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

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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 NH2-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 NH2 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 and 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 CO2 (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-diameter 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% CO2 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% CO2 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).

**Erythrocyte 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 Fya(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 NH2-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-
region II of the P. vivax protein at concentrations up to 100 
M. HPEP35 also inhibits the binding of human erythro-
cytes to region II of the P. knowlesi Duffy antigen—binding 
protein with 50% inhibition at a concentration of 4.9 ± 2.3 
M. It thus appears that the P. vivax and P. knowlesi ligands 
bond the same site on the human Duffy blood group anti-
gen. In addition, HPEP35 also inhibits the binding of 
rhesus erythrocytes to region II of the P. knowlesi Duffy an-
tigen—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 erythro-
cytes 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-uniden-
tified receptor on rhesus erythrocytes. HPEP35 had no ef-
flect 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 
M (Table 1).

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 evi-
dent in the rhesus sequence in this region (17). To deter-
mine 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 bind-
ing 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 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 | 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 | >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 | >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 
concentration 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.

Figure 1. Peptides from the NH₂-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.

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 hu-
man 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.
we studied the binding of normal and N-glycanase-treated
to the region II of the EBA-175 receptor molecule. It has been demonstrated that the hu-
man sequence (17). To determine whether glycosylation of
the same amino acid sequence signals NXS/T as the hu-
tman Duffy blood group antigen has asparagine-linked gly-
cosylation, since it contains
amino acid residues, which bind to the EBA-175 receptor.
region II was expressed on the Cos cell surface. Transfection efficiencies
were in the range of 2–5%.

| 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-bind-
ing ligand were tested for binding to rhesus and human erythrocytes.

ficiency, 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 eryth-
rocytes 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 hu-
manspecific Duffy group antigen has asparagine-linked gly-
cosylation (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 hu-
manspecific 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-gly-
canase–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
region II (Table 2). In the same experiment,
region II did not bind the
Duffy antigen to Cos cells expressing
P. vivax Duffy-binding ligand
region II, and P. knowlesi–treated
squirrel monkey erythrocytes, like rhesus erythro-
region II. Numbers represent concentrations at which 50% inhibition
ofbinding was achieved. Two separate experiments were performed with
P. vivax region II and N-glycanase–treated rhesus erythrocytes.

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 | -       |

*Normal and N-glycanase–treated rhesus erythrocytes were preincu-
bated 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.

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) |
|----------------------------------------|--------------|-----------|----------------------------------|
| Human Fy(a+b+)                          | Rhesus       | None      | 5 nM                             |
| Human Fy(a+b+)                          | Rhesus       | N-glycanase | 5 nM, 6 nM                      |
| Human Fy(a+b+)                          | Rhesus       | N-glycanase | >1 μM                           |

**Normal and N-glycanase–treated rhesus erythrocytes were preincu-
bated 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.

It can be argued that enzymatic treatment with N-glyca-
nase reduces the negative surface charge density on erythro-
cytes and leads to nonspecific binding interactions. Another
possibility that must be considered is that N-glycanase treat-
ment 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 re-
region II of EBA-175, which requires sialic acid for binding,
indicating that sialic acid was removed from the erythro-
cytes. Reduction of negative charge on the surface cannot,
therefore, account for the binding of N-glycanase–treated

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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 glycoporphin A (8, 14), the *P. vivax* Duffy–binding ligand requires only the peptide backbone of the Duffy blood group antigen. Both tryptic fragments of glycoporphin 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 glycoporphin A; the *P. vivax* Duffy–binding ligand interacts only with the peptide backbone of the receptor molecule.

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