Protection against Malaria by Sickle-Cell Trait

HANS LASER and ROGER KLEIN

Agricultural Research Council, Institute of Animal Physiology, Babraham CB2 4AT, U.K., and Medical Research Council, Moulteno Institute, Downing Street, Cambridge CB2 3EE, U.K.

We have shown that the haemolytic activity of fatty acids may be implicated in the destruction of erythrocytes by malaria parasites (Laser et al., 1975a,b). Under normal conditions free fatty acids are buffered by albumin in the serum and haemoglobin within the erythrocyte. During a malarial infection, however, the presence of the Plasmodium leads to an increase in both serum and erythrocyte fatty acid concentrations which may exceed the buffering capacity of the available protein.

The possession of the gene for haemoglobin S and relative resistance to the lethal effects of infection with Plasmodium falciparum (malignant tertian malaria) have been shown to be related, giving rise to the phenomenon of balanced polymorphism (Lehmann & Huntsman, 1966). The high incidence of heterozygotes for the HbS gene in certain areas of the world which are, or have been been endemic for P. falciparum malaria, has been attributed to the selective advantage conferred by this gene (Allison, 1954), even though the homozygous condition is generally lethal.

We became interested in possible mechanisms by which this selective advantage might be brought about, and have for this reason examined possible differences in the fatty acid-buffering capacity of normal and sickle-cell haemoglobins. The buffering capacity of haemoglobin was estimated by measuring the extent to which added haemoglobin delayed the fatty acid-induced haemolysis of erythrocytes. Haemoglobin was prepared from normal (AA), heterozygous (AS) and homozygous (SS) human blood as follows. Erythrocytes were freed of serum by centrifugation followed by repeated washing with phosphate-buffered saline (at least three times with 10 vol.) containing 121 mM-NaCl, 4.9 mM-KCl, 1.2 mM-KH$_2$PO$_4$, 16.5 mM-Na$_2$HPO$_4$ and 1.2 mM-MgSO$_4$, adjusted to pH 7.2. The washed cells were lysed by alternate freezing and thawing, and the haemolysate was centrifuged at 74000 g, for 45 min (Beckman type 50 swinging-bucket rotor) to remove cell stroma and membrane fragments. The clear supernatant was removed and used in the experiments described below. Material was freshly prepared for each set of experiments in order to limit the formation of methaemoglobin. The time taken for haemolysis to occur in the presence of various concentrations of the different haemoglobins was determined in the following manner. The potassium salt of oleic acid (17.6 µM) was dissolved in 10 ml of phosphate-buffered saline. This solution was allowed to equilibrate at 37°C for 10-15 min before the addition of sufficient erythrocyte suspension to give a haemoglobin concentration of 30 µg/ml, measured spectrophotometrically after complete lysis. The suspension was incubated at 37°C, and haemolysis was detected visually by clearing of the suspension when viewed against a ruled grid (Laser, 1950); the end-point corresponded to more than 90% haemolysis. Similar results were obtained by spectrophotometric recording. The haemoglobins under test were added to the system before the addition of the erythrocyte suspension.

The results shown in Table 1 demonstrate that haemolysis is delayed by the presence of haemoglobin S, as compared with the same concentration of haemoglobin A, with the homozygous haemoglobin (SS) being more effective than the heterozygous (AS) material. Haemoglobin from patients with SC disease was also found to delay haemolysis and appears to be more effective than homozygous sickle-cell haemoglobin.

A possible molecular interpretation for these results may be based on the well-known differences in electrophoretic mobility, and hence the net charge carried by these haemoglobins, and the amino acid mutation at the sixth position of the β residue, which involves a change from glutamic acid in haemoglobin A (α2β$_2$Glu6) to valine in haemoglobin S (α2β$_2$Val6) and lysine in haemoglobin C (α2β$_2$Lys6). This could increase the buffering capacity of the protein for fatty acid anions as the total charge carried became more positive.
Table 1. Relative times for the haemolysis of human erythrocytes by oleic acid in the presence of various added haemoglobins

The time for haemolysis to occur in the absence of added haemoglobin, other than that contained within the erythrocytes, is arbitrarily assigned a value of 1. Conditions were as specified in the text. Columns (a) and (b) are results from two separate sets of experiments.

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One possible mechanism for the protective effect of sickle-cell trait (the heterozygous condition) was described by Luzatto et al. (1970), in which parasitized cells 'sickled' more readily than non-parasitized cells, and were thus removed from the circulation before completion of schizogony; it has been pointed out, however, that this mechanism is unlikely, since the incidence of gametocytes is similar in both 'sicklers' and 'non-sicklers' (Edington & Gilles, 1976).

We would like to propose that a mechanism involving the increased buffering by haemoglobin of fatty acids produced within the erythrocyte by the parasite, could provide a simple explanation of the selective advantage of sickle-cell trait (AS) in man, by preventing or retarding the release of merozoites and the associated intravascular haemolysis. Although haemoglobin from homozygotes (SS) is more effective at fatty acid buffering than that from heterozygotes (AS), the homozygous condition is itself a major evolutionary disadvantage.

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