Genotypic variations may elucidate resistance to \textit{Plasmodium falciparum} infection \textit{In vitro}

Abstract

\textit{Plasmodium falciparum} malaria remains a significant cause of human suffering, and most malaria-related morbidity and mortality occurs in children living in sub-Saharan Africa. Evolutionary pressure has explained that various erythrocyte polymorphisms could protect against severe complications and death from \textit{Plasmodium falciparum} malaria. Several mechanisms have been proposed to explain the protection of hemoglobin AS and SS from severe \textit{Plasmodium falciparum} malaria. Sickle trait; the heterozygous and homozygous state of normal hemoglobin A (HbA) could confer protection against malaria in Africa. In the present study, we cultured \textit{Plasmodium falciparum} infected red blood cells from AA, AS and SS for six days. During the six days period, the level of parasite load (Parasitemia) and the activity of arginase released by the parasite were monitored on daily basis. Result obtained shows a significant (P<0.05) increase in both the level of parasite load and the activity of arginase. This increase was found to be higher in AA genotype while lower in both SS and AS, but with AS been much lower. The mechanisms by which sickle trait confer such malaria protection might be as a result of change in structural conformation that alter with the parasite ability to invade into the cells through the membrane protein receptors and hence a decrease in its activity in both AS and SS respectively.

Keywords: genotype, infection, \textit{Plasmodium falciparum}, arginase, parasitemia

Introduction

Malaria is still among the most important public health problems with about 3 billion at risk and 781,000 estimated deaths annually.\textsuperscript{1} Human malaria is caused majorly by two species of parasites: \textit{Plasmodium falciparum} and \textit{Plasmodium vivax}.\textsuperscript{1} \textit{Plasmodium falciparum} malaria is more often with high morbidity and mortality rate among children living in sub-Saharan Africa.\textsuperscript{2} In highly endemic regions, the majority of children with malaria present with a mild form with only a small percentage often develop severe disease and subsequently death.\textsuperscript{3} This is due to resistance developed over several years of exposure.\textsuperscript{3} While children susceptible to severe malaria die before reproductive age, therefore, genes that confer resistance to severe disease should be in the population living in sub-Saharan Africa.\textsuperscript{4,5} Red blood cell polymorphisms, such as hemoglobin S (HbS), \textalpha{-}thalassemia, \textbeta{-}thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hemoglobin E, and ovalocytosis shows some level of protections against severe \textit{P. Falciparum} malaria because epidemiological evidence shows a relationship between areas highly endemic to malaria and distribution of these polymorphisms.\textsuperscript{1,4}

Haemoglobinopathies are group of genetic deficiencies which result in synthesis of defective haemoglobin such as haemoglobin S.\textsuperscript{6} High prevalence (20 to 25%) of hemoglobin S (HBS) is known to be found in Africa and may reach up to 40% in some regions.\textsuperscript{7} Several studies suggested this polymorphism to confer some protection against severe form of malaria.\textsuperscript{7} The sickle cell trait (HBAS) comes as a result of a valine substitution with glutamic acid at position 6 of the hemoglobin chain.\textsuperscript{8} In mostly children, HBAS provides carriers with protection against severe \textit{Plasmodium falciparum} malaria and this can explains the relatively high penetrance of this mutation in some areas reaching 30% in areas endemic to \textit{P. Falciparum} infections.\textsuperscript{9,10,11}

In this study, we attempted to reconcile some molecular mechanism proposed to explain this protections\textsuperscript{12,13} through comparative study on survival of \textit{P. Falciparum} in some selected and most common red blood cell polymorphism. Since, the pathogenic course of \textit{Plasmodium falciparum} infection is characterized with some molecular changes which include hypoglycemia, lactic acidosis, hemolytic anemia, hemoglobinuria, and hypoxinemia which all depend on the level of parasite load.\textsuperscript{14} In this study, we monitored the changes of parasite load and the level of arginase activities. The depletion of L-arginine is achieved by the help of arginase from the malarial parasite, which catalyzes the hydrolysis of the side chain guanidinium group to form L-ornithine and urea.\textsuperscript{15} Decrease in the level of L-arginine correlate with decreased level of immunity and nitric oxide (NO) production,\textsuperscript{14,16} thus favors parasite proliferations.\textsuperscript{17,18} In our work, we examine the activity of this enzyme in cultured blood cells from all the three most common genotypes alongside parasite proliferation.

Materials and methods

Materials and reagents

RPMI 1640 media (Sigma Aldrich, Germany), Giemsa stain (Sigma Aldrich, Germany), Ninhydrin (sigma Aldrich, Germany), L-arginine (Sigma Aldrich, Germany), L-arginine (Sigma Aldrich, Germany).

Sample collection

\textit{Plasmodium falciparum} infected and an uninfected blood sample (AA, AS, SS) from healthy and infected patient was obtained from Ahmadu Bello University Sickbay, Zaria. The sample was collected with the help of certified medical personnel.

Media preparation

O\textdegree{} blood was collected, centrifuged at 10000 rpm for 20 min at 4\textdegree{}C. Serum was kept in aliquots and inactivated by keeping at 56\textdegree{}C water bath (30min). 10ml inactivated O\textdegree{} human serum was added to 90ml of incomplete media (96 ml of stock RPMI 1640 media containing 4ml of 5% sodium carbonate solution) making 100ml of working media.
Preparation of red blood cells and culture

O+ blood was collected, centrifuged (1500rpm for 10min) at room temperature. Plasma and buffy coat layer was removed and this washing process was repeated 3times in an incomplete media. Equal amount of complete media was added to the washed red blood cells and stored at 4˚C in refrigerator.

Infected red blood cell culture

50% suspension of infected cells (Plasmodium falciparum) in complete media (containing 15% serum) was added to equal amount of prepared uninfected O+ red blood cells to obtained an initial parasitemia ranged from 0.5 to 1.0%. The mixture was diluted with complete media to get 5% cell suspension (5% hematocrit). Culture was kept in CO2 incubator at 37˚C in Mary Hallaway Teaching Laboratories (MHTL).

Estimation of parasite load

After every 24 hours a portion of the cultured cells was collected (by removing the media without disturbing the cells) and a thin smear was prepared using Giemsa stain for examination of parasite load under light microscope. Fresh complete media (containing 10% serum) was added, mixed properly, and kept in the incubator. And % parasite load was counted using the formula below and erythrocyte containing ≥2 parasite is still counted as one infected erythrocyte.

\[
\% \text{parasitemia} = \frac{\text{no. of infected RBCs}}{\text{no. of uninfected RBCs}} \times 100
\]

Estimation of arginase activity

This is based on the facts that arginase act on its substrate (arginine) to produce ornithine and urea, under boiling temperature. A portion of the cultured cells was used to determine the activity of arginase spectrophotometrically by measuring the formation of L-Ornithine at 515nm with ninhydrin.

Statistical analysis

The data was analyzed using one way ANOVA followed with a multiple comparison test (Bonferroni) using statistical package for social science (SPSS) and the result was presented as mean ± standard deviation.

Results

Progressive increased parasite load in P. Falciparum infected cells

There was a significant (P<0.05) increase in the level of parasite load in all the genotypes (AA, AS, SS) from day 1 to day 5 of the experiment period. This increase was observed to be higher in AA followed by SS and very slower in AS genotype (Figure 1-4). This could simply explain the ease at which the parasite proliferate in AA genotype and its ability to further invade into the uninfected cells present in the culture medium.

Progressive increased arginase activity in P. Falciparum infected cells

The activity of arginase was also found to increase with increasing parasite load and in accordance to the previous order with AA, SS and AS respectively. This could also explain in addition, the competitive advantage by the parasite to source more of its nutrient requirement for survival and further weakens the immune power by suppressing the activity of nitric oxide synthase (Figure 5).

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Abo LC, Melo GC, et al. Potential immune adjuvants in the treatment of malarial infections. University teaching hospital (ABUZ). Individual written informed consent was provided by all study participants or their parents.

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Conflict of interest
Author declares that there is no conflict of interest.

References


