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Identification and typing of Cameroonian isolates of *P. malariae* using monoclonal antibodies against *P. brasilianum*

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Abstract

In the present study, monoclonal antibodies raised against *Plasmodium brasilianum* were used to demonstrate, for the first time, antigenic diversity in natural populations of *Plasmodium malariae* isolates and as diagnostic tool to detect low parasitaemia *P. malariae* infection. Seventeen McAbs reacting by indirect immunoflorescence antibody (IFA) assay with no other Plasmodium species than *P. brasilianum*, were shown to react with *P. malariae* and were used for typing 29 *P. malariae* isolates from hyperendemic areas in Yaounde and in three villages of South Cameroon with parasitaemia ranging from 0.01% to 1.8%. All 29 isolates were distinguished by their ability to react with certain antibodies and considered as representing different isolates of *P. malariae*. One of these McAbs (No. 14) recognized *P. malariae* isolates to both in Yaounde and from Mengang but not in Edou or in Nkol Mvae, which may recognize a specific epitope that is less common in strains found in these villages and provide evidence of regional variation within the *P. malariae* parasites. The McAbs Nos. 16 and 17 were used to determine their usefulness as diagnostic tools for 30 suspected blood samples that were collected from patients with fever and it became clear that they could detect sub-microscopical infections of *P. malariae*. This study supports the concept of using of *P. brasilianum* as a substitute for *P. malariae* during immunodiagnosis of malaria in endemic areas where PCR assay cannot be used for identification of the *P. malariae* parasites. In addition our results for the first time provide evidence of considerable antigenic diversity of clinical *P. malariae* isolates in Cameroon.

Keywords: *P. malariae*; *P. brasilianum*; Monoclonal antibody; Antigenic diversity; Diagnosis

1. Introduction

Plasmodium malariae is a cosmopolitan human malaria parasite, which has been poorly studied, mainly because it seldom causes acute disease and cannot yet be propagated *in vitro*. Blood stage forms build up slowly and parasitaemia levels remain low, a feature that may be explained by a lower number of merozoites per schizonts, a longer asexual cycle (72 h compared to 48 h for all other human malaria parasites) and a preference for older erythrocytes. Acute attacks of *P. malariae* infection are seldom life threatening, even in non-immunes. The infection is chronic, with the parasite remaining in the peripheral blood for years, possibly for life, with occasional clinical recrudescences. The chronic nature of the infection may be responsible for extensive nephropathies, which may progress to renal failure

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due to deposition of immune complexes. Nephrotic syndrome occurs in many parts of Africa, where it is about 100 times more frequent than in Europe and America (Abdurrahman et al., 1983; Greenwood and Whittle, 1981; Hendrickse et al., 1972; Kibukamusoke and Voller, 1970; Kibukamusoke et al., 1967). Because of the frequent low parasitaemia, with occasional recrudescence, and its prevalence in asymptomatic carriers (Black et al., 1994; Trape et al., 1985), blood film surveys may not give a true indication of its prevalence, and fever surveys may also give an underestimation.

Parasite variability is a major factor in parasite survival strategies. Some parasites rely on random mutations to create variation and this may create difficulties for the host to eliminate them. Despite extensive studies on diversity in *Plasmodium falciparum* and *Plasmodium vivax* isolates from patients, no such studies have so far, been carried out in wild isolates of *P. malariae*. The chronic nature of *P. malariae* infection may be due to variation, which may occur in antigens involved in protective immunity against this parasite.

Plasmodium brasilianum is a quartan malaria parasite of New World monkeys and is closely related to *P. malariae*. In fact, it has been proposed that *P. brasilianum* may be a strain of *P. malariae*, which became adapted to New World monkeys sometimes the early 1600 s (Contacos et al., 1963). *P. brasilianum* is often used as antigen during immunological studies, when *P. malariae* antigen is difficult to obtain. A close evolutionary relationship between *P. brasilianum* and the human malaria parasite *P. malariae* is suggested by analogies in the morphology and the course of development of the erythrocytic and exoerythrocytic stages of these two parasites in primate hosts (Contacos et al., 1963). Immunological data on the immunodominant circumsporozoite protein of these parasites (Cochrane et al., 1985, 1984) and amino acid sequence data on this protein (Lal et al., 1987) strongly support this relationship. In addition, *P. brasilianum* is readily transmitted under experimental conditions to human by both mosquito bites (Contacos et al., 1963) and inoculation of parasitized blood cells (Coatney et al., 1971).

A study for typing clinical isolates of *P. malariae* by indirect immunofluorescent test (IFAT) was conducted using a subset of monoclonal antibodies (McAbs) raised against a *P. brasilianum*, as the antigenic diversity in natural population of *P. malariae* had not previously been investigated. When using these McAbs to recognize isolates of *P. malariae* in patient samples collected in south Cameroon, it became clear that they could also be used as tools for the detection of sub-microscopical infections of *P. malariae*.

2. Materials and methods

2.1. Study areas and P. malariae isolates

Bloods samples were collected from patients of various ages both in Yaoundé, the capital of Cameroon, and in Mengang, Edou and Nkol Mvae, villages located approximately 100 km east of Yaoundé. The ethical concerns were cleared by the Cameroonian Ministry of Health and the local district health and administrative officials.

In Yaoundé, blood was mainly collected from patients visiting the Dispensaire de Messa, a primary health care centre in central Yaoundé. These patients had different degrees of malaria symptoms i.e. fever and tiredness, but were not necessarily slide positive. In the rural villages, blood was mainly obtained from school children aged 6–17 years of age or from younger children who inhabited the villages and were brought to the study team by their mothers.

P. vivax is basically non-existant in Cameroon and *Plasmodium ovale* is very rare while both *P. falciparum* and *P. malariae* are widespread. Hence, Giemsa stained thick and thin blood smears were used to determine the Plasmodium species.

2.2. Monoclonal antibodies (McAbs)

The antigen used for immunization was prepared from squirrel monkeys infected with *P. brasilinanum* (Peru strain). Initial immunizations were performed with antigens (equal to 10^6 parasitized erythrocytes) emulsified in complete Freund's adjuvant and injected intravenously into Biozzi x Balb/c mice. Mice received intense course of twice weekly intravenous injections, for 3 weeks consisting of the same amount of antigen in incomplete Freund's adjuvant. Mice were bled and plasma was tested for the presence of anti-parasite antibody by indirect immunoflorescence antibody (IFA) assay. The mouse with the highest titer was boosted with the antigens intravenously without adjuvant, and splenocytes were used for hybridization 4 days later.

Hybridomas were produced by fusing immune spleen cells with the NS-1/1-Ag4-1 (NS-1) myeloma cell line, using the modified procedure of Kohler and Milstein (1975). Culture supernatants were screened for reactivity with asexual-stage of *P. brasilianum* antigens by immunofluorescence antibody (IFA) assay. Some of the