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Antibody responses to *Plasmodium falciparum* vaccine candidate antigens in three areas distinct with respect to altitude

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Abstract

Antibody levels against malaria antigens were measured among patients presenting with uncomplicated malaria at health centers from three locations in Zimbabwe (Bindura, Chiredzi and Kariba) that are distinct with regard to altitude and climatic conditions. Antibody levels were determined by ELISA using the antigens, apical membrane antigen 1 (AMA-1), erythrocyte binding antigen 175 (EBA-175), circumsporozoite surface protein (CSP), merozoite surface protein 1 (MSP-1) and Pfg27. For all the antigens tested, IgG and IgM levels were higher for Bindura (altitude 1100 m) compared to Kariba (<600 m, altitude) and Chiredzi (∼600 m, altitude) with the exception of IgG and IgM to AMA-1 and EBA-175 which were similar between Chiredzi and Bindura. Plasma samples were further analyzed for their functional activity by testing their ability to inhibit the growth of *Plasmodium falciparum* in culture. Our results, determined by microscopy and verified by the LDH assay revealed that plasma from the three locations had similar inhibitory activity against the growth of *P. falciparum in vitro*. Our data revealed that highest growth inhibition correlated with the highest levels of MSP-1 antibody values.

Keywords: *P. falciparum*; Antibody responses; Malaria vaccine; Transmission; Zimbabwe

1. Introduction

Malaria remains a disease of major public health importance especially in sub-Saharan Africa (Keiser et al., 2004; Donnelly et al., 2005; Wang et al., 2005). People exposed to malaria parasites mount immune responses that can be measured for example by antibodies to specific antigens corresponding to various life cycle stages of the parasite. With the advent of genomic approaches and availability of recombinant antigens, it has become possible to employ defined antigens corresponding to different life cycle stages considered to be vaccine candidates. Functional characterization of

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such immune responses is critical for validation of a set of antigens as good vaccine candidates. Some of the proteins routinely employed have included MSP-1, EBA-175 and AMA-1 that are expressed during the asexual blood stage and CSP expressed during the sporozoite stage of the parasite. The MSP-1 protein has been studied extensively and antibodies to this protein have been found abundantly in naturally exposed people from endemic areas (Cavanagh et al., 2004; John et al., 2004). Monoclonal and polyclonal antibodies inhibit parasite growth *in vitro* and immunization/challenge experiments in animals have all established MSP-1 as a strong vaccine candidate (Hui et al., 1994; Chang et al., 1996; Lyon et al., 1997; Good et al., 1998). Furthermore, phase I clinical trials demonstrated that recombinant MSP-1 (MSP-1_{19}, MSP-1_{42} or N-terminal blocks 3 and 4) is safe and immunogenic in both adults (Ockenhouse et al., 2006; Stoute et al., 2007) and children (Genton et al., 2003). Phase 2 clinical trials are currently underway to determine the efficacy of the MSP-1 (MSP-1_{19} or MSP-1_{42}) vaccine against clinical illness.

Studies have shown that antibodies directed against AMA-1, a protein localized in the rhoptry organelles of merozoites (Crewther et al., 1990) neutralize the invasion of merozoites (Narum et al., 2000). Further significance of AMA-1 as a potential vaccine candidate was demonstrated by protection induced by immunization with recombinant AMA-1 against *P. chabaudi* and *P. yoelii* infections in rodents (Narum et al., 2000), and in monkeys against *P. knowlesi* (Stowers et al., 2002) and by human studies where antibodies to the full length AMA-1 were associated with protection in Kenyans across all age groups (Polley et al., 2004). Likewise, erythrocyte binding antigen 175 (EBA-175), a 175 kDa merozoite expressed protein located in the micronemes, that mediates sialic acid-dependent invasion of red blood cells (RBC) (Camus and Hadley, 1985; Sim et al., 1994) has also been shown to elicit potentially protective antibody responses (Okenu et al., 2000; Singh et al., 2002; John et al., 2005).

Antigens in the sexual stages are also potent in eliciting immune responses and may be used to measure transmission potential. Gametocyte surface proteins like Pfs230 and Pfs48/45 have been shown to be targets of antibody mediated immunity and are potential candidates for transmission blocking vaccines (Healer et al., 1997; Lobo and Kumar, 1998). The immune system also recognizes intracellular proteins of sexual stage parasites such as Pfg27 and antibodies to this protein were detected in more than 95% of plasma from people in malaria endemic areas (Riley et al., 1994). Though antibodies to intracellular proteins are not associated with transmission blocking activity, they can be used as markers of malaria transmission (Healer et al., 1997).

In addition to understanding mechanisms of immune acquisition, antibodies may also act as surrogate markers of transmission intensity. Since antibodies persist for periods ranging from months to years, such markers may have an added advantage in that they can reveal and predict long term trends that do not reflect seasonal variation. Drakeley et al. (2005) found that antibodies to MSP-1_{19} correlated positively with malaria endemicity and negatively with altitude in a study conducted in Tanzania. Thus antibody measurements may provide a quick tool for estimating malaria exposure and transmission. We examined plasma samples from symptomatic patients in three different endemic areas of Zimbabwe and tested them for antibody responses against *Plasmodium falciparum* stage specific antigens. In addition, the functional activity of these antibodies were tested through growth inhibition assays. The three areas chosen for immune response analysis differ not only with respect to altitude but also for key environmental variants such as temperature and rainfall, significantly impacting malaria transmission dynamics (Ebi et al., 2005).

2. Materials and methods

2.1. Source of specimens and ethical considerations

Samples for this immunoepidemiology study were collected under the auspices of the National Institute of Health Research in Harare, Zimbabwe (formerly, Blair Research Institute). The samples included blood slides, thick and thin smears stained with Giemsa and blood plasma collected from people presenting with uncomplicated malaria (presentation of fever, headaches, chills or general malaise in the absence of severe symptoms such as coma, seizures and anemia) at health centers (Rutope clinic in Bindura, Rupangwana clinic in Chiredzi and Nyamhunga clinic in Kariba, the health centers will latter be referred to by the locations they represent) during peak transmission months of March and April 2003. Bindura is 60 km north of Harare the capital city and the clinic where samples came from serves a catchment of about 5000 people. The area studied is typically peri-urban and most of the people live in brick-houses and they earn a living through subsistence farming. Chiredzi is 580 km south-east of Harare, near the border with Mozambique. Samples were collected from Rupangwana clinic which has a catchment size of 8000 people. The area is rural with the majority of the people living in thatched houses made up of clay and who are primarily subsistence farmers. Kariba is a tourist resort town 366 km north west