Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Tropica 97 (2006) 259–264
ACTA TROPICA
www.elsevier.com/locate/actatropica

Wild isolates of *Plasmodium falciparum* from India show restricted polymorphism in T-helper cell epitopes of the circumsporozoite protein

Vinay Bhatia, Pramatha R. Bhattacharya *

Malaria Research Centre, 22, Sham Nath Marg, Delhi 110054, India

Received 14 December 2004; received in revised form 6 September 2005; accepted 11 October 2005
Available online 24 January 2006

Abstract

Genetic polymorphism in T-helper cell epitopic regions of circumsporozoite protein of 148 *Plasmodium falciparum* isolates from different epidemic and endemic regions of India has been analyzed by polymerase chain reaction and sequencing. The variation has been found to be regionally unbiased in the sense that identical sequence variation has been found in different regions of India. The variation has also been found to be restricted and could be categorized into four groups. Since the variation is restricted, prototype variants could be included in a subunit polyvalent vaccine against sporozoites.

Keywords: *Plasmodium falciparum*; Circumsporozoite protein; T-helper cell epitope; India

1. Introduction

Circumsporozoite protein (CSP) of *Plasmodium falciparum* is a potential candidate for human malaria vaccine (Etlinger et al., 1988; Good et al., 1988). The importance of T-cells in malaria immunity has been appreciated for a long time. However, the variation in T-cell epitopes is a serious impediment for vaccine development. Two T-cell epitope (Th2R and Th3R) have been identified at the C-terminal end of CSP flanking the highly conserved RII region (de la Cruz et al., 1987; Good et al., 1987). T-cell epitope variants could be included in a subunit polyvalent sporozoite vaccine for endemic areas to elicit natural boosting of antibody and cellular immune responses. However, the feasibility of such a strategy will largely depend on the extent of polymorphism in these epitopes exhibited by parasites. A recent vaccine formulation (Stout et al., 1997) RTS, S, which include C-terminal end of CSP including T-cell epitope and Hepatitis B virus surface antigen, showed promising results. In the present investigation we have studied the variation in Th2R and Th3R of CSP of *P. falciparum* isolates from different endemic and epidemic areas of India, which are few hundred kilometers apart. Our study shows that the variation is regionally unbiased in the sense that similar type of variation have been found in different geographical regions of India, restricted and could be categorized into groups. Since the variants are small in number, the prototype variants could be included in a subunit polyvalent anti-sporozoite vaccine.

* Corresponding author. Tel.: +91 11 23979998/23928804; fax: +91 11 23943743.
E-mail address: prbbh@yahoo.co.in (P.R. Bhattacharya).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2005.10.007

2. Materials and methods

2.1. Parasites

P. falciparum infected (microscopically examined positive) blood samples were collected from patients with their consent in sterile heparinized tube from different widely separated epidemic and endemic areas of India. The blood samples were immediately frozen and were kept frozen until use. The areas, from where the blood samples were collected, covered broad regions of India.

2.2. Preparations of genomic DNA

Blood samples were thawed and genomic DNA was prepared by the method of Foley et al. (1992) with following modification (Bhattacharya, 1999). In brief, 50 μl of parasitized blood samples were washed thrice with 1 ml of ice-cold 5 mM Na_2HPO_4 buffer (pH 8.0) by vortexing vigorously and centrifugation at 10,000 × *g* for 10 min. The supernatant was discarded completely and the pellet was resuspended in 50 μl of sterile water and heated in a boiling water bath for 10 min, cooled slowly at room temperature and centrifuged at 10,000 × *g* for 10 min. Then, 40 μl of supernatant was taken and 5 μl of this supernatant was used in a 50 μl polymerase chain reaction (PCR) (Saiki et al., 1998).

2.3. Polymerase chain reaction (PCR) amplification and DNA sequencing

The oligonucleotide primers used for PCR amplification of T-helper cell epitopic regions (Th2R and Th3R) correspond to nucleotides 1008–1028 and 1323–1347 in the sequence of 7G8 clone (Lockyer et al., 1989; Doolan et al., 1992). In addition to the template DNA, each reaction mixture contained 50 pM of each of the forward and reverse primers, 10 mM deoxy nucleotide triphosphates and 2.5 U of Taq polymerase (Bangalore Genei, Bangalore, India). Amplification consisted of denaturation at 95 °C for 2 min, annealing at 55 °C for 1 min, amplification at 72 °C for 3 min for 30 cycles and final extension at 72 °C for 10 min all with a Techne thermal cycler. To prevent contamination, DNA template was always added at the last moment. Negative controls were always included, some without template, some without enzyme and some with mismatched primers and none of these amplified DNA. The amplified PCR products were purified by PCR clean-up kit (Boehringer Mannheim, Mannheim, Germany) according to manufacturer's protocol. The sequencing was carried out using the same primers used to amplify the T-helper cell epitopic regions by automated sequencer (Genetic Analyser 310 and ABI-PRISM 377, Applied Biosystem, USA) according to manufacturer's protocol. Sequencing was carried out from three independent PCR. The DNA and the deduced amino acid sequences were compared by DNA star programme (DNASTAR Inc., USA).

3. Results

P. falciparum infected blood samples were collected from malaria patients living in different epidemic and endemic areas of India, viz., Uttarpradesh (UP), Delhi (DL) Rajasthan (RJ), Orissa (OR), Assam (AS), Madhya Pradesh (MP), Karnatka (KT) and West Bengal (WB). These regions are separated by few hundred kilometers from each other. The nucleotide and deduced amino acid sequences of Th2R and Th3R regions of 148 isolates were analyzed and compared (Tables 1 and 2). The deduced amino acid sequence of LE5 sequence was compared with 7G8 sequence (Lockyer et al., 1989; Doolan et al., 1992) and the deduced amino acid sequences of Indian isolates were compared with 7G8 sequence. The isolates could be categorized according to the deduced amino acid sequence of Th2R and Th3R regions into four groups (Tables 1 and 2). It is interesting to note that the isolates belong to the same group (Tables 1 and 2) in their sequence variation in Th2R and Th3R regions, respectively, i.e. the sequence variation in Th2R and Th3R are linked.

Fig. 1 denotes the relative distribution of allelic variants in different geographical regions of India.

It is appealing to note that the restricted polymorphism observed in T-cell epitopic regions was not regionally biased and did not restrict to isolates from one region only and the same group included isolates from other regions also, which are separated by hundreds of kilometers.

The mutational changes observed, mostly occurred at the first base of the codons and less frequently at the second and third base of the codons. However, all these base substitutions were found to be non-synonymous mutations resulting in amino acid changes and no synonymous mutations were observed.

4. Discussion

Several studies have demonstrated the importance of T-cell mediated immunity to malaria (Grun and Weidanz, 1981; Brown et al., 1986; Jensen et al., 1983). CSP, which contains both B-cell and T-cell epitopes and a potential candidate for vaccine development (Etlinger et al.,