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Glucose-6-phosphate dehydrogenase deficiency inhibits *in vitro* growth of *Plasmodium falciparum*

(malaria/β^0-thalassemia)

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ABSTRACT Glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49)-deficient red blood cells from male hemizygotes and female heterozygotes from the island of Sardinia were studied for their ability to support growth *in vitro* of the malaria-causing organism *Plasmodium falciparum*. Parasite growth was approximately one-third of normal in both hemi- and heterozygotes for G6PD deficiency. In Sardinians with the β^0-thalassemia trait, parasite growth was normal except when G6PD deficiency occurred together with the thalassemia trait. The data support the hypothesis that G6PD deficiency may confer a selective advantage in a malarious area; the female heterozygote may be at a particular advantage because resistance to malaria equals that of male hemizygotes, but the risk of fatal hemolysis may be less. However, more female heterozygotes must be studied to confirm this hypothesis. No protective effect of β^0-thalassemia trait could be demonstrated *in vitro*.

Red blood cell glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) deficiency has been postulated for many years to provide a selective advantage in the presence of falciparum malaria infection. Efforts to show abnormalities in the growth of the parasite *Plasmodium falciparum* in G6PD-deficient cells by using *in vitro* culture have succeeded in demonstrating inhibition of growth when an oxidative stress is applied. However, growth under optimal culture conditions was not decreased (1, 2).

We report here that G6PD-deficient red blood cells from Sardinian subjects inhibit the growth of *P. falciparum* under normal culture conditions. On the other hand, the presence of the β^0-thalassemia trait either separately or together with G6PD deficiency appeared to have no inhibitory influence on parasite growth rates.

METHODS

The Sardinian blood samples consisted of 12 normal subjects (8 males and 4 females), 7 male G6PD-deficient hemizygotes, 3 female G6PD-deficient heterozygotes, 5 male heterozygotes for β^0-thalassemia, and 5 male G6PD-deficient hemizygotes, who were also heterozygotes for β^0-thalassemia—a total of 32 samples. Some biochemical and hematological characteristics of the sample are shown in Table 1. No subjects had fetal hemoglobin levels in excess of 1.0%, but subjects with the β^0-thalassemia trait displayed significantly elevated hemoglobin A_2 values and severe microcytosis (3). No samples contained abnormal hemoglobins in this study.

All blood samples were collected in citrate/phosphate/dextrose anticoagulant, maintained at 4°C, and shipped by air to New York, where they were used 3–4 days after collection. G6PD activity was determined with the aid of Sigma kit no. 345 UV and a Gilford Recording Spectrophotometer model 240. Red blood cell indices were obtained from a Coulter Counter model S. Hemoglobin A_2 was determined by means of an Isolab (Akron, Ohio) ion exchange chromatographic column; the upper limit of normal is 3.5% Hb A_2. Hemoglobin F was determined as the alkali-resistant fraction (normal adult value, <1.0%). Hemoglobin electrophoresis on cellulose acetate (pH 8.6) was performed and no abnormal hemoglobins were found. Data on the histochemically evaluated G6PD female heterozygote red blood cells were obtained from Rinaldi *et al.* as described (4).

The method of Trager and Jensen (5) was used for the growth of *P. falciparum* in red blood cells at a packed cell volume of 5.0%. The FCR3/Gambia strain obtained from Trager and Jensen was used for this study. Infected cells on day 0 were diluted 1:100 with red blood cells to be used for the experiment. A blood cell suspension of 1.5 ml was placed in Petri dishes, which, in turn, were placed in a candle jar at 37°C. Oxygen tension in candle jar cultures has been shown in previous studies to be equivalent to 17% oxygen (6). Cultures were set up in triplicate; medium was changed daily and 1,000 Giemsa-stained cells were evaluated each day for the percentage of infected cells. Each culture always contained both normal and enzyme-deficient cells that were run with the same inoculum in the same candle jar by using the same batch of medium. The data were evaluated by means of the F test and the Student t test with correction for small sample size (7).

Table 1. Biochemical and hematological characteristics of blood samples from Sardinia, Italy

Sample	n	G6PD, units/g of Hb	MCV, femtoliters*	% Hb A_2
Normals	12	8.98 ± 0.77 (7.70–10.60)	90.3 ± 2.6 (86.5–95.2)	2.73 ± 0.19 (2.3–3.0)
G6PD-deficient hemizygotes	7	0.27 ± 0.25† (0.00–0.60)	91.8 ± 6.5 (83.9–101.5)	2.84 ± 0.33 (2.4–3.5)
G6PD-deficient heterozygotes	3	4.40 ± 0.30†‡ (4.10–4.70)	92.1 ± 3.0 (88.9–95.0)	3.06 ± 0.28 (2.9–3.4)
β^0-Thalassemia trait	5	15.40 ± 0.54† (14.7–16.1)	64.0 ± 3.29† (62.3–68.0)	6.18 ± 0.28† (5.7–6.4)
β^0-Thalassemia trait with G6PD deficiency	5	0.30 ± 0.27† (0.00–0.70)	66.1 ± 4.5† (59.5–72.0)	5.7 ± 0.39† (5.2–6.2)

Each value is shown as the mean ± SD with the range in parenthesis.
* MCV, mean corpuscular volume.
† $P < 0.001$.
‡ % G6PD normal cells = 37.6 ± 5.5% (range, 32–43).

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Abbreviation: G6PD, glucose-6-phosphate dehydrogenase.

RESULTS

Five days after inoculation of *P. falciparum* into the culture, both G6PD-deficient and G6PD-deficient β^0-thalassemia trait red blood cells demonstrated parasitemias about one-third that of controls (Fig. 1). Interestingly, female heterozygotes for G6PD deficiency showed parasitemias that were indistinguishable from G6PD-deficient male hemizygotes; this finding occurred despite the presence of 30–40% histochemically normal cells (Table 1). The β^0-thalassemia trait alone or with G6PD deficiency appeared to play no inhibitory role in parasite growth.

When cultures were observed daily throughout a 5-day culture period, inhibition of parasite growth in G6PD-deficient cells could be detected by the third day (Fig. 2). In the enzyme-deficient cells, all stages of parasite growth were seen (ring forms, trophozoites, and schizonts), but many of the forms appeared degenerate and abnormal in morphology, especially after day 3. The morphology of parasites growing in β^0-thalassemia cells appeared normal. We were unable to reverse the growth inhibition in these cultures with dithiothreitol (data not shown).

These results differ somewhat from those of Friedman (1), who was unable to find growth inhibition in G6PD-deficient cells unless oxidative stress was applied. Under these conditions, dithiothreitol was partially protective. The discrepancy with our results could be easily explained if one assumes that the primary factor for growth inhibition is the degree of red blood cell G6PD deficiency. The G6PD A^- mutant presumably used in Friedman's study is known to involve a much milder degree of deficiency.

On the other hand, our results with the β^0-thalassemia trait cells are similar to those of Pasvol *et al.* (8) and Friedman (1) and suggest that extraerythrocytic determinants may play a role in protecting thalassemic heterozygotes from lethal malaria infection.

The epidemiological evidence for G6PD deficiency and the malaria hypothesis is quite extensive and especially detailed and well worked out for the island of Sardinia (9). Although only a small sample of female G6PD-deficient heterozygotes were included in this study, a consistent protection for this group is suggested. Further studies on additional blood samples with a variety of percentages of normal cells will be required to confirm the hypothesis put forth by Luzzatto *et al.* (10), in which female heterozygotes were postulated to be the main beneficiaries of G6PD deficiency.

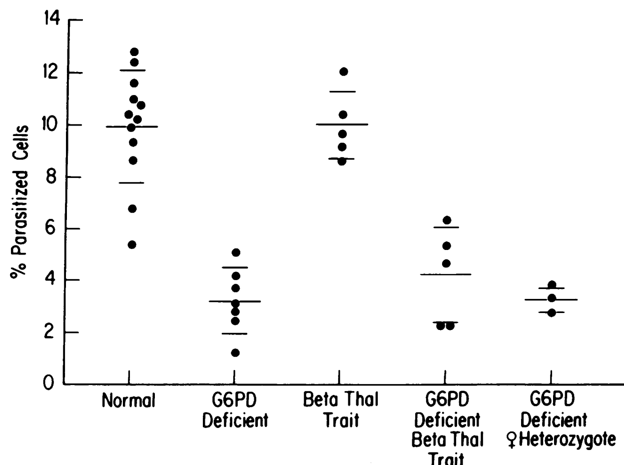

FIG. 1. Parasitemia of red blood cells of various genotypes observed on day 5 of culture. Each point represents the mean of three separate cultures set up simultaneously with normal controls. Beta Thal = β^0-thalassemia.

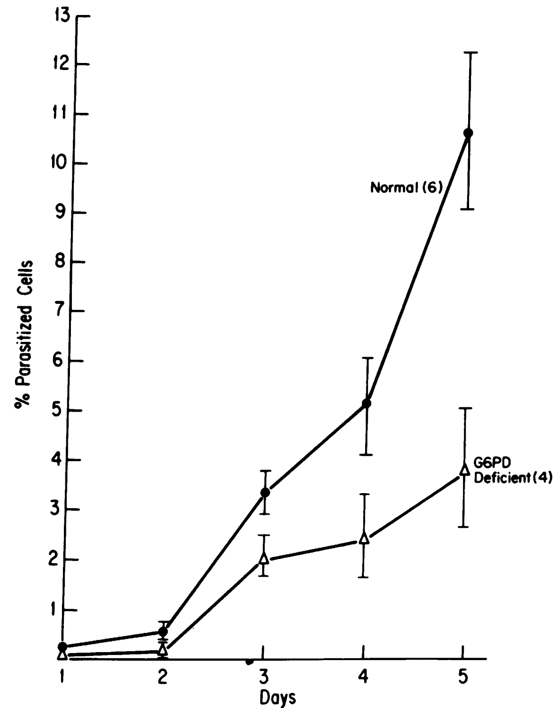

FIG. 2. Growth curves of *P. falciparum* in six representative control samples (●) and four G6PD-deficient samples from male hemizygotes (△). Cultures were performed as in Fig. 1. Data are expressed as the mean ± SD.

Finally, these results by themselves do not suggest a biochemical mechanism whereby G6PD deficiency may be inhibitory for malaria. In addition to the differences in the ability of G6PD-deficient cells to maintain glutathione in the reduced form, G6PD-deficient red blood cells also may be unable to generate ribose for purine nucleotide synthesis (11). The relative importance of these two distinct metabolic activities to the malaria-causing parasite remains to be explored.

Note Added in Proof. Luzzatto *et al.* have independently described growth inhibition of *P. falciparum* by G6PD-deficient red blood cells in culture (12).

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