Reduced Deformability of Thalassemic Erythrocytes and Erythrocytes With Abnormal Hemoglobins and Relation With Susceptibility to *Plasmodium falciparum* Invasion

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A number of genetically variant erythrocytes showed decreased deformability of both intact cells and membranes prepared therefrom as measured by laser diffractometry. Erythrocytes associated with minor or no clinical symptoms (e.g., α-thalassemia traits, hemoglobin Hb E trait, Hb Constant Spring trait), which showed only a minimal decrease in deformability, were, in general, invaded efficiently by the malarial parasite *Plasmodium falciparum*. Other variant erythrocytes (β-thalassemia/Hb E, homozygous Hb E, homozygous Hb Constant Spring, Hb H, Hb H/Hb Constant Spring) with low deformability showed different degrees of reduction in invasion susceptibility, most of which were less than proportional with deformability decrease. It is concluded that parasite invasion is only weakly related to gross cell deformability, which in turn depends on various factors other than membrane deformability.

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RHEOLOGIC AND other mechanical properties of many genetically variant erythrocytes with abnormal hemoglobin (Hb) synthesis or structure are known to be altered and are likely to contribute to the shortened lifespan of such cells and other pathologic complications. Micropore filtration studies show moderately diminished cell flexibility of erythrocytes from heterozygous β-thalassemic patients ascribed to microcytosis, rigidity of erythrocytes in homozygous patients due to altered cell shape, diminished Hb fluidity and, for splenectomized patients, the presence of inclusion bodies. Both α- and β-thalassemic erythrocytes have excess surface area in relation to cell volume, increased membrane rigidity, and decreased ability to undergo cell deformation under hypertonic osmotic stress as measured by ektacytometry. However, while the mechanical stability of α-thalassemic membranes was normal or slightly enhanced, that of β-thalassemic membranes was markedly decreased. Different defects were also noted in the skeletal proteins of α- and β-thalassemic erythrocytes.

The altered mechanical properties of variant erythrocytes may have a consequence on their interactions with the malarial parasite *Plasmodium falciparum*, helping to protect the variant individuals from infection. Malayan ovalocytes have been shown to have rigid membranes, likely to be the main cause for the inhibition of parasite growth in these erythrocytes, and consequently providing an explanation for the high frequency of ovalocytosis in this population. The underlying molecular defect for this type of ovalocytosis is probably abnormal structure and function of band 3 protein. Other erythrocytes with decreased deformability, such as sickle erythrocytes under low oxygen tension, erythrocytes deficient in band 4.1, and glycoporphin-β and -γ, all resist parasite invasion to differing degrees. The importance of erythrocyte deformability is further demonstrated by the fact that normal cells treated with glutaraldehyde or diamide, which cross-link membrane components, show a decrease in parasite invasion that is correlated with a decrease in deformability. Externally bound agents such as monoclonal antibodies to glycoporphin-α also inhibit invasion in parallel with decreased deformability. These results implicate an important role of cell deformability, or factors closely related with deformability, on parasite invasion. However, erythrocytes with grossly reduced deformability from treatment with N-ethylmaleimide are still efficiently invaded, as are highly undeformable stomatocytes formed by chlorpromazine treatment. In addition, hereditary pyropoikilocytes, elliptocytes, and spherocytes were reported to retain considerable susceptibility to invasion despite their reduced deformability. In another study, hereditary elliptocytes and spherocytes (due to spectrin or band 4.1 defects) were also found to be invaded normally. Therefore, it is unclear whether deformability in itself is a major determinant in invasion or whether other underlying membrane properties are more important determinants.

Many thalassemic erythrocytes and those containing abnormal Hbs have reduced susceptibility to *P falciparum* infection. In this report, we investigate the possible relationship between deformability of these erythrocytes and their susceptibility to malarial invasion. Variant erythrocytes with small or no decrease in cell deformability compared with normal cells tended to be invaded efficiently, while those with larger deformability decrease tended also to have reduced invasion. However, the fact that these erythrocytes in general showed less than proportionate decrease in invasion susceptibility with respect to their cell deformability decrease implies that other factors also play a significant role in controlling invasion.

MATERIALS AND METHODS

**Patients.** Blood samples were obtained as heparinized or citrated samples from the Faculty of Medicine, Siriraj Hospital.
Criteria for identification of the variations were as described previously.20 No blood transfusion had been administered to the patients for at least 3 months before this study.

Measurement of erythrocyte cell and membrane deformability by laser diffractometry. The laser diffractometer used for measuring deformability has been described elsewhere.21 It consists of a flat glass cell (0.21 × 7 × 30 mm²) and a helium-neon laser. The flat glass cell was placed vertically with its longest side horizontal, and the laser beam traversed the gap of the cell perpendicularly. Erythrocyte suspensions were subjected to defined shear stresses in the cell, and the resulting changes in shape were monitored by laser diffraction. The extent of shape change was used as the measure of deformability: when the cells were deformed by shear stress (74 to 232 dyne/cm²), the diffraction patterns became elliptical. These were photographed and the deformability index (DI) was calculated from the equation(di) = (L - W)/(L + W), where L is the measured vertical length of the inner dimension of the first ring in the pattern, and W is the horizontal length.

To prepare erythrocytes for deformability measurement, the samples were centrifuged at 700g for 10 minutes at 25°C. The plasma and buffy coat were discarded and the erythrocytes were resuspended in isotonic phosphate-buffered saline (PBS) (7.0128 g/L sodium chloride, 2.842 g/L disodium hydrogenphosphate, 0.6085 g/L potassium dihydrogenphosphate, pH 7.4) at a hematocrit of 50%. Fifty microliters of PBS was suspended in 10 mL of 15 g% dextran T40 (average molecular weight 40,000; Pharmacia Fine Chemicals, Uppsala, Sweden) in isotonic PBS for deformability measurement. The viscosity of the suspending medium was 11.5 cp.

Parasite invasion assay. The K1 strain of P falciparum was maintained in continuous culture according to the method of Trager and Jensen.22 The culture was synchronized with sorbitol23 and erythrocytes infected with mature schizonts were concentrated by Percoll gradient centrifugation24 to about 80% to 90% parasitemia. The invasion assay was performed in duplicate with flat-bottom 24-well plates (Nunclon, Delta, Denmark) by incubating 100 µL of schizont-infected erythrocytes with 400 µL of 50% suspension of test cells or resealed membranes in a total volume of 500 µL. Parasitemia levels were determined immediately and the numbers of ring forms were determined 20 hours later. The results are expressed as invasion index, ie, the ratio of ring-form parasitemia to initial schizont parasitemia.

RESULTS

DI for whole cells for various types of thalassemic erythrocytes and erythrocytes with abnormal hemoglobins at three different values of shear stress are shown in Fig 1 and Table 1. The susceptibilities of different variant erythrocytes to P falciparum invasion are also shown in Table 1, and the relationship with deformabilities is shown in Fig 2. In general, the variant erythrocytes associated with minor or no clinical symptoms such as α-thal 1 trait (−/−/αα), α-thal 2 trait (−/−/αα), Hb Constant Spring (CS) trait, and Hb E trait erythrocytes showed small to moderate decrease in DI, compared with the larger decrease found for erythrocytes associated with more severe clinical symptoms. There was a general correlation between DI and susceptibility to invasion of the variant erythrocytes, as seen most clearly for both β-thal/Hb E erythrocytes from both splenectomized and non-splenectomized patients, and also for β-trait, Hb H (α-thal 1/α-thal 2), and Hb H/Hb CS (α-thal 1/Hb CS) erythrocytes. However, taken together, the results in Fig 2 show, in general, a relatively less decrease in invasion susceptibility of the variant erythrocytes than expected were there a proportionate relationship with deformability. There was no obvious correlation between deformability or susceptibility to invasion and the hematologic parameters (data not shown).

DISCUSSION

We have shown that many genetically variant erythrocytes involving abnormal Hb synthesis or structure have decreased cell deformability as measured by laser diffractometry (Fig 1 and Table 1). Although our experimental approach measured deformability in response to shear stress, whereas parasite invasion may be related with bending rather than shear deformability, it is likely that there is general decrease in both bending and shear deformability of these variant erythrocytes compared with
normal cells. Deformability decrease in β-thal trait erythrocytes have been previously reported by Tillmann and Schroter,5 who used a filtration technique to measure deformability change. Our results are also in line with the more detailed ektacytometric analysis on α- and β-thalassemic erythrocytes and membranes by Schreier et al.3 who showed that interaction of excess α- and β-globin chains with membranes produces different cellular changes, probably leading to different pathophysiologic consequences. Apart from membrane properties, the decreased deformability of the variant erythrocytes reported here is probably caused by other factors also, such as the state of the cell contents and geometry of the cells. Therefore, a correlation of cell deformability and parasite invasion would indicate that the latter is dependent on the former, irrespective of its contributing factors. The results in Table 1 and Fig 2 show that there is a general correlation between cell deformability and susceptibility to parasite invasion. However, except for Hb H erythrocytes, other variant erythrocytes studied tended to have a lower decrease in invasion susceptibility than expected from a proportionate relationship. In contrast, an approximate proportionality between deformability and invasion in glutaraldehyde-treated cells was previously observed by Mohandas et al.,8 who concluded from similarity to Malayan ovalocytes that these cells are protected from invasion by their increased rigidity. Pasvol et al.15 also recently showed a linear correlation between the effect of MoAbs to glycophorin-α and Fab fragments on the deformability and invasion of human erythrocytes.

Rangachari et al.16 found that modification of deformability of erythrocytes by various means did not lead to parallel effects on susceptibility to parasite invasion. For example, treatment of cells with N-ethylmaleimide (NEM), which grossly reduces deformation and greatly increases the shear elastic modulus, results in only a minor inhibition of invasion. Cells from subjects with hereditary pyropoikilocytosis and two types of elliptocytosis are also efficiently invaded, despite their reduced deformability. They suggested from these results that cell and membrane deformability per se are unlikely to be the primary determinant of malarial invasion, except at very high levels of rigidity, but the freedom of membrane proteins to migrate in the course of entry of the parasite may be a more important determinant. While the movement of the membrane proteins and the integrity of the cytoskeleton that controls this movement are likely of importance, ambiguity remains concerning the role of deformability in such studies where modifiers of deformability also exert an effect on the cytoskeleton, in turn influencing the membrane molecular movement. The dissociation of spectrin tetramers into dimers by NEM treatment, or defect in dimer self association in hereditary elliptocytosis, both of which are accompanied by reduction in deformability, may also be accompanied by a compensating increase in the freedom of movement of membrane proteins. Both α- and β-thalassemic erythrocytes of various types have also been shown to have defective dimer self association, possibly affecting membrane protein movement as well as deformability.25 Compensating changes of deformability decrease and increase in freedom of movement of membrane protein molecules may explain the less than proportionate decrease in invasion of most variant erythrocytes studied (Fig 2). Furthermore, it is possible that differences other than membrane deformability, such as cell viscosity and geometry, contribute to decrease in cell deformability of these variant erythrocytes, and therefore may have an effect on parasite invasion. We have found (results not shown) that membranes prepared from the variant erythrocytes were invaded relatively efficiently irre-
spective of their deformability decrease, indicating that membrane deformability per se may play only a minor role in determining invasion susceptibility. In addition, membrane stability, another factor distinct from membrane deformability but also regulated by skeletal protein associations, may also play a role in determining invasion susceptibility. With decreased membrane stability, the cell may lyse during the invasion process. For such variants as Hb H and β-thal/Hb E erythrocytes, the reduction in invasion reported here may be sufficient to explain the inhibition of the parasite in culture, whereas for other variants such as sickle erythrocytes, inhibition of malarial parasite invasion by monoclonal antibodies against glycoporphin A correlates with reduction in red cell deformability. Blood 74:1836, 1989

Mawby WJ, Merry AH: Inhibition of malarial parasite invasion by hereditary spherocytes, and elliptocytes, inhibition of intracellular growth may be the major factor. Furthermore, erythrocyte membrane deformability and stability may play a role in growth and release of the merozoites. For example, premature release of the merozoites from variant erythrocytes would hamper their ability to invade new erythrocytes. The rheologic properties and the interaction of infected variant erythrocytes with the host immune system are also likely to be modulated by deformability changes. These factors may be important in the mechanisms for protection of variant individuals from falciparum malaria infection.

REFERENCES

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