Brief Definitive Report

EVIDENCE FOR DIFFERENCES IN ERYTHROCYTE SURFACE RECEPTORS FOR THE MALARIAL PARASITES, *PLASMODIUM FALCIPARUM* AND *PLASMODIUM KNOWLESI*

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The asexual erythrocytic cycle responsible for the clinical manifestations of malaria is perpetuated by merozoites which rupture out of infected erythrocytes and invade uninfected ones. Interaction between the merozoites and receptors on the erythrocyte surface initiate invasion (1-3). Chemical or immunologic interference between the merozoite and its receptor could block the infection at this point in the life cycle. Previously, we studied the characteristics of the erythroycte surface that are required for interaction and subsequent invasion by *Plasmodium knowlesi* merozoites (3, 4). Erythrocytes which lacked Duffy blood group determinants (Duffy negative erythrocytes) (3) or had been treated with chymotrypsin (4) were resistant to invasion. With the recent advances in in vitro cultivation of *Plasmodium falciparum* (5, 6), it was possible to compare the erythrocyte surface determinants required for invasion by *P. falciparum*, the major malaria of man, and *P. knowlesi*, a monkey malaria that infects man.

Materials and Methods

Human erythrocytes (test cells) which either lacked a blood group determinant (Table I) or had been treated with various enzymes (Table II) were exposed to *P. falciparum* and *P. knowlesi* merozoites in vitro, and the percentage of test cells infected was determined. Chimpanzee erythrocytes infected with *P. falciparum* (Camp strain) and frozen in liquid nitrogen were thawed (7) on the day of the experiment. They contained approximately 50% infected erythrocytes and varied in viability from experiment to experiment. The infected cells were centrifuged in microhematocrit tubes. Cells from the upper half of the tubes, containing about 90% infected erythrocytes were mixed with test erythrocytes in a ratio of 1 to 25 and cultured as previously described (6). Erythrocytes were removed from culture 48 h later, spread in a thin film on a slide, stained with Giemsa, and the percent of erythrocytes infected with young trophozoites (ring forms) determined. Rhesus erythrocytes that are refractory to invasion by *P. falciparum* were included in each experiment (Table I). In parallel experiments, samples of test erythrocytes were cultured with *P. knowlesi*-infected cells (3). Since *P. falciparum* merozoites were released after 48 h in culture and *P. knowlesi* within 1 h, test erythrocytes were incubated at 35°C for 48 h before addition of *P. knowlesi*-infected cells.

Source of Human Erythrocytes. Erythrocytes were collected in acid citrate dextrose (ACD) and maintained at 4 C. Erythrocytes from the Finnish En(a−) (G.W.) and two controls arrived in ice

from Finland and were cultured on 2 separate days with each parasite. Some erythrocytes (Kell null, Jk[a−b−], Lu[a−b−], Ge[a−], S-s-U-, En[a−] [A.P., England], and their controls) were obtained as frozen droplets in liquid nitrogen. Control erythrocytes were from healthy individuals with a common blood group phenotype. Erythrocytes were washed three times in culture media before use.

Enzyme Treatment of Human Erythrocytes. Erythrocytes were treated with chymotrypsin and trypsin, and the enzymes were inhibited after treatment as described previously (3, 4, 8). For neuraminidase treatment, a 20% suspension of erythrocytes in Tris-buffered saline with 3.6 mM KCl, 1.6 mM $CaCl_2$, and 1.2 mM $MgSO_4$, pH 7.4, was incubated with 25 U/ml of neuraminidase (*Vibrio cholerae*, B grade, Calbiochem, San Diego, Calif.) at 37°C for 30 min. Control erythrocytes from the same individual were treated identically except that no enzyme was used. The enzyme effect on the erythrocytes was monitored in each experiment: (*a*) chymotrypsin, removal of Duffy blood group activity; (*b*) neuraminidase, 76 ± 1.4 (SEM) percent reduction in electrophoretic mobility; (*c*) trypsin, 34 ± 1.4 (SEM) percent reduction in electrophoretic mobility and removal of MN blood group activity.

Results

Human erythrocytes lacking specific blood group determinants were all susceptible to invasion by *P. falciparum*, including erythrocytes with blood group phenotypes (Fy[a−b−] and S-s-U-) characteristic of black African populations (Table I). The two erythrocyte samples of the En(a−) phenotype showed a reduction in invasion by *P. falciparum*. Since a twofold decrease in invasion is within the range of variation between different individuals, it is not known if the reduced invasion was caused by the En(a−) phenotype or some other unrelated erythrocyte factor. In contrast to the small reduction in susceptibility of En(a−) cells to *P. falciparum*, Duffy negative cells (Fy[a−b−]) are refractory to infection by *P. knowlesi* and rhesus cells are refractory to infection by *P. falciparum* (Table I).

The pattern of resistance of enzyme-treated human erythrocytes to infection differed for *P. falciparum* and *P. knowlesi* (Table II). Chymotrypsin (0.1 mg/ml) treatment blocked infection by *P. knowlesi* but had no influence on infection by *P. falciparum*. Conversely, trypsin or trypsin plus neuraminidase caused a marked reduction in infection by *P. falciparum* but little or no change in susceptibility to *P. knowlesi*. Neuraminidase also caused a significant reduction in infection by *P. falciparum*, although the effect was less than that of trypsin or trypsin plus neuraminidase. Neuraminidase treatment did not block infection by *P. knowlesi*.

Discussion

In a previous study we observed directly the interaction between erythrocytes and *P. knowlesi* merozoites (9). It consisted of attachment of the merozoite to the erythrocyte membrane followed by deformation and invasion. None of these events occurred when susceptible human erythrocytes were treated with chymotrypsin (Mason, S. J., L. H. Miller, and J. A. Dvorak. Unpublished data.), indicating that the receptor necessary for attachment of *P. knowlesi* was removed. In the present study chymotrypsin treatment (0.1 mg/ml) that blocks invasion by *P. knowlesi* had no effect on invasion by *P. falciparum*. On the other hand, treatment of human erythrocytes with trypsin or neuraminidase reduces invasion by *P. falciparum* but not by *P. knowlesi*. If *P. falciparum* invades erythrocytes by a similar mechanism to *P. knowlesi*, then infection of enzyme-

TABLE I

The Susceptibility of Human Erythrocytes Lacking Various Blood Group Antigens to Invasion by P. falciparum and P. knowlesi

Blood group of (number studied) Test cells	Percent infected erythrocytes			
	P. falciparum		*P. knowlesi*	
	Test cell	Control	Test cell	Control
ABO null (1)*	2.1	2.2	10.3	7.9
Rh null (1)*	2.0	2.2	10.7	7.9
Kell null (1)*	1.8	1.4	20.3	14.6
Jk (a−b−) (1)*	2.6	1.4	21.3	14.6
Lu (a−b−) (1)*	1.9	1.4	13.3	14.6
Tn (1)	5.7	5.4	5.2	4.4
Ge (a−) (1)	10.1	12.7	7.3	7.3
S-s-U- (3)	7.4	1.3(8.5)‡	6.5	7.7
En (a−) (G.W., Finland)	4.8	10.3	6.8	9.9
En (a−) (A.P., England)	4.9	12.7	6.3	7.3
Fy (a−b−) (4)	8.4	7.7	<0.1	13.0
Rhesus (*Macaca mulatta*) (5)	≤0.1	9.6	20.8	7.5

* The lower percent infection of ABO null, Rh null, Kell null, Jk(a−b−), Lu(a−b−), and their controls by *P. falciparum* resulted because the chimpanzee erythrocytes, containing 50% *P. falciparum*-infected cells, were not concentrated by centrifugation before mixing with these cells.

‡ Frozen cells from one control were run on that day. The number in parenthesis is the mean percent infection for fresh cells from six people run on that same day.

TABLE II

The Effect of Enzyme Treatment of Human Erythrocytes on Invasion by P. falciparum and P. knowlesi

Enzyme	Reduction of erythrocyte invasion* mean ± SEM (no. of exp)	
	P. falciparum	*P. knowlesi*
	%	
Chymotrypsin, 0.1 mg/ml	−2.3 ± 4 (3)	90 (1)‡
Neuraminidase, 25 U/ml	54 ± 8 (6)§	−9 ± 21 (3)
Trypsin, 1 mg/ml	78 ± 3 (6)§	2.5 ± 11 (4)
Trypsin plus neuraminidase	93 ± 2 (2)	26 ± 15 (2)

* Percent reduction $= \frac{\text{(Percent infection of control cells)} - \text{(percent infection of enzyme-treated cells)}}{\text{(percent infection of control cells)}} \times 100.$

‡ The resistance of chymotrypsin (0.1 mg/ml) treated erythrocytes to invasion by *P. knowlesi* in one parallel experiment corresponded to the results of published data for six experiments under similar conditions (95 ± 2.5 [SD] percent reduction in invasion) (3, 4, 8).

§ $P < 0.01$ Comparison of invasion of control and enzyme-treated erythrocytes by the paired t test.

treated erythrocytes by one but not the other parasite would suggest that the initial recognition phase is blocked by treatment with proteolytic enzymes and that different proteins or glycoproteins are involved in the recognition of each parasite. Moreover, the observation that erythrocytes remain susceptible to one of the two parasites after enzyme treatment indicates that the enzyme does not cause a nonspecific toxic effect.

We can speculate as to the nature of the *P. falciparum*-specific determinant(s) from the changes in the erythrocyte surface after enzyme treatments. As indicated by periodic acid Schiff staining of sodium dodecyl sulfate-polyacrylamide gels of enzyme-treated erythrocytes, trypsin (1 mg/ml) removes more of the surface sialoglycopeptides (e.g., from glycophorins A and B) than does chymotrypsin (0.1 mg/ml) (8, 10). Trypsin (1 mg/ml) but not chymotrypsin (0.1 mg/ml) causes reduction of invasion by *P. falciparum*. Removal of sialic acid by neura-

minidase treatment also reduced infection. It is possible, therefore, that a sialoglycopeptide is the erythrocyte receptor for *P. falciparum* merozoites. Glycophorin A cannot be the sole receptor for *P. falciparum*, since infection of En(a−) cells, which lack this sialoglycoprotein (11, 12), was not completely inhibited. Further investigations with purified erythrocyte membrane glycoproteins may resolve the nature of the receptor for *P. falciparum*.

Summary

Human erythrocytes lacking various blood group determinants were susceptible to invasion by *Plasmodium falciparum* including Duffy-negative erythrocytes that are refractory to invasion by *Plasmodium knowlesi*. Erythrocytes treated with trypsin or neuraminidase had reduced susceptibility to *P. falciparum* and normal susceptibility to *P. knowlesi*. Chymotrypsin treatment (0.1 mg/ml) blocked invasion only by *P. knowlesi*. The differential effect of enzymatic cleavage of determinants from the erythrocyte surface on invasion by these parasites suggests that *P. falciparum* and *P. knowlesi* interact with different determinants on the erythrocyte surface.

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