Brief Definitive Report

The Domain on the Duffy Blood Group Antigen for Binding *Plasmodium vivax* and *P. knowlesi* Malarial Parasites to Erythrocytes

By Chetan E. Chitnis,* Asok Chaudhuri,‡ Richard Horuk,§ A. Oscar Pogo,‡ and Louis H. Miller*

*From the * Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; ‡Department of Cell Biology, New York Blood Center, New York 10021; and §Department of Immunology, Berlex Biosciences Inc., Richmond, California 94804*

Summary

Plasmodium vivax and the related simian malarial parasite *P. knowlesi* use the Duffy blood group antigen as a receptor to invade human erythrocytes and region II of the parasite ligands for binding to this erythrocyte receptor. Here, we identify the peptide within the Duffy blood group antigen of human and rhesus erythrocytes to which the *P. vivax* and *P. knowlesi* ligands bind. Peptides from the NH_2-terminal extracellular region of the Duffy antigen were tested for their ability to block the binding of erythrocytes to transfected Cos cells expressing on their surface region II of the Duffy-binding ligands. The binding site on the human Duffy antigen used by both the *P. vivax* and *P. knowlesi* ligands maps to a 35–amino acid region. A 34–amino acid peptide from the equivalent region of the rhesus Duffy antigen blocked the binding of *P. vivax* to human erythrocytes, although the *P. vivax* ligand expressed on Cos cells does not bind rhesus erythrocytes. The binding of the rhesus peptide, but not the rhesus erythrocyte, to the *P. vivax* ligand was explained by interference of carbohydrate with the binding process. Rhesus erythrocytes, treated with *N*-glycanase, bound specifically to *P. vivax* region II. Thus, the interaction of *P. vivax* ligand with human and rhesus erythrocytes appears to be mediated by a peptide–peptide interaction. Glycosylation of the rhesus Duffy antigen appears to block binding of the *P. vivax* ligand to rhesus erythrocytes.

Invasion of erythrocytes by *Plasmodium* merozoites is a multistep process that requires a series of specific molecular interactions between the invading merozoite and the target erythrocyte. *P. vivax* and the related simian malarial parasite, *P. knowlesi*, require interaction with the erythrocyte chemokine receptor, also known as the Duffy blood group antigen, to invade human erythrocytes (1–5). Duffy-negative human erythrocytes, which lack the Duffy blood group antigen, are completely resistant to invasion by these parasites. Although *P. knowlesi* is absolutely dependent on the Duffy blood group antigen for invasion of human erythrocytes, *P. knowlesi* can efficiently invade rhesus erythrocytes by Duffy antigen–independent pathways (4). The erythrocyte receptors for *P. knowlesi* involved in these Duffy antigen–independent invasion pathways are not known.

The *P. vivax* and *P. knowlesi* ligands that bind to the Duffy blood group antigen contain a cysteine-rich domain that occurs in a superfamily of *Plasmodium* proteins that serve as host-binding ligands (6). These include ligands that bind erythrocytes during invasion (7, 8), as well as ligands that mediate binding of *P. falciparum*–infected erythrocytes to the endothelium (6, 9, 10). This cysteine-rich domain, referred to as region II, is found in the Duffy-binding proteins of *P. vivax* and *P. knowlesi* (the α gene), in other erythrocyte-binding proteins of *P. knowlesi* (the β and γ genes), and in the *P. falciparum* glycophorin A–binding protein EBA-175 (11). When expressed in Cos cells, region II in the Duffy-binding ligands of *P. vivax* and *P. knowlesi* binds human Duffy-positive erythrocytes with the same specificity as the protein from which it derives (7).

In this paper, we identify the epitope in the Duffy blood group antigen that blocks the binding of Duffy-positive human erythrocytes to *P. vivax* region II that is expressed on the surface of Cos cells. Sequence analysis suggests that the human and rhesus Duffy antigens contain multiple transmembrane stretches with an ~64–amino acid hydrophilic region at the NH_2 terminus that is extracellular (12). Here, we demonstrate that a 35–amino acid peptide from this extracellular portion of the human Duffy blood group antigen blocks the binding of Duffy-positive erythrocytes to region II of the Duffy-binding proteins of *P. vivax* and *P. knowlesi*. We find that the same region of the rhesus Duffy antigen

also blocks binding of *P. vivax* region II to Duffy-positive human erythrocytes, although rhesus erythrocytes do not bind to *P. vivax* region II. Rhesus erythrocytes, however, can bind *P. vivax* region II after treatment with *N*-glycanase, which removes N-linked sugars, indicating that carbohydrates block the receptor on rhesus erythrocytes for *P. vivax*. These studies suggest that the *P. vivax* ligand can bind the peptide backbones of both the human and rhesus Duffy antigens.

Materials and Methods

Recombinant Plasmids for Surface Expression in Cos7 Cells. The plasmid constructs used to express region II of the *P. vivax* Duffy-binding protein (pHVDR22), the *P. knowlesi* Duffy-binding protein (pHKADR22), the *P. knowlesi* β protein (pHKBDR22), and *P. falciparum* EBA-175 (EBA-175 RII) on the Cos cell surface have been described previously (7, 8). Each of these constructs contains DNA sequences encoding region II of the parasite ligands fused with the signal sequence and transmembrane segment of herpes simplex virus glycoprotein D (HSV gD). The fusion proteins are targeted to the Cos cell surface by the signal sequence of HSV gD and are anchored to the surface by the transmembrane segment. These expression plasmids contain a SV40 origin of replication that allows replication in Cos7 cells, as well as a Rous sarcoma virus long terminal repeat that serves as a promoter for expression in Cos7 cells (13).

Cell Culture and Transfection of Cos7 Cells. Cos7 cells (CRL 1651; American Type Culture Collection, Rockville, MD) were cultured in DMEM with 10% heat-inactivated FCS (both from GIBCO BRL, Gaithersburg, MD) in a humidified CO_2 (5%) incubator at 37°C. Fresh monolayers of Cos7 cells were transfected in 3.5-cm-diameter wells with 5 μg of plasmid DNA by the calcium phosphate precipitation method, as described earlier (7). Cells were washed three times in PBS 12–16 h after transfection. Transfection efficiencies were determined by immunofluorescence assays 48–60 h after transfection, as described earlier (7). Ascites containing the mAb DL6 (kindly provided by Drs. Gary Cohen and Roselyn Eisenberg, University of Pennsylvania, Philadelphia, PA) that reacts against amino acids 272–279 of the mature HSV gD protein were used as the primary antibody in the immunofluorescence assays as described earlier (13).

Erythrocyte-binding Assays. Cos7 cells were transfected in 3.5-cm-diam wells and used for erythrocyte-binding assays 40–60 h after transfection, as described earlier (7). Briefly, 100 μl of a 10% erythrocyte suspension was added to 0.9 ml of media in wells containing transfected cells. The plates were swirled to mix the erythrocytes well, and the erythrocytes were allowed to settle for 2 h at 37°C. Nonadherent erythrocytes were removed by washing the Cos7 cells three times with PBS, and the number of transfected Cos7 cells with rosettes of erythrocytes was scored in 20 fields at a magnification of 40 using an inverted microscope.

To study the ability of peptides from the Duffy antigen to inhibit erythrocyte binding, transfected Cos cells were preincubated for 1 h in a 5% CO_2 incubator at 37°C in 0.9 ml of complete DMEM with 10% FCS containing different concentrations (0–100 μM) of peptides. Human or rhesus erythrocytes (100 μl) at a hematocrit of 10% were added to wells containing different concentrations of the peptides and were allowed to bind for 2 h at 37°C in a 5% CO_2 incubator. Nonadherent erythrocytes were removed by washing the Cos7 cells three times with PBS, and the number of rosettes was scored in 20 fields viewed at a magnification of 400 using an inverted microscope. Inhibition curves were drawn for three independent experiments and used to determine concentrations for 50% inhibition.

To study the ability of the chemokine MGSA (melanoma growth–stimulating activity) in inhibiting binding, erythrocytes were preincubated in media containing different concentrations of MGSA (0–1,000 nM) for 1 h at room temperature before they were used in erythrocyte-binding assays, as described earlier (7).

Erythrocytes and Pretreatments with Enzymes. Blood was collected in 10% citrate phosphate dextrose (Baxter, Deerfield, IL) and stored at 4°C for up to 4 wk. Standard blood banking methods using two antisera (anti-Fya and anti-Fyb) were used to determine the Duffy phenotypes. Duffy-positive erythrocytes used in the binding assays had the Fy(a^+b^+) phenotype. Erythrocytes were washed three times in RPMI 1640 (GIBCO BRL) and resuspended to a hematocrit of 10% in RPMI 1640 for use in the erythrocyte-binding assays. Washed human and rhesus erythrocytes were treated with neuraminidase, as described earlier (14). Human, rhesus, and squirrel monkey erythrocytes were deglycosylated with the *N*-glycanase peptide-*N*-glycosidase F from *Flavobacterium meningosepticum* (Oxford Glycosystems, Oxford, U.K.). Washed erythrocytes (100 μl) were incubated with mixing for 1 h at 37°C in 500 μl of PBS containing 10 U of *N*-glycanase and reaction buffer supplied by the manufacturer. The erythrocytes were washed extensively in PBS to remove the enzyme and stop the deglycosylation.

Peptide Synthesis. Peptides were synthesized using an automated synthesizer, as described earlier (15). Mass spectrophotometric and analytical reverse-phase HPLC analyses and amino acid composition were performed to confirm the purity and sequence of the synthetic peptides. In some cases, protein sequence analysis was also performed to check the sequence of the peptides.

Results and Discussion

Region II, the 5′ cysteine-rich region of the *P. vivax* Duffy antigen–binding protein, has been shown to possess erythrocyte-binding properties (7). Transfected Cos cells expressing *P. vivax* region II on the cell surface bind Duffy-positive, but not Duffy-negative, human erythrocytes (7). Here, we have used the Cos cell–binding assay to identify the binding site on the human Duffy antigen that is used in this interaction. A 35–amino acid peptide (HPEP35) from the NH_2-terminal extracellular domain of the human Duffy antigen (Fig. 1 *a*) was tested for its ability to inhibit binding of *P. vivax* region II to human erythrocytes in the Cos cell–binding assay. HPEP35 was chosen because it is recognized by anti-Fy6 (Chaudhuri, A., unpublished data), an mAb to the human Duffy antigen that can block erythrocyte invasion by *P. vivax* in vitro (16). To test the ability of HPEP35 to inhibit binding of human erythrocytes to *P. vivax* region II, Cos cell–binding assays were performed in the presence of increasing concentrations (0–100 μM) of HPEP35. Fig. 2 shows the inhibition curve from one such experiment. HPEP35 inhibits the binding of Duffy-positive human erythrocytes to Cos cells expressing *P. vivax* region II with 50% inhibition at a concentration of 2.9 ± 1.4 μM (Table 1). Three smaller peptides from the 35–amino acid region of HPEP35 were also tested in the inhibition assays (Fig. 1 *b*). The smaller peptides (HPEP13, HPEP22, and HPEP3850) had no effect on the binding of human erythrocytes to re-

a

```
HPEP35      AELSPSTENSSQLDFEDVWNSSYGVNDSFPDGDYD
RHPEP34     AELSPSTQNSSQL_NSDLWNFSYDGNDSFPDVDYD
```

b

```
HPEP35      AELSPSTENSSQLDFEDVWNSSYGVNDSFPDGDYD
HPEP13      AELSPSTENSSQL
HPEP22                        DFEDVWNSSYGVNDSFPDGDYD
HPEP3850        PSTENSSQLDFEDVWNSSYGVNDS
```

Figure 1. Peptides from the NH_2-terminal, extracellular segment of the human and rhesus Duffy blood group antigens. (*b*) Amino acid sequence of peptides from the human Duffy antigen (HPEP35, HPEP13, HPEP22, and HPEP3850). (*a*) Comparison of the rhesus peptide RHPEP34 and the human peptide HPEP35.

gion II of the *P. vivax* protein at concentrations up to 100 μM. HPEP35 also inhibits the binding of human erythrocytes to region II of the *P. knowlesi* Duffy antigen–binding protein with 50% inhibition at a concentration of 4.9 ± 2.3 μM. It thus appears that the *P. vivax* and *P. knowlesi* ligands bind the same site on the human Duffy blood group antigen. In addition, HPEP35 also inhibits the binding of rhesus erythrocytes to region II of the *P. knowlesi* Duffy antigen–binding protein (the α gene), suggesting that the same site within the parasite domain is used for binding to both human and rhesus Duffy blood group antigens.

To rule out the possibility that the inhibition of binding observed with HPEP35 is a nonspecific effect, we tested the ability of HPEP35 to inhibit the binding of erythrocytes to region II of *P. falciparum* EBA-175 and region II of the *P. knowlesi* β protein, neither of which binds the Duffy antigen. Region II of EBA-175 binds sialic acid residues in the context of the glycophorin A peptide backbone, and region II of the *P. knowlesi* β protein binds an as-yet-unidentified receptor on rhesus erythrocytes. HPEP35 had no effect on the binding of human erythrocytes to region II of EBA-175 or the binding of rhesus erythrocytes to region II of the *P. knowlesi* β protein at concentrations up to 100 μM (Table 1).

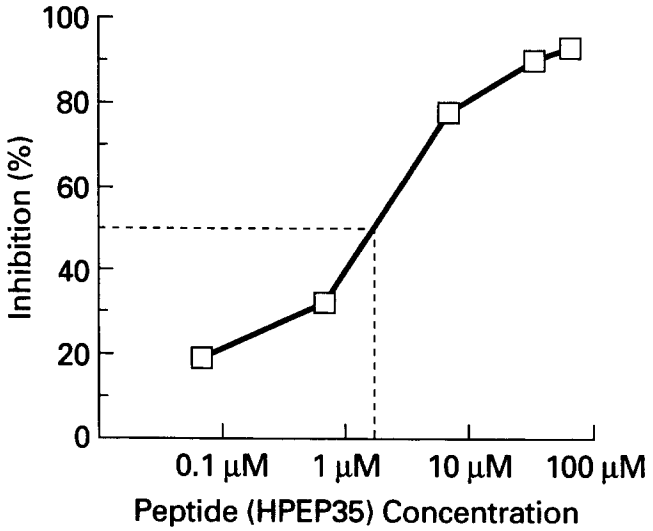

Figure 2. Inhibition of erythrocyte binding to *P. vivax* region II with the peptide HPEP35. An inhibition curve for one experiment in which the peptide HPEP35 is used to inhibit the binding of Duffy-positive human erythrocytes to transfected Cos cells expressing *P. vivax* region II is shown. The concentration for 50% inhibition of erythrocyte binding was determined for each experiment from the inhibition curve as shown.

Table 1. *Inhibition of Erythrocyte Binding to Transfected Cos Cells Expressing Region II Using Peptides from the Human (HPEP35) and Rhesus (RHPEP34) Duffy Antigens**

Region II expressed on Cos cell surface	Erythrocytes	50% inhibition concentration: Mean ± SD	(No. of studies)
A Inhibition of erythrocyte binding with HPEP35			
P. vivax	Human Fy(a$^+$b$^+$)	2.9 ± 1.4 μM	(3)
P. knowlesi α	Human Fy(a$^+$b$^+$)	4.1 ± 2.7 μM	(3)
P. knowlesi α	Rhesus	13.1 ± 6.0 μM	(3)
P. knowlesi β	Rhesus	>100 μM	(2)
P. falciparum EBA-175	Human Fy(a$^+$b$^+$)	>100 μM	(2)
B Inhibition of erythrocyte binding with RHPEP34			
P. vivax	Human Fy(a$^+$b$^+$)	2.1 ± 2.1 μM	(3)
P. knowlesi α	Human Fy(a$^+$b$^+$)	5.5 ± 3.9 μM	(3)
P. knowlesi α	Rhesus	4.9 ± 2.3 μM	(3)
P. knowlesi β	Rhesus	>100 μM	(2)
P. falciparum EBA-175	Human Fy(a$^+$b$^+$)	>100 μM	(2)

*Average concentrations (± SD) for the peptides HPEP35 (Fig. 1 *b*) and RHPEP34 (Fig. 1 *a*) at which 50% inhibition of binding is achieved are shown. Inhibition curves from two to three independent experiments were used to determine the average concentrations at which 50% inhibition is achieved. The highest concentration at which the peptides were tested was 100 μM. For cases where the average concentrations for 50% inhibition is reported as >100 μM, the inhibition was <5% at the highest peptide concentration used (100 μM). Numbers in brackets (No. of studies) show the number of independent experiments used for the determination of the 50% inhibition concentrations.

The amino acid differences between the human and rhesus Duffy antigens in the 35–amino acid region that has been identified as the binding site are shown in Fig. 1 *a*. Compared with the human Duffy antigen, eight amino acid substitutions and a single amino acid deletion are evident in the rhesus sequence in this region (17). To determine whether the corresponding 34–amino acid region of the rhesus Duffy antigen (RHPEP34) serves as the binding site for the *P. knowlesi* Duffy antigen–binding protein, the ability of RHPEP34 to inhibit erythrocyte binding in Cos cell assays was tested (Table 1 *B*). RHPEP34 inhibits binding of rhesus as well as human erythrocytes to region II of the *P. knowlesi* Duffy-binding protein, indicating that RHPEP34 indeed serves as the binding site on the rhesus Duffy antigen for the *P. knowlesi* ligand. As a test for speci-

Table 2. *Attachment of N-Glycanase–treated Rhesus Erythrocytes to Region II of the P. vivax Duffy-binding Ligand**

Erythrocytes	Treatment	Binding
Rhesus	None	−
Rhesus	*N*-glycanase	+
Rhesus	Neuraminidase	−
Squirrel monkey	None	−
Squirrel monkey	*N*-glycanase	+
Human Fy(a^+b^+)	None	+
Human Fy(a^+b^+)	*N*-glycanase	+
Human Fy(a^-b^-)	None	−
Human Fy(a^-b^-)	*N*-glycanase	−

*Transfected Cos cells expressing region II of the *P. vivax* Duffy-binding ligand were tested for binding to rhesus and human erythrocytes. Binding is reported as negative (−) when no rosettes were seen in the entire well. Where binding is reported as positive (+), ~100–200 rosettes are seen in 20 fields viewed at a magnification of 40. In each case, immunofluorescence assays were performed to ensure that *P. vivax* region II was expressed on the Cos cell surface. Transfection efficiencies were in the range of 2–5%.

Table 3. *Inhibition by MGSA of the Attachment of N-Glycanase–treated Rhesus Erythrocytes to Region II of the P. vivax Duffy-binding Ligand**

Region II expressed on Cos cell surface	Erythrocytes	Treatment	50% inhibition concentration (MGSA)
P. knowlesi α	Rhesus	None	5 nM
P. vivax	Rhesus	*N*-glycanase	5 nM, 6 nM
P. knowlesi β	Rhesus	*N*-glycanase	>1 μM

*Normal and *N*-glycanase–treated rhesus erythrocytes were preincubated with different concentrations (0, 0.1, 1, 10, 100, and 1,000 nM) of the chemokine MGSA (melanoma growth–stimulating activity) and used in binding assays with transfected Cos cells expressing *P. vivax* region II. Numbers represent concentrations at which 50% inhibition of binding was achieved. Two separate experiments were performed with *P. vivax* region II and *N*-glycanase–treated rhesus erythrocytes.

ficity, we confirmed that RHPEP34 does not inhibit the binding of human erythrocytes to region II of *P. falciparum* EBA-175 or the binding of rhesus erythrocytes to region II of the *P. knowlesi* β protein, neither of which binds the Duffy antigen (Table 1 *B*). We also tested the ability of RHPEP34 to inhibit the binding of human erythrocytes to region II of the *P. vivax* Duffy antigen–binding protein. Since *P. vivax* region II does not bind rhesus erythrocytes, we expected that RHPEP34, a peptide from the rhesus Duffy antigen, would not inhibit the binding of human erythrocytes to *P. vivax* region II. Unexpectedly, we found that RHPEP34 inhibits the binding of human Duffy-positive erythrocytes to *P. vivax* region II with 50% inhibition at concentrations of 2.1 ± 2.1 μM (Table 1 *B*). Thus, it appears that although *P. vivax* region II does not bind the Duffy antigen on rhesus erythrocytes, a peptide from the rhesus Duffy antigen can block the binding of human erythrocytes to *P. vivax* region II.

One possible reason for this anomalous result with the synthetic peptide is that carbohydrates on the rhesus, but not the human, Duffy blood group antigen may block access of the parasite ligand to the peptide backbone of the receptor molecule. It has been demonstrated that the human Duffy blood group antigen has asparagine-linked glycosylation (18, 19). Presumably, the rhesus Duffy antigen also has asparagine-linked glycosylation, since it contains the same amino acid sequence signals NXS/T as the human sequence (17). To determine whether glycosylation of the rhesus Duffy blood group antigen influences binding, we studied the binding of normal and *N*-glycanase–treated rhesus erythrocytes to *P. vivax* region II. Whereas normal rhesus erythrocytes do not bind *P. vivax* region II, *N*-glycanase–treated rhesus erythrocytes bind the *P. vivax* ligand (Table 2). This indicates that, despite the differences in amino acid sequences, *P. vivax* can bind the peptide backbones of both the human and rhesus Duffy antigens. This explains why RHPEP34, a peptide derived from the rhesus Duffy antigen, inhibits the binding of human erythrocytes to *P. vivax* region II.

Erythrocytes from a new world monkey, the squirrel monkey (*Saimiri sciureus*), do not bind the *P. vivax* Duffy-binding protein (5), although they express the Duffy blood group antigen (16, 17). We found that the squirrel monkey erythrocytes fail to bind to Cos cells expressing *P. vivax* region II, but do bind to Cos cells expressing the *P. knowlesi* Duffy binding protein (α gene) region II (7). *N*-glycanase–treated squirrel monkey erythrocytes, like rhesus erythrocytes, bind to Cos cells expressing *P. vivax* region II (Table 2), indicating that carbohydrates on the Duffy blood group antigen of squirrel monkey erythrocytes also block its binding to the *P. vivax* Duffy-binding protein.

It can be argued that enzymatic treatment with *N*-glycanase reduces the negative surface charge density on erythrocytes and leads to nonspecific binding interactions. Another possibility that must be considered is that *N*-glycanase treatment may create a novel binding epitope independent of the Duffy blood group antigen. To test these possibilities, the following experiments were performed. Since sialic acid residues are the most important source of negative charge on the erythrocyte surface, neuraminidase-treated rhesus erythrocytes were tested for binding to *P. vivax* region II. Neuraminidase-treated rhesus erythrocytes did not bind *P. vivax* region II (Table 2). In the same experiment, neuraminidase-treated human erythrocytes did not bind region II of EBA-175, which requires sialic acid for binding, indicating that sialic acid was removed from the erythrocytes. Reduction of negative charge on the surface cannot, therefore, account for the binding of *N*-glycanase–treated

rhesus erythrocytes to *P. vivax* region II. It was also found that *N*-glycanase–treated, Duffy-negative human erythrocytes do not bind *P. vivax* region II (Table 2). This rules out the possibility that *N*-glycanase treatment may create a novel epitope that binds *P. vivax* region II.

We have previously shown that the chemokine, MGSA, binds the Duffy blood group antigen and can be used to inhibit the binding of *P. vivax* region II to Duffy-positive human erythrocytes (3, 7). MGSA also binds the rhesus Duffy antigen (Horuk, R., unpublished data). Indeed, MGSA inhibits the binding of rhesus erythrocytes to Cos cells expressing region II of the *P. knowlesi* Duffy-binding ligand (the α gene; Table 3). To confirm that *P. vivax* region II binds the peptide backbone of the deglycosylated rhesus Duffy blood group antigen, we tested whether MGSA could inhibit the binding of *N*-glycanase–treated rhesus erythrocytes to *P. vivax* region II. MGSA inhibits the binding of *N*-glycanase–treated rhesus erythrocytes to region II of the *P. vivax* Duffy antigen–binding protein, with 50% inhibition at nanomolar concentrations (Table 3). Inhibition of erythrocyte binding indicates that MGSA and *P. vivax* region II bind the same molecule on *N*-glycanase–treated rhesus erythrocytes, namely the deglycosylated rhesus Duffy antigen. Chemokine and the *P. vivax* Duffy-binding protein, however, do not recognize the same epitope because the 35 mer from the human Duffy antigen does not block binding of chemokines to human erythrocytes (Horuk, R., unpublished data).

One puzzle is why *P. vivax* infects squirrel monkeys, although squirrel monkey erythrocytes do not bind *P. vivax* Duffy-binding protein (5) and also do not bind to Cos cells expressing *P. vivax* region II (this study). Squirrel monkey erythrocytes do contain the reticulocyte receptor to which the *P. vivax* reticulocyte-binding proteins bind (20). Binding to the reticulocyte receptors is responsible for the preferential invasion of reticulocytes by *P. vivax* (20). These interactions are not, however, sufficient for invasion. For example, Duffy-negative human erythrocytes that carry the reticulocyte receptor are not invaded by *P. vivax*. We can speculate that the Duffy blood group antigen on squirrel monkey erythrocytes, despite not being ideal for binding because of carbohydrate modifications, still function in invasion. The erythrocyte-binding assays used may not be sensitive enough to detect this weaker interaction. How does one then explain the refractoriness of rhesus erythrocytes to invasion by *P. vivax* when *P. vivax* region II binds *N*-glycanase–treated rhesus erythrocytes? Rhesus erythrocytes are known to lack the reticulocyte receptor (20) for the *P. vivax* reticulocyte-binding proteins. It is possible that, like the Duffy antigen, the reticulocyte receptor is also absolutely required for invasion. Their absence on rhesus erythrocytes may be the reason for their refractoriness to *P. vivax*.

Differences exist between the nature of the receptor-binding specificities for region II of *P. falciparum* EBA-175 and of the *P. vivax* Duffy-binding ligand. Whereas *P. falciparum* EBA-175 requires both sialic acid and the peptide backbone of glycophorin A (8, 14), the *P. vivax* Duffy-binding ligand requires only the peptide backbone of the Duffy blood group antigen. Both tryptic fragments of glycophorin A containing amino acids 1–64 and the 35–amino acid synthetic peptide from the extracellular domain of the Duffy antigen inhibit binding of erythrocytes to their respective ligands with 50% inhibition at concentrations of ~5 μM (reference 8, and this paper). Thus, peptides derived from the Duffy antigen (in the absence of any carbohydrates) appear to effectively inhibit erythrocyte binding by the *P. vivax* ligand. Furthermore, rhesus erythrocytes can bind *P. vivax* region II after treatment with *N*-glycanase. We conclude that the receptor–ligand interactions that mediate erythrocyte invasion differ in the protein to which they bind and in the requirement for carbohydrate. The receptor for *P. falciparum* EBA-175 consists of both carbohydrate and the peptide backbone of glycophorin A; the *P. vivax* Duffy-binding ligand interacts only with the peptide backbone of the receptor molecule.

We thank Dr. John Coligan and the Biological Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, for synthesizing the peptides used in this study and Diana Hudson-Taylor for performing some of the erythrocyte binding assays.

This research was supported in part by a grant to A.O. Pogo (HL 53927) from the National Heart, Lung and Blood Institute, National Institutes of Health.

Address correspondence to Louis H. Miller, Building 4, Room 10, 9000 Rockville Pike, National Institutes of Health, Bethesda, MD 20892. The present address for Chetan Chitnis is International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi, India 110067.

Received for publication 16 April 1996 and in revised form 25 July 1996.

References

1. Miller, L.H., S.J. Mason, J.A. Dvorak, M.H. McGinnis, and I.K. Rothman. 1975. Erythrocyte receptors for (*Plasmodium knowlesi*) malaria: Duffy blood group determinants. *Science (Wash. DC).* 189:561–563.
2. Miller, L.H., S.J. Mason, D.F. Clyde, and M.H. McGinnis. 1976. The resistance factor to *Plasmodium vivax* in blacks:

Duffy blood group genotype, FyFy. *N. Engl. J. Med.* 295: 302–304.

3. Horuk, R., C.E. Chitnis, W.C. Darbonne, T.J. Colby, A. Rybicki, T.J. Hadley, and L.H. Miller. 1993. A receptor for the malaria parasite *Plasmodium vivax*: the erythrocyte chemokine receptor. *Science (Wash. DC).* 261:1182–1184.
4. Haynes, D.J., J.P. Dalton, F.W. Klotz, M.H. McGinnis, T.J. Hadley, D.E. Hudson, and L.H. Miller. 1988. Receptor-like specificity of a *Plasmodium knowlesi* malarial protein that binds to Duffy antigen ligands on erythrocytes. *J. Exp. Med.* 167: 1873–1881.
5. Wertheimer, S.P., and J.W. Barnwell. 1989. *Plasmodium vivax* interaction with the human blood group glycoprotein: identification of a parasite receptor-like protein. *Exp.Parasitol.* 69: 340–350.
6. Su, X.Z., V. Heatwole, S.P. Wetheimer, F. Guinet, J.A. Herrfeldt, D.S. Peterson, J.A. Ravetch, and T.E. Wellems. 1995. The large diverse gene family *var* encodes proteins involved in cytoadherence and antigenic variation of *Plasmodium falciparum*-infected erythrocytes. *Cell.* 82:89–100.
7. Chitnis, C.E., and L.H. Miller. 1994. Identification of the erythrocyte binding domains of *Plasmodium vivax* and *Plasmodium knowlesi* proteins involved in erythrocyte invasion. *J. Exp. Med.* 180:497–506.
8. Sim, B.K.L., C.E. Chitnis, K. Wasniowska, T.J. Hadley, and L.H. Miller. 1994. Receptor and ligand domains for *Plasmodium falciparum* malaria invasion of erythrocytes. *Science (Wash. DC).* 264:1941–1944.
9. Smith, J.D., C.E. Chitnis, A.G. Craig, D.J. Roberts, D.E. Hudson-Taylor, D.S. Peterson, R. Pinches, C.I. Newbold, and L.H. Miller. 1995. Switches in expression of *Plasmodium falciparum var* genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. *Cell.* 82: 101–110.
10. Baruch, D.I., B.L. Pasloske, H.B. Singh, B. Xiahui, X.C. Ma, M. Feldman, T.F. Taraschi, and R.J. Howard. 1995. Cloning the *Plasmodium falciparum* gene encoding PfEMP-1, a malarial variant antigen and adherence receptor on the surface of parasitized human erythrocytes. *Cell.* 82:77–87.
11. Adams, J.H., B.K.L. Sim, S.A. Dolan, X. Fang, D.C. Kaslow, and L.H. Miller. 1992. A family of erythrocyte binding proteins of malaria parasites. *Proc. Natl. Acad. Sci. USA.* 89:7085–7089.
12. Chaudhuri, A., J. Polyakova, V. Zbrezna, K. Williams, S. Gulati, and A.O. Pogo. 1993. Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for the *Plasmodium vivax* malaria parasite. *Proc. Natl. Acad. Sci. USA.* 90:10793–10797.
13. Cohen, G.H., W.C. Wilcox, D.L. Sodora, D. Long, J.Z. Levin, and R.J. Eisenberg. 1988. Expression of herpes simplex virus type1 glycoprotein D gene deletion mutants in mammalian cells. *J. Virol.* 62:1932–1940.
14. Camus, D., and T.J. Hadley. 1985. A *Plasmodium falciparum* antigen that binds to host erythrocytes and merozoites. *Science (Wash. DC).* 230:553–556.
15. Parker, K.C., B.M. Carreno, L. Sestak, U. Utz, W.E. Biddison, and J.E. Coligan. 1992. Peptide binding to HLA-A2 and HLA-B27 isolated from *Escherichia coli. J. Biol. Chem.* 267: 5451–5459.
16. Barnwell, J.W., M.E. Nichols, and P. Rubenstein. 1989. In vitro evaluation of the role of the Duffy blood group in erythrocyte invasion by *Plasmodium vivax. J. Exp. Med.* 169: 1795–1802.
17. Chaudhuri, A., J. Polyakova, V. Zbrzezna, and A.O. Pogo. 1995. The coding sequence of Duffy blood gene in humans and simians: restriction fragment length polymorphism, antibody and malarial parasite specificities, and expression in nonerythroid tissues in Duffy-negative individuals. *Blood.* 85: 615–621.
18. Tanner, M.J.A., D.J. Anstee, G. Mallinson, K. Ridgwell, P.G. Martin, N.D. Aventi, and S.F. Parsons. 1988. Effect of endoglycosidase *F*-peptidyl *N*-glyosidase F preparations on the surface components of the human erythrocyte. *Carbohydr. Res.* 178:203–212.
19. Chaudhuri, A., and A.O. Pogo. 1995. Blood Cell Chemistry. Vol. 6. Molecular Basis of Major Human Blood Group Antigens. J.-P. Cartron and P. Rouger, editors. Plenum Press, New York. 243–265.
20. Galinski, M.R., C.C. Medina, P. Ingravallo, and J.W. Barnwell. 1992. A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites. *Cell.* 69:1213–1226.