Plasmodium Falciparum In Vitro: Diminished Growth in Hemoglobin H Disease Erythrocytes

By Titus C. Ifediba, Arnold Stern, Amal Ibrahim, and Ronald F. Rieder

Studies of the ability of Plasmodium falciparum to grow in vitro in the red blood cells of subjects with certain β-thalassemia syndromes are often difficult to interpret because of the known inhibitory effect of an elevated cellular content of human fetal hemoglobin (HbF). P falciparum therefore was cultured in vitro in the erythrocytes of subjects with hemoglobin H (HbH) disease and various other α-thalassemia genotypes that are unaccompanied by increased levels of HbF. Growth of the malaria parasite was markedly retarded in HbH red blood cells, when compared with growth in blood from normal control subjects. No consistent impairment of growth was seen in the erythrocytes of subjects having deletion of only one or two α-globin genes. These results indicate that erythrocytes with a severe thalassemia phenotype provide a less hospitable growth environment for P falciparum than normally hemoglobinized red blood cells, even in the absence of increased levels of HbF.

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The high frequencies of sickle cell anemia, glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, and the thalassemia syndromes in areas historically endemic for falciparum malaria have been postulated to be due to balanced polymorphisms resulting from protection provided by the hematologic disorders against this parasitic infestation. Recently developed techniques for the cultivation of the malarial parasite in vitro have allowed experimental assessment of the ability of the parasite to grow in normal and various types of abnormal human red cells. Impaired growth of Plasmodium falciparum has been demonstrated in sickle, G-6-PD-deficient, hemoglobin CC (HbCC), and hemoglobin EE (HbEE) erythrocytes.

In thalassemia trait cells, growth of the parasite has been reported as unimpaired except in the presence of oxidant stress. The interpretation of such studies in the more severe β-thalassemia syndromes has been complicated by the presence of increased amounts of human fetal hemoglobin (HbF). There is evidence that HbF by itself exerts an inhibiting effect on the growth of plasmodia.

Erythrocytes from subjects with α-thalassemia generally do not exhibit increased levels of HbF; they therefore provide an opportunity to assess the effect of varying degrees of hypochromia and microcytosis on the growth of P falciparum in the absence of increased HbF.

Most α-thalassemia syndromes are the result of deletion of one or more of the normal complement of four α-globin genes. In general, the clinical stigmata of thalassemia in these disorders increases with increasing loss of α-gene activity. We have therefore assayed the growth in vitro of the malarial parasite in erythrocytes from subjects with several of the α-thalassemia syndromes.

MATERIALS AND METHODS

Studies were conducted with the NF-77 strain of P falciparum isolated from East Africa and supplied through the generosity of Professor J.H.E. Th. Meuwissen, Katholieke University, Nijmegen, The Netherlands. Stock malarial cultures were maintained with type A, Rh+ human erythrocytes, collected in citrate-phosphate-dextrose (obtained from New York Blood Center, New York). These cultures were grown in RPMI 1640 (Gibco, Grand Island, NY) containing 25 mmol/L N-2-hydroxyethyl piperazine-N-1-ethanesulfonate (HEPES; Sigma Chemical Co, St Louis), 50 μg/mL hypoxanthine, and 10% type A, Rh+ human serum with 2,3-diphosphoglycerate (2,3-DPG) sequestered with 5% CO₂ to maintain a partial pressure of CO₂ of 50 mm Hg.

To prepare parasites for growth in test erythrocytes, stock cultures were synchronized by the method of Lambros and Vanderberg. Cultures were centrifuged at 200 g for five minutes. The supernatant was discarded, and the pellet (approximately 1 mL) was resuspended in 5 mL of 5% D-sorbitol (0.274 mol/L) for five minutes at 37 °C. 200 μL of the resuspended cells were then placed in culture for 24 hours.

Test erythrocytes were collected in heparinized tubes and used within four hours. The erythrocytes were washed twice in RPMI 1640 and resuspended in the culture medium, initially containing 15% type AB human serum for one day and 10% serum for the
mean corpuscular hemoglobin.

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P falciparum support the growth of (Fig 1). As growth continued beyond the first 48 hours, been reported previously.'6

had deletion of two of the four a-globin genes (genotype -a/a; previously reported as subjects III-10 and III-12, respectively18) and one subject (C, Fig 2 and Table I had deletion of one a-globin gene with the presence of one nonfunctional a-globin gene (genotype -aThal/aa; previously reported as subject III-11). Results in these studies were varied. Erythrocytes of subject B were tested on two separate occasions and supported parasite growth as well as the control erythrocytes (Fig 2, panel B). Cells from subject A supported growth at a lower level (peak parasitemia 7.2% and 5.7% on day 4) than the control red blood cells (peak parasitemia 11.6%, 12.0% on day 4) (Fig 2, panel A). Little growth of P falciparum was exhibited in the experiment with blood cells from subject III-1 (Fig 2, panel C).

RESULTS

Subjects with a variety of a-thalassemia syndromes were tested for the ability of their erythrocytes to support the growth of P falciparum in vitro. The genotypes of these subjects were determined by restriction endonuclease gene-mapping methods and have been reported previously.16-19 The genotypes and hematologic values of the tested subjects are shown in Table 1.

In all five subjects with HbH disease (genotype: --/a), growth of the parasite was diminished markedly compared with growth in control erythrocytes (Fig 1). As growth continued beyond the first 48 hours, the trophozoites and schizonts appeared pycnotic and shrunken in HbH disease cultures. Reinvasion was markedly decreased, as evidenced by absence of ring forms during five to ten days of culture. Investigation of subjects B, C, and D (Fig 1 and Table 1) on two separate occasions gave similar results.

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PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

*HbF of HbH2;
In two subjects (A and B, Fig 3 and Table 1) with three functional α-globin genes (silent carrier state, genotype αα/-α), one subject (C, Fig 3 and Table 1) with one α-gene on one chromosome 16 and three α-genes present on the homologous chromosome (genotype ααα/-α), and one subject (D, Fig 3 and Table 1) with five α-globin genes (αααα/αα), growth of the parasite was normal (Fig 3). Subjects A, C, and D were tested on two separate occasions with similar results.

DISCUSSION

In two subjects (A and B, Fig 3 and Table 1) with three functional α-globin genes (silent carrier state, genotype αα/-α), one subject (C, Fig 3 and Table 1) with one α-gene on one chromosome 16 and three α-genes present on the homologous chromosome (genotype ααα/-α), and one subject (D, Fig 3 and Table 1) with five α-globin genes (αααα/αα), growth of the parasite was normal (Fig 3). Subjects A, C, and D were tested on two separate occasions with similar results.

In the present series of experiments, the ability of P falciparum to grow in erythrocytes of subjects with HbH disease was consistently and markedly diminished compared to controls.

The parasitemias established at the initiation of incubation were essentially equal in the experimental and control cultures. Although absolute distinction between defects in invasion and growth was not possible in the present experiments, invasion of erythrocytes by the parasite, as gauged by the degree of parasitemia 24 hours after initiation of culture, was slightly, but consistently lower in the HbH erythrocytes. During the course of the incubations, the increase in parasitemia was lower in the HbH erythrocytes than in the normal red blood cells, as indicated by the growth curves (Fig 1). The peak parasitemias were markedly higher in the normal cells (up to six times in some experiments). The decreased mean cellular volume of the thalassemic cells accompanied by an increase in red cell concentration could contribute to some decrease in the observed percentage of parasitized HbH cells compared with normal cells but not to the degree found in these experiments. Such an explanation for the differences in peak parasitemias is also unlikely in view of the similar parasitemias at zero time. In addition, growth of the parasite within the test erythrocytes was clearly defective, as judged by the higher frequency of dead forms noted within the HbH red blood cells. These results show that a severe thalassemic phenotype, without an accompanying increase in the level of HbF, can be inhibitory to development of P falciparum in vitro.

Whether there is impairment of growth in vitro in α-thalassemia trait (-α/-α) erythrocytes is unclear from the present study. One experiment showed marked impairment of the development of parasitemia, while in another subject growth in the thalassemic cells clearly was equal to the control on two occasions. Further studies on additional subjects are needed. In one previous study, the ability of P falciparum to grow in vitro in uncharacterized α-thalassemia trait erythrocytes was reported as normal except under oxidant stress. In our experiments, with the milder forms of α-thalassemia having deletion of only a single α-globin gene, there was consistently normal development of the parasite in vitro.

The present observations of defective growth in vitro of P falciparum in HbH disease erythrocytes are consistent with epidemiologic studies on the thalassemia syndromes that have linked the high frequency of these genetic disorders in certain populations to endemic malaria in the geographic regions inhabited by those groups. However, the mechanism(s) for the present experimental observations is (are) totally unknown, and any linkage with the observed clinical epidemiologic data is highly speculative. Such factors as increased susceptibility of HbH erythrocytes to deleterious metabolic alterations incurred by conditions of culture in vitro could result in defective parasite growth.

All our subjects with HbH disease are black, and they have all exhibited mild clinical courses despite moderate anemia. In this aspect, the clinical disorder may be different from that observed in subjects from Southeast Asia, where HbH disease has been reported as being frequently accompanied by severe anemia, hepatosplenomegaly, and debilitating illness. Reproduction appears to be normally successful in our subjects, and thus in black subjects with HbH disease, increased resistance to malarial infection theoretically could result in selection for the -α/ -α haplotypes in populations living in areas endemic for malaria. However, the extremely low incidence of HbH disease and the chromosome lacking both α genes in black subjects makes this consideration a highly unlikely explanation for the historical persistence of α thalassemia in that population.
REFERENCES

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