rich antigen homologous. Conformational as well as antigenic predictions were performed using the ANTHEPROT package. Chemical synthesis was carried out by the multiple manual synthesis using the t-boc strategy. The peptides were used as antigens for the preparation of polyclonal antibodies in rabbits. Out of the 14 peptide constructs, eight by ELISA and, six by MABA elicited antibodies that showed correspondence between the predictive study and the immunogenicity obtained in rabbits. All antipeptide (GLURP, HRP2, and FIRA) antisera were found to bind to the corresponding synthetic sequence in an ELISA assay. The binding activity and specificity of antibodies were determined by Western blot with supernatant culture from *P. falciparum*. Anti-GLURP (IMT-94 and IMT-200) antisera bound to five molecules present in supernatant with molecular weight of 73, 82, 116, 124, and 128 kDa. Anti-HRP2 (IMT-192) antisera recognized a band of 58 kDa. In both cases, the specific molecules were inhibited by preincubation with the homologous peptide. Anti-HRP3, anti-FIRA neither anti-SERPH antisera showed reactivity. Antipeptides HRP2 antibodies recognized the recombinant protein present in Parasight™-F test. The same way, synthetic peptides from HRPII molecule were recognized by monoclonal antibody present in the Parasight™-F assay. Our results confirm the potential value of synthetic peptides when inducing monospecific polyclonal antibodies for the development of diagnostic tests based on the capture of antigens.

Index Descriptors and Abbreviations: Immunogenicity; Peptides; MABA; *Plasmodium falciparum*

1. Introduction

There is a worldwide increasing concern about malaria, not only because of the high morbidity and mortality, but also, because of the progressive increase of its prevalence in tropical areas, where control measures were effective in the past. Malaria causes more than three hundred million acute illnesses and kills at least one million people every year. Ninety percent of deaths due to malaria occur in sub-Saharan Africa and most of them occur in children under the age of five (WHO/TDR Malaria Database).

Changes in the strategy of control such as preventing and controlling malaria during pregnancy, promoting the use of insecticide-treated mosquito nets, and prompt access to effective treatment, had been strategically emphasized by WHO (Roll Back Malaria). Prompt treatment is dependent

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on immediate and accurate malaria diagnosis. The classical thin and thick smears have certain limitations (personnel with expertise, microscopes, etc.), which have stimulated the development of new diagnostic methods suitable for field conditions. Therefore, alternative diagnostic methods suitable for use in malaria endemic areas are needed. Several novel, non-microscopical diagnostic techniques have been developed. These methods include different PCR-based techniques (nested PCR and RT-PCR). The limits of detection of these methods (2.5–10 parasites/μL) are equal to (or better than), those provided by light microscopy, but in general, these are technically difficult to perform and their use is restricted to reference centers (Malhotra et al., 2005; Ndao et al., 2004; Siribal et al., 2004; Snounou et al., 1993a,b). Other techniques for the laboratory diagnosis of malaria have been sought for use in endemic and non-endemic areas. Immunodiagnostic approaches based on antibody capture of circulating antigens to *Plasmodium spp* have also been developed and are promising, since they are fast and reliable. The simplest, and therefore most suitable for use in the field, is an immunochromatographic strip tests. These currently marketed strips are often too expensive for routine clinical diagnostic use or for malaria control programmes in endemic areas. There are three methods based on the capture of histidine-rich protein (HRP2): two that use IgG monoclonal antibody, the Parasight™-F (Beckton and Dickinson) (Shiff et al., 1993) and, Now® ICT™ Malaria Pf/Pv (Binax, Inc., Portland, ME), which capture *Plasmodium* aldolase, in addition to the HRP2 protein (Moody, 2002) and, PATH Falciparum Malaria IC test (Mills et al., 1999), that uses IgM monoclonal antibody, which binds to the HRP2 antigen. The other tests detect parasite lactate dehydrogenase (pLDH) (OptiMAL®, Flow Inc., Portland, Org.) (Piper et al., 1996). These tests have adequate sensitivity of >90% and near 100% at high parasite densities under field conditions and specificities of >90% (Arróspide et al., 2004; Coleman et al., 2002; Fryauff et al., 2000; Mills et al., 1999; Palmer et al., 1998; Vanderjagt et al., 2005; Wongsrichanalai et al., 2003; WHO, 1996). However, some methods lack enough specificity under certain conditions (such as Parasight™-F) specially in cases of persons having positive rheumatoid factor (Laferi et al., 1997). Another problem is the cost of each test, still too high for the majority of endemic countries (US $ 2–10/test) (Makler et al., 1995). Based on these facts, we have identified, synthesized, and evaluated a series of immunogenic peptides from different *Plasmodium falciparum* excretory–secretory proteins, to obtain specific antibodies suitable for an antigen-capture assay.

2. Materials and methods

2.1. Peptide design and synthesis

The selected excretory and secretory proteins from the *P. falciparum* were analyzed by software "ANTHEPROT V.4.0. (Deleage, G. Institut Biologie et Chimie des Proteines 7, Lyon. France), and by Hoops and Woods (1981). Fourteen peptides were synthesized from the whole sequences of the: GLURP (glutamate-rich protein, GenBank Accession No. A45555); HRP2 (histidine-rich protein 2, GenBank Accession No. AAC47453); HRP3 (histidine-rich protein 3, GenBank Accession No. AAC47454); FIRA (Falciparum Interspersed Repeat Antigen, GenBank Accession No. A54641); SERPH (serine-rich-antigen homologous, GenBank Accession No. AAA29764). These peptides were synthesized by the multiple manual synthesis described by Merrifield (1963) and modified by Hougthen et al. (1986), using the t-boc strategy. To allow co-polymerization of peptides it was added Cys-Gly and Gly-Cys at the amino- and carboxy-terminal ends.

2.2. Immunization

New Zealand white rabbits (Instituto Nacional de Higiene, MSDS, Venezuela) were immunized subcutaneously on the back with 250 μg of different peptides emulsified (1/1 v/v) with complete Freund's adjuvant (CFA). Half of that amount of peptides were dissolved in incomplete Freund's adjuvant (IFA) and used to boost immunization on days 15 and 30. Rabbit sera were collected on days 0 and 40 for immunological assays. Bleeding of rabbits was made under anesthesia with intramuscular 10 mg/kg ketamine.

2.3. Plasmodium falciparum in vitro culture

A *P. falciparum* strain (FCB2) was maintained in continuous culture using RPMI 1640 medium supplemented with 10% human serum, under reduced oxygen tension, according to the methods described by Trager and Jensen (1976). The supernatant was removed, and aliquots were stored at −70 °C or in liquid nitrogen and used as an antigen in assays.

2.4. ELISA measurements

The level of antibodies to peptide constructs were measured by enzyme linked immunosorbent assay (ELISA) according Voller et al. (1974). Briefly, microtiter plates (Dynex, Virginia, USA) were coated with the supernatant culture from *P. falciparum* (parasitemia 10%) diluted in carbonate–bicarbonate buffer (pH 9.6) to a concentration of 10 μg/mL, or the different peptides at 5 μg/mL. The plates were incubated overnight at 4 °C and blocked with 1% BSA for 2 h. Rabbits sera diluted 1:100 were added in triplicate, and the mixture were incubated at room temperature for 1 h. The plates were washed three times with 0.05% phosphate-buffered saline (PBS)–Tween 20 (PBS-T) buffer between each incubation step. Color was developed with peroxidase-conjugated goat anti-rabbit IgG (Sigma, St. Louis, MO), followed by H_2O_2 with *o*-phenylenediamine (Pierce Biotechnology, Rockford, IL). Absorbance was read at 492 nm on a Synergy™ HT reader (Bio-Tek, Vermont, USA).